

**OPPORTUNISTIC AND OTHER INTESTINAL  
PARASITES AMONG HIV/AIDS PATIENTS IN  
ETHIOPIA**

**BY  
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## **Declaration**

I, the under signed declare that this thesis is my original work and has not been presented for a degree in any other university.

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## II. ABBREVIATIONS

A	Adenine
AA	Adenine- Adenine
ACC	Adenine-Cytosine-Cytosine
ACT	Adenine-Cytosine-Thymine
AE	Elusion buffer
AG	Adenine-Guanine
AGC	Adenine- Guanine-Cytosine
AGT	Adenine-Guanine-Thymine
ARG	Adenine-Arginine- Guanine
ATT	Adenine-Thymine-Thymine
AIDS	Acquired Immunodeficiency Syndrome
Al	<i>Ascaris lumbricoides</i>
ART	Antiretroviral therapy
AW1 buffer	Washing buffer 1
AW2 buffer	Washing buffer 2
bp	Base pair
BM/D	Bowel motion per day
C	Cytosine
CA	California
CAA	Cytosine-Adenine-Adenine
CAC	Cytosine-Adenine-Cytosine
CAT	Cytosine-Adenine-Thymine
CCA	Cytosine-Cytosine-Adenine
CCC	Cytosine- Cytosine- Cytosine

CD4	Cluster of Differentiation (cells)
CGT	Cytosine-Guanine-Thymine
CRY	Cytosine-Arginine-Tyrosine
CTC	Cytosine-Thymine- Cytosine
CIM	<i>Cryptosporidium-Isospora-Microsporidia</i>
DNA	Deoxyribo Nucleic Acid
D&Conc.	Direct and Concentration Method
EDTA	Ethylenediamine Tetraacetic Acid
ENARP	Ethio-Netherlands AIDS Research Project
Eh/d	<i>Entamoeba histolytica/dispar</i>
EHNRI	Ethiopian Health and Nutrition Research Institute
ELISA	Enzyme Linked Immunosorbent Assay
ESTC	Ethiopian Science and Technology Commission
G	Guanine
GCC	Guanine Cytosine- Cytosine
GCC	Guanine Cytosine- Cytosine
GCT	Guanine Cytosine- Thymine
GeneAmp	Gene amplifier
GGA	Guanine-Guanine-Adenine
GGG	Guanine-Guanine- Guanine
GGT	Guanine-Guanine-Thymine
GTT	Guanine-Thymine-Thymine
GI	<i>Giardia lamblia</i>
gm	gram
HAART	Highly Active Antiretroviral Therapy

HC	High Concentration (Super Taq)
HCl	Hydrochloric acid
HIV	Human Immunodeficiency Virus
HPF	High Power Field
HT	Hoffmann's biotechnology
IDSA	Infectious Diseases Society of America
KCl	Potassium chloride
KGT	Lysine- Guanine-Thymine
KH <sub>2</sub> PO <sub>4</sub>	Potassium dihydrogen Phosphate
HW	Hookworm
LUMC	Leiden University Medical Centre
MgCl <sub>2</sub>	Magnesium Chloride
MOH	Ministry of Health
MSP	Microsporidia Specific Primers
MZN	Modified Ziehl Neelsen
μl	Microlitre
μm	Micrometre
NaCl	Sodium Chloride
Na <sub>2</sub> HPO <sub>4</sub>	Disodium hydrogen Phosphate
NASBA	Nucleic Acid Sequence Based Assay
NEC	National Ethical Committee
nm	nano metre
nm	nano mole
OIP	Opportunistic Intestinal Parasites
PBS	Phosphate Buffer Saline

PCR	Polymerase Chain Reaction
Pmol	Pico mole
PVPP	Polyvinylpolypyrrolidone
RBC	Red Blood Cells
RNA	Ribonucleic acid
rpm	Revolution per minute
SAF	Sodium acetate-Acetic acid-Formaldehyde
SHELIA	Soluble Hybridisation Enzyme Linked Immunosorbent Assay
SM	<i>Schistosoma mansoni</i>
Sstr	<i>Strongyloides stercoralis</i>
T	Thymine
TAC	Thymine-Adenine-Cytosine
TAT	Thymine-Adenine-Thymine
TBE	Tris buffer with Boric acid and EDTA
TCA	Thymine-Cytosine-Adenine
TCG	Thymine-Cytosine-Guanine
TGA	Thymine-Guanine-Adenine
TGC	Thymine-Guanine-Cytosine
TGT	Thymine-Guanine-Thymine
TTA	Thymine- Thymine-Adenine
Tt	<i>Trichuris trichiura</i>
UK	United Kingdom
UNAIDS	Joint United Nations program on HIV/AIDS
UNICEF	United Nations Children's Fund
USPHS	United States Public Health Services

USA	United States of America
UV	Ultra Violet
WHO	World Health Organisation

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## VI. ABSTRACT

### OPPORTUNISTIC AND OTHER INTESTINAL PARASITES AMONG HIV/AIDS PATIENTS IN ETHIOPIA

Rapid expansion of HIV/AIDS pandemic has brought about a dramatic change in the fauna of intestinal parasites worldwide. In HIV/AIDS patients, opportunistic intestinal parasites (OIP) are seriously causing hard- to-control diarrhoea in Africa. The well-known OIP that cause diarrhoea at latest stage of HIV infection are *Cryptosporidium parvum*, *Isospora belli*, *Enterocytozoon bieneusi*, *Encephalitozoon intestinalis* and *Cyclospora cayetenensis*. The determination and recognition of these newly emerging parasites in immunocompetent, immunocompromised and HIV/AIDS patients was facilitated and increased with the advent of newly improved diagnostic methods. However, no detailed investigation of these parasites exists in Ethiopia. HIV/AIDS is also one of the major public health problems in Ethiopia severely affecting the productive and reproductive age groups of the society. The present study was aimed at investigating the relationship of OIP and HIV/AIDS in diarrhoea patients in Ethiopia . The assessment of the chemotherapeutic effect of intestinal parasites in the Wonji HIV/AIDS natural history study cohort showed that follow up visits of the cohort population based on scheduled regular visit diagnosis and treatment resulted in an impressive sustainable control of intestinal parasitic infections as compared to patients that visited Wonji hospital without such follow up. The Hospital setting was found more appropriate to study the relationship of intestinal parasites with emphasis on OIP in diarrhoeal patients with HIV/AIDS. After informed consent was obtained, 330 diarrhoeal patients were recruited. Clinical data and biological samples were collected. Blood was processed for HIV-testing by using ELISA and reactive samples were confirmed by western blot. CD4+cell count was done by FACScan, and viral load by NASBA. Stool was processed for parasites including ova, by direct and formol-ether method, Modified Ziehl Neelsen for *Cryptosporidium*, *Isospora* and

*Cyclospora*, Autofluorescence for *Cyclospora* and Flurochrome Uvtitex-2B and nested PCR for intestinal microsporidia. Out of 330 diarrhoeal patients examined for intestinal parasites 268(81.2%) were positive for one or more parasites; and of these 74.2% were HIV positive. The major clinical presentations such as chronic diarrhoea lasted > 4weeks, severe weight loss>10%, and anorexia were more common in HIV positive than HIV negative patients. 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* were significantly higher in HIV negatives than HIV positives (P<0.001). Among the intestinal protozoan, *Blastocystis hominis* was frequently observed in HIV positive (36.3%) and HIV negative (31.8%) with no significant difference. The OIP were significantly higher in HIV positive diarrhoea patients: *C. parvum* 28.6%, *I. belli* 22.5% and intestinal microsporidia 18.2% (P<0.001). Except 6 cases (7.1%) of *C. parvum* no other OIP were detected in HIV negative patients. Based on PCR and microscopic analysis of the stool; microsporidia species involved as single and double infection of *Enterocytozoon bienersi* and *Encephalitozoon intestinalis* were identified for the first time from HIV/AIDS patients in Ethiopia. The OIP were more frequently found with the CD4+ cell count below 50 cells/mm<sup>3</sup> and except for a few cases of *C. parvum* and *I. belli*, the majority were found at CD4+ below 200 cells/mm<sup>3</sup>. Most of the OIP were found in association with high viral load (above 10000 copies/ml); *Cryptosporidium* 90%, *Isospora* 82.4% and intestinal microsporidia 89.3%. Most *C. parvum* and *I. belli* infected cases were detected from AIDS patients at stage IV while intestinal microsporidia were from both stage III and IV cases. From this study, it is suggested that early diagnosis of diarrhoeal patients for HIV and OIP is important to understand and management of diarrhoeal illness. This study also revealed that the majority

of OIP were noted at CD4+ below 50 cells/mm<sup>3</sup> and below 100cells/mm<sup>3</sup> .Thus, it is advisable to initiate HAART (in this cases most likely at 200 cells/mm<sup>3</sup>) for HIV/AIDS patients in order to control the risk of developing diarrhoea disease by OIP.

## 1. INTRODUCTION

Current estimates showed 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 (Bundy et.al., 1992; Saviola et.al., 1996; Chan, 1997; Albonico,et.al., 1999; Fincham, et.al., 2003; De Siliva 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 (Lockwood and Weber, 1989; Gomez Morales, et.al., 1995). 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 developing countries are some of the main factors (WHO, 1987; Savioli, et.al., 1992; Franzer and Muller, 1999).

An increasing number of population mainly in Africa and many parts of the developing world is severely immunocompromised because of human immunodeficiency virus (HIV) infection. As a result, some intestinal parasites are among the main health problems in HIV/AIDS patients as concomitant infections due to depleted immunity. The opportunistic intestinal parasites are the major problems in such group of patients (Lockwood and Weber, 1989; Goodgame, 1996; Kaplan, et.al., 1996). And the advent of newly improved diagnostic techniques to identify opportunistic intestinal parasites has increased intestinal parasite detection and recognition both in immunosuppressed and immunocompetent individuals.

### **1.1. Intestinal Parasitic Infections in Ethiopia**

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 (WHO, 1981; Teka, 1984; WHO, 1987). In addition, lack of awareness of simple health promotion practices is also a contributing factor ( Zein, 1988; Kloos and Tesfayohannes, 1993). According to the Ethiopian Ministry of Health (MOH, 1996), more than half a million annual visits of the out patient services of the health institutions are due to intestinal parasitic infections. However, this report may be an underestimate, 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 to peripheral health institutions.

Even though inadequate data exists on the prevalence and transmission dynamics of intestinal parasites in different eco-epidemiological and geographical regions of the country, the presence of all human intestinal parasites that are commonly encountered in developing countries, with the exception of some parasites of Asia and the Far East have been recorded by different investigators (MacConnel and Armstrong, 1976; Tatichefi et al., 1981, Tedla, 1986; Tedla, 1989; Lo, et.al., 1989; Kloos and Tesfayohannes, 1993; Melakberhan, et.al., 1993; Birrie and Ericko, 1995; Jemaneh, 2000). However, some of the investigators mainly focused on helminth parasites whereas others on both intestinal protozoa and helminth parasites.

Based on variation in climatic and geographic zones in Ethiopia, it should be evident that there are macro and micro- environmental factors contributing to the differences in the

distribution and prevalence of intestinal parasites (Tedla and Ayele, 1986; Jemaneh, 2000). Various investigations conducted on *Schistosomiasis mansoni* and geohelminths in Ethiopia have shown the far reaching implication of helminth infections to public health in many Ethiopian communities ( Tedla,et.al.1982; Tesfayohannes, 1987; Tedla and Yimam, 1987; Tedla, 1989; MelakeBerhan, et.al.,1993; Jemaneh, 2000 ). It was also reported that intestinal helminthiasis is either the first (Teka, 1984) or the second (Tedla, 1989 ) reason for hospital visits in Ethiopia. Furthermore, varying degrees of prevalence rates of intestinal parasites have been reported depending on the climatic, altitudinal and the microenvironmental factors of different ecological zones in different communities; a 93% prevalence among Falasha immigrants in Israel being the highest ever reported ( Berger et.al., 1989 ). This could be attributed to the application of different modern stool diagnostic techniques, stool collection methods and experience of the technical staff.

Among the common intestinal parasites, *Schistosoma mansoni* has been recorded in all regions of the country and about 19 to 20 million people are assumed to live at risk of infection (Birrie, et.al, 1989; Woldemichael and Kebede,1996; Woldemichael, et.al., 1999; Fontanet et.al., 2000b; Jemaneh, 2000; Tiruneh, et.al., 2001; Ericko, et.al.,2002 ). The two soil-transmitted intestinal parasites, *Ascaris lumbricoides* and *Trichuris trichiura* are frequently reported as co-existing and are highly prevalent in the country; their prevalence increasing with altitude (Tedla and Ayele, 1986; Jemaneh, 1998). Their highest prevalence was reported at an altitude more than 2400 meters above sea level, commonly affecting children of school age (Jemaneh, 1998 ).

The other soil transmitted helminth parasites widespread in Ethiopia are the two human hookworm species *Necator americanus* and *Ancylostoma doudenale* (Armstrong and Tadesse,

1975). Both species occur sympatrically although *Necator americanus* has a high preponderance over *Ancylostoma duodenale* (Jemaneh and Tedla, 1984; Tedla and Jemaneh, 1985). The highest prevalence of hookworm infection is reported from low lands of the country with 60-80 % prevalence in the altitudinal range of 800-1200 meters above sea level (Tedla and Jemaneh, 1985; Jemaneh, 1998). The other helminth parasite that occupies almost the same geographical range as the hookworms in Ethiopia is *Strongyloides stercoralis*. *S. stercoralis* infection was reported in 41 out of 50 communities surveyed with its prevalence ranging from 0% at 2800m to 44.0% at 2100m (McConnell and Armstrong, 1976). In a cohort intake population in Wonji Sugar Estate, central Ethiopia, Fontanet et.al (2000a) had reported a prevalence of 8% and 13% in HIV-negative and HIV positive subjects, respectively. The infection is rare or absent in the arid low land areas ( Tesfayohannes, 1987; Jemaneh, 2000 ).

In addition, Tatichef et.al. (1981) had reported 1.0% of *Fasciola* spp. and 0.6% for *Isospora belli* infection among pre-school children in Addis Ababa. Based on the national referral diagnostic result of 11,737 stool samples, the Annual Report of the Ethiopian Health and Nutrition Research Institute (1994/95), had indicated 0.3% for *Fasciola* spp., 0.03% for *Cryptosporidium parvum*, 0.02% for *Isospora belli* and 0.02% for *Sarcocystis* spp.

Among the common diarrhoeogenic intestinal protozoan parasites, amoebiasis caused by *Entamoeba histolytica* and giardiasis caused by *Giardia lamblia* are the commonly reported infections in Ethiopia (McConnell and Armstrong, 1976; Kloos and Tesfayohannes, 1993). A wide range in prevalence of *E. histolytica* infection was reported: an infection rate of 55% among the isolated Say Say shifting cultivators in the Blue Nile gorge (Fuller-Torrey,1966) and in a study conducted in 50 farming communities on central and northern plateaus of the

country, the parasite was identified in 94% of the study communities with infection rates ranging from 3% to 50% with an average of 19% of the study population infected (McConnell and Armstrong, 1976).

In a recent study on the epidemiology of infections with intestinal parasites and HIV-1 in Wonji, *E.histolytica/dispar* was the most common parasite with a prevalence of 25% (Fontanet et.al., 2000a). In these studies no differentiation was made between *E.histolytica* and *E.dispar*. However, the study conducted in the same locality by Kebede et.al (2003) using PCR-SHELIA reported 21 cases of *E. dispar* out of 232 diarrhoea patients, and no case of pathogenic *E.histolytica* was detected. Gatti et.al., (1998 ) by using isoenzyme characterization, had reported 27 cases of *E. dispar* zymodemes and 2 cases of *E.histolytica* zymodemes from 123 individuals from Wonji. As this study indicates, the true *E.histolytica* infection may be rare and needs an in-depth study including an upgrading of the diagnostic techniques in different health institutions.

*G.lamblia* caused diarrhoea is widely reported with different infection rates in Ethiopia. Ninety-eight percent of the 50 communities studied by McConnell and Armstrong (1976) in the central and northern highlands of Ethiopia had *G.lamblia* infection; with the overall prevalence of 11%, ranging from 3 to 23%. A Study conducted in pre-school children indicated a prevalence of 9.3% (Tatichef et.al.,1981). In addition, a country wide survey of giardiasis in conjunction with *Schistosomiasis mansoni* was conducted between 1979 and 1993 including a total of 93 communities in various parts of Ethiopia among school children and other residents. The study indicated that there is no variation in prevalence with altitude. The result showed an overall prevalence of 8.9% among school children and 3.1% among non-school residents (Birrie and Eriko,1995).

## **1.2. Human Immunodeficiency Virus (HIV-1) Infection in Ethiopia.**

The HIV-1 epidemic began in the early 80's in Ethiopia. The first positive case was identified in 1984, based on the retrospective analysis of sera collected for other purposes (Tsige, et.al., 1988 ). The first AIDS cases were diagnosed in Addis Ababa Hospitals in 1986 ( Lester,et.al., 1988 ). Since then a few records have been available on the magnitude of the HIV-1 epidemic in Addis Ababa, and the prevalence recorded was 25% in commercial sex workers in 1989 (Mihret, et.al., 1990), 11% in pregnant women of the inner city in 1991. At present, HIV epidemic is highly distributed through out the country affecting the population severely (Mekonnen, 2003). The prevalence of HIV-1 epidemic in Ethiopia is approximately 4.4% (with lowest prevalence 2.8% to highest prevalence 6.7%) in the adult urban population (UNAIDS/UNICEF/WHO, 2004).

In 2000 and 2001, various studies estimated that HIV infection in Ethiopia range from 2.1 to 3.0 million people. An estimated 117,000 to 208,000 people in the age range 15 to 49 died of AIDS in 2001 alone (UNAIDS, 2002). However, these are crude estimates and the incidence of HIV infections and AIDS cases apparently continue to rise rapidly (Kloos et.al., 2003). Recent information indicated that 1.5 to 2.1 million people are living with HIV infection (UNAIDS/UNICEF/WHO, 2004). HIV/AIDS is now a major cause of mortality in the age group of 15-49 years old, affecting the most productive and reproductive group of the society.

In 1997, the Ethio-Netherlands AIDS Research Project (ENARP) was established at the Ethiopian Health and Nutrition Research Institute. The study had two cohort sites ; Wonji 110 Km South East of Addis Ababa and Akaki 30 km from Addis Ababa. The study aimed at understanding the natural history and spread of HIV/AIDS disease in Ethiopia. Up to 2002, a

total of about 1670 subjects joined the cohort from both sites. Study on intestinal parasitic infections in relation to HIV/AIDS was initially planned to be conducted in these cohort sites. But because of the following reasons the Wonji and Akaki cohorts were found to be inappropriate population to evaluate the relation between intestinal parasites and HIV/AIDS in the Ethiopian context:

- a) The base-line information concerning intestinal parasites collected from both cohorts sites were not obtained by using the appropriate techniques required to determine specific infections, especially opportunistic intestinal parasitic infections,
- b) Starting from the intake, cohort participants were repeatedly treated whenever they had complaints of intestinal ailments; thus interfering with relevant base-line information collection on intestinal parasites,
- c) The number of HIV positive individuals and actual AIDS cases at the two cohort sites were too few for follow up.

For these reasons it was decided to shift the study population on diarrhoeal HIV/AIDS patients in a hospital setting.

Nevertheless, it was learnt that repeated treatment intervention had impacts on intestinal parasites among cohort populations. Thus, the data obtained so far from Wonji cohort is considered for the assessment of repeated treatment intervention that brought about drastic reduction in intestinal parasites.

### **1.3. Intestinal Parasitic Infections in HIV/AIDS Patients**

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 to be sporadic or zoonotic infections, have become opportunistic parasites causing uncontrollable life-

threatening diarrhoea (Wittner et.al, 1993; Weiss and Keohane, 1997; Lindo, et.al.,1998 ). As compared to developed countries, the prevalence of opportunistic intestinal parasites is expected to be higher in developing countries among HIV infected population. This is also reflected by the prevalence of opportunistic intestinal parasites in a given geographical locality among the general population (Lindo et.al, 1998, Cimerman et.al., 1999). HIV infection has been shown to predispose the patient to intracellular opportunistic intestinal parasites such as *Cryptosporidium parvum*, *Isospora belli*, *Cyclospora cayetanensis*, *Enterocytozoon bieneusi*, *Encephalitozoon intestinalis* (Wittner et.al.,1993; Goodgame, 1996; Ortega and Sterling, 1996; Weiss and Keohane, 1997). This does not seem to be the case with extracellular intestinal parasites such as *Ascaris lumbricoides*, *Trichuris trichiura*, Hookworm Spp., *Giardia lamblia*, and others.

Some studies have indicated that compared to the general population, there is relatively lower prevalence of non-opportunistic extracellular intestinal parasites in HIV/AIDS patients ( Gomze Morales, et.al., 1995; Lindo, et.al, 1998 ). Although differences in exposure may not be ruled out, it is suggested that HIV- induced enteropathy may not create conducive environment for the establishment of extracellular intestinal parasites (Debellatta and Moitti, 1992; Wittner, 1993). Others have also argued that mucosa dwelling parasites may benefit from HIV-induced pathological changes and the reduced immune response due to HIV infection (Goodgame, 1996), which creates suitable environment for opportunistic intestinal parasites in HIV/AIDS patients (Lindo, et.al. 1998). Most clinical manifestations of HIV/AIDS patients results either from the reactivation of pre-existing latent pathogens, as the individuals become immunosuppressed or is caused by exposure to locally predominant pathogens. Consequently, clinical presentations of AIDS and the pathogens responsible in

different geographical areas reflect the differing prevalence of opportunistic intestinal parasitic infections in a given community (Colebunders et.al., 1988; Lindo, et.al., 1998).

### **1.3.1. Diarrhoea and HIV/AIDS**

Diarrhoea is a common clinical manifestation of HIV infections both in the developing (90%) and the developed (30-50%) countries (Colebunders, et.al., 1988). Its cause could be quite variable: bacterial, viral or parasitic (commonly opportunistic intestinal parasites) (Germani, et.al., 1998; Kelly, 1998 ). Chronic diarrhoea lasting for more than one month is one of the major complaints of AIDS patients occurring in about 40% of cases and it is one of the WHO-staging criteria for AIDS (WHO, 1993). It has been shown that at least 40-80% of AIDS patients report diarrhoeal episodes during their illness (Kelly, 1998). About 50% of chronic diarrhoea in AIDS patients may be explained by enteric infections with one or more species of pathogenic organisms, commonly opportunistic ones (Bartlett et.al, 1992). Gut architectural alteration secondary to local HIV infection, (usually referred to as HIV enteropathy) a condition characterizing chronic diarrhoea in AIDS patients in whom no identifiable aetiological agent has been found for the diarrhoea (Kotler et.al.,1984; Bartlett, et.al, 1992). Patients with this syndrome have malabsorption, and small bowel histology revealing villous blunting and chronic inflammation (Kotler, et.al., 1984). There is another view that enteropathy syndrome may not represent a direct effect of HIV on gut mucosa, but rather could be due to opportunistic enteric pathogens that are difficult to detect and therefore as yet undiagnosed (Colebunders, et.al., 1988; Bartlett et.al, 1992; Dallabetta and Miotti, 1992).

It is also held that HIV related enteropathy is not only the cause of unexplained diarrhoea, but may also create favourable environment for the invasion of intracellular opportunistic intestinal parasites (Dallabetta and Miotti, 1992; Germani et.al., 1998).

### **1.3.2. Spectrum of intestinal parasites in HIV/AIDS patients**

HIV/AIDS pandemic has brought about a great change in intestinal parasite fauna. As the spectrum of immunodeficiency progresses, HIV infected individuals become susceptible to a variety of opportunistic parasite infections that occur with greater frequency and severity. Almost 80% of AIDS patients die from AIDS-related infections including intestinal parasites rather than HIV infection itself (Kelly, 1998). Several intestinal parasites previously considered non- pathogenic or with transient pathogenic potential in immunocompetent individuals are opportunistically becoming aggressive and causing debilitating illness in HIV/AIDS patients. Most of these infections are caused by organisms that do not normally affect immunocompetent individuals (Kaplan, et.al, 1996).

The principal pathogenic intestinal parasites commonly reported as opportunistic and that cause chronic diarrhoea in HIV/AIDS patients are *Cryptosporidium parvum*, *Isospora belli*, *Cyclospora cayatanensis* and intestinal microsporidia (*Enterocytozoon bieneusi* and *Encephalitozoon intestinalis*). These infections usually occur late in the course of HIV infection when CD4+ T-cell count has been severely depleted ( mostly below 200 cells/mm<sup>3</sup> and in case of intestinal microsporidia below 100 cells/mm<sup>3</sup>).

**a) *Cryptosporidium parvum***

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; Ungar, 1990). The first case of human infection with *Cryptosporidium parvum* was reported in 1976 (Dubey, 1990; Mosier and Oberst, 2000). Since then, it is an increasingly recognised agent of intestinal infection as a common cause of severe diarrhoea in immunocompetent and immunocompromised humans and domestic animals (Zu et.al.1992; Hunter and Nicholes, 2002). The oocyst measures 4-6µm. in diameter.

The major clinical symptoms are watery diarrhoea, malabsorption and wasting syndromes. The severity of the disease depends on the immune status of the individuals (Current et.al., 1983; Martins and Guerrant, 1995). The nature of diarrhoea is usually secretory or malabsorptive, voluminous, intractable, watery and often cholera-like (Zu,et.al.,1992; Clark and Sears, 1996). 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 (Ungar, 1990; Martins and Guerrant, 1995; Manabe, et.al., 1998). Severe weight loss (as much as 25kg) has been reported due to debilitating diarrhoea (Ungar, 1990; Mengesha, 1994 ). Abdominal pain with cramps, low-grade fever (less than 39<sup>0</sup>C), vomiting, nausea and anorexia accompanies the diarrhoea (Clark and Sears, 1996 ). The diarrhoea can be bright yellowish-green in colour, offensive and may contain mucus ( Bartlett et.al, 1992; Martins and Guerrant, 1995 ).

It has been estimated that in AIDS patients with diarrhoea, the association of *Cryptosporidium parvum* ranges from 10 to 30% in the developed countries and 30 to 50% in the developing world (Petersen, 1993). *Cryptosporidium parvum* has also been implicated in the etiology of extraintestinal cryptosporidiosis causing acalculous cholecystitis, sclerosing cholangitis and pancreatitis in HIV-infected patients (Lopez-velez, et.al., 1995). In general, *Cryptosporidium parvum* is the most common opportunistic protozoan parasite that is at present well established and documented as a worldwide cause of diarrhoea in immunosuppressed individuals in general and HIV/AIDS patients in particular. It is now an AIDS -defining illness as it is involved with chronic diarrhoea (longer than one month) and the most common cause of enteric disease in HIV/AIDS patients (WHO, 1993).

The development of chronic cryptosporidiosis in AIDS patients has been correlated with reduced CD4+cell count (Flanigan et.al., 1992; Flanigan,1994 ). That is, the severity of the disease is manifested in AIDS patients usually when the CD+4 cell count is below 200cells/mm<sup>3</sup> (Lopez-Velez et.al.,1995; McDonald, 2000). The number of CD4+ cell count is higher in AIDS patients with diarrhoea when intestinal cryptosporidiosis is not involved. CD4+ counts are particularly low in patients with extraintestinal cryptosporidiosis, cell count of 55 cells/mm<sup>3</sup> (Lopez-Velez et.al.,1995; Theodos, 1998). Flanigan et.al., (1992) had reported that patients with CD4+ cell count of less than 180 cells/mm<sup>3</sup> have persistent infection, while patients with CD4+ cell count greater than 200cells/mm<sup>3</sup> have a transient or self- limited infection. However, some studies have indicated that self-limited cryptosporidiosis might be associated with a more intact immune system as reflected by a higher absolute CD4+ cell count. Flanigan (1994) had also demonstrated that individuals with CD4+ cell count above 500/mm<sup>3</sup> do spontaneously clear *Cryptosporidium* infection, while it is only patients with CD4+ cell count 140/ mm<sup>3</sup> or less that develop chronic life

threatening diarrhoea. Thus, the CD4+ cell count is used as a marker of the ability of an individual's immune system to respond appropriately to cryptosporidial infection at the mucosal surface ( Bartlett et.al, 1992; Martins and Guerrant, 1995 ; McDonald, 2000). *Cryptosporidium parvum* is also an increasingly recognized agent of intestinal infections in immunocompetent humans (Kuhls et.al., 1994). Human infections in healthy individuals, are manifested with symptoms that are self-limited and usually last less than a month. In HIV/AIDS patients and immunocompromised individuals, diarrhoea can persist for months (or even for life) and eventually may become life threatening (Clark and Sears, 1996).

Transmission of *Cryptosporidium parvum* occurs by ingestion of infective oocysts through mainly faecal-oral route of contamination, by human-to-human, animal-to-human or environmentally such as in water-borne outbreaks (Griffiths, 1998). Nosocomial transmission (i.e. in hospital room mates ) has also been described (Ravn, et.al., 1991). An outbreak of cryptosporidiosis in a hospital in Copenhagen had affected some 18 HIV-positive patients. The source of the outbreak was identified as ice from an ice machine in the ward, contaminated by an incontinent psychotic patient with cryptosporidiosis who was using his hands to pick out ice for cold drinks (Bruce, et.al., 2000).

In different parts of the world, *Cryptosporidium parvum* has reached the public health domain when it became widely recognised as the most serious and difficult to control cause of water-borne diarrhoea. This was confirmed by the major outbreak in Milwaukee, Wisconsin in 1993 (Adal, 1994; MacKenzie, et.al, 1995; Tzipori and Griffiths, 1998). Cryptosporidiosis infection in humans has been identified in more than 60 countries in six continents (Ungar, 1990). In Ethiopia, the existence of cryptosporidial infections has been reported in children under five and in HIV/ AIDS patients (Table 2).

**b) *Cyclospora cayetanensis***

Another newly defined coccidian opportunistic intestinal parasite in humans is *Cyclospora cayetanensis*. Cyclosporiosis is characterized by mild to severe watery diarrhoea, nausea, anorexia and abdominal cramps. It has been described from HIV/AIDS patients with protracted diarrhoea ( Wurtz, 1994; Sifuenes-Osonio, et.al., 1995). In immunocompetent individuals diarrhoea appears to be prolonged but self-limited, lasting from just over one week to a mean duration of about three weeks. Although infections appear eventually to resolve spontaneously, in some cases, both in immunosuppressed and immunocompetent individuals, patients have been successfully treated with oral Trimethoprim-Sulfamethoxazole that produces a rapid improvement of the symptoms. But in AIDS patient recrudescence of the symptoms is a major problem (Sifuenes-Osonio, et.al., 1995; Curry and smith, 1998) *Cyclospora* resembles *Cryptosporidium*, but the size varies ranging from 8-10µm with 2 sporocysts and having 2 sporozoites in each sporocyst, whereas *Cryptosporidium* measures 4-6µm in size and has 4 naked sporozoites (Curry and smith, 1998).

**c) *Isospora belli***

*I.belli* is another well-defined coccidian opportunistic intestinal parasite in HIV/AIDS patients and in some areas it is the cause of gastroenteritis (Lumb and hardiman, 1991 ). It mostly causes watery diarrhoea and weight loss. Diarrhoea produced by *Isospora belli* infection in AIDS patients is often secretory-like, without blood and leads to dehydration; low grade fever, eosinophilia, abdominal pain, vomiting and malaise are some of the symptoms reported ( Apt, 1986; Bartlett et.al, 1992 ; Lindsay, et.al., 1997 ). .

*Isospora belli* infections are essentially cosmopolitan 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 ( Faust, et.al., 1961; Sorvillo, et.al., 1995, 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.

Most oocysts 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  $\mu\text{m}$  by 10-19  $\mu\text{m}$ . They are elongated and ellipsoidal ( Sarvillo,et.al., 1995; Curry and smith, 1998 ). Transmission in humans occurs via faecal-oral route, mainly by ingestion of infectious oocysts from contaminated food and/or water. Diagnosis depends on microscopic identification of oocysts in the stool. Like *Cryptosporidium*, the oocysts of *Isospora* are acid fast during staining, but differ from oocyst of *Cryptosporidium* by their size and shape. The treatment of *Isospora belli* is by use of Trimethoprim-Sulfamethoxazole. Prophylaxis for *Pneumocystis carinii* pneumonia in HIV/AIDS patients may effectively prevent the acquisition of primary *Isospora belli* infection or the recrudescence of existing infection ( Lindsay et.al., 1997). In HIV/AIDS patients recurrence of infections are commonly reported (Curry and smith, 1998; Verdier, et.al., 2000 ) after treatment of *Isospora belli*.

**d) Intestinal Microsporidia (*Enterocytozoon bieneusi* and *Encephalitozoon intestinalis*)**

Other important intestinal parasites in HIV/AIDS patient are the microsporidia. They are ubiquitous, obligate intracellular spore forming protozoan parasites increasingly detected as opportunistic pathogens in HIV/AIDS patients. Since the onset of HIV/AIDS pandemic, a number of parasitic microsporidian pathogens of humans have been recognised. The frequency with which they are encountered and reported in clinical practice and the intensity of infections with opportunistic microsporidian parasites in AIDS patients have tremendously increased (Weber, et.al, 1994; Bryan, 1995; Chukwuma, 1996; Goodgame et.al, 1996). To date, at least, thirteen species of microsporidia have been reported to infect animals (Didier et.al., 1998). Among these are six known genera that have been associated with human disease, namely *Enterocytozoon*, *Encephalitozoon*, *Nosema*, *Pleistophora*, *Trachipleistophora*, *Vittaforma* (Weber et.al., 1994; Bryan, 1995; DeGirolami et.al., 1995).

Infection in humans can occur in different tissues including the small intestine, kidney, cornea and liver with various clinical manifestations (Table 1). Before 1985, however reports of clinical disease related to intestinal microsporidial infection in humans were rare. Since 1985, when *Enterocytozoon bieneusi* was identified as an aetiological agent of diarrhoea (Desportes, et.al., 1985), clinical syndromes associated with intestinal microsporidiosis in AIDS patients were frequently reported (Bryan, 1995; Kotler and Orenstein, 1998). Diarrhoea and malabsorption are the most common clinical syndromes associated with intestinal microsporidial infections in AIDS patients (Weber et.al., 1994; Bryan, 1995). Infection by *Enterocytozoon bieneusi* and/or *Encephalitozoon intestinalis* have been identified as main causes for watery diarrhoea and wasting syndrome in AIDS patients, when the CD4+ cell count is below 100 cells/mm<sup>3</sup> (Asmuth, et.al., 1994). In Ethiopia, so far, there is no information concerning intestinal microsporidial infection in HIV/AIDS patients.

Before the occurrence of HIV/AIDS pandemic, microsporidial infection in humans were only sporadically reported, and the first case was detected in 1959. And until 1985 only six cases were documented. The genera microsporidia incriminated and number cases were *Encephalitozoon* (2 cases), *Nosema* (1 case), *Pleistophora* (1case) and unidentified genera (2cases) (Desportes et.al, 1985; Weber and Bryan, 1994). Since then, different reports of microsporidial infection in HIV sero- negative and immunocompetent individuals have appeared. In a study conducted by Desportes et.al. (1998) in Bamako 3 cases of HIV-seronegative patients were found infected with microsporidia. The patients had wasting syndrome, persistent diarrhoea, anorexia, vomiting, nausea, fever, and vitamin B12 deficiency and the microsporidia species involved was *Enterocytozoon bieneusi*.

Intestinal microsporidiosis has also been reported from young African children confirmed to be HIV negative (Hautvast et.al., 1997). Eight cases of *Enterocytozoon bieneusi* were detected in children in Niamey and the infection was associated with persistent diarrhoea in 6 of the cases (Bretagne et.al., 1993). *Enterocytozoon bieneusi* was also detected in one of 176 children from rural Zambia. The infection was symptomatic in this child who was immunocompetent. It has also been noted that *Enterocytozoon bieneusi* infection was a potential cause of traveller's diarrhoea in immunocompetent subjects (Sobottka et.al., 1995).

The detection of microsporidia in HIV- negative subjects suggests that these parasites could be an underestimated cause of intestinal infection in the tropics. In addition to HIV/AIDS infection, other sources of immunodeficiency also are risk factors for intestinal microsporidiosis. This has been shown by the determination that microsporidiosis is a major cause of chronic diarrhoea in patients that have organ transplant and those taking drugs for

cancer treatment. Thus, in developed countries, the increasing use of potent chemotherapeutic and immunosuppressive agents to prevent transplanted tissue rejection in human allograft recipients have made patients susceptible to chronic and debilitating intestinal opportunistic infections such as microsporidiosis and cryptosporidiosis (Kaplan et.al., 1996). Thymic dysfunction contributes to the depression of cell-mediated immunity in severely malnourished children (Parent et.al.,1994; Cegielski et.al., 1999), suggesting that malnutrition can also be a risk factor for developing opportunistic intestinal parasitic infections. Thus, the presence of opportunistic intestinal parasites outside HIV/AIDS patients may have great epidemiological significance among the poor and malnourished.

**Table 1. Microsporidia species and associated clinical manifestations reported in HIV- infected patients ( after Weber et.al., 1994 and Kotler and Orenstein, 1998).**

	<b>Species</b>	<b>Clinical manifestations</b>
1	<i>Enterocytozoon bieneusi</i>	Diarrhoea, wasting syndrome, cholecystitis, cholangitis, bronchitis, pneumonia, sinusitis, rhinitis
2	<i>Encephalitozoon intestinalis</i>	Diarrhoea, disseminated infection (tubulointerstitial nephritis, Cholecystitis)
3	<i>Encephalitozoon hellem</i>	Keratoconjunctivitis, conjunctivitis, disseminated infection (tubulointerstitial nephritis, ureteritis, cystitis, Bronchiolitis, Pneumonia, colonization of bronchial epithelium)
4	<i>Encephalitozoon cuniculi</i>	Fulminant hepatitis, peritonitis
5	<i>Encephalitozoon</i> spp. (species not designated)	Keratoconjunctivitis, sinusitis, nasal polyps
6	<i>Pleistophora</i> spp.	Myositis

e) *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 diarrhoea is a controversial and not well-documented one (Zierdt, 1988; Libre, et.al., 1989; Jelinek et.al., 1997). Although *Blastocystis 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 ultrastructural and structural evidences, *Blastocystis hominis* has now been classified under protozoa (Zierdt, 1988; Stenzel and Boreham, 1996). Some investigators have presented strong evidence for its pathogenicity (Garcia et.al.1984; Garavelli, et.al., 1991) while others have considered it to be a commensal ( Udknow and Markell, 1993; Kaneda et.al., 2002). Reports of asymptomatic and symptomatic *Blastocystis hominis* infections in humans are worldwide. *Blastocystis hominis* infections are predominantly reported from developing countries of tropical and subtropical regions. Travellers from the developed countries might be affected with *Blastocystis hominis* infection when they travel to these regions of the world. (Keystone, 1995; Shlim et.al., 1995; 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 (Stenzel and Boreham, 1996; Gericke, et al.,1997).

Well recognized clinical signs due to *Blastocystis hominis* in symptomatic individuals, include abdominal discomfort or pain, diarrhoea, nausea, vomiting, flatulence, gastroenteritis, colitis and other minor complaints ( Boreham and Stenzel, 1993; Boreham et.al., 1996; Stenzel and Boreham, 1996). However, whether or not *Blastocystis hominis* can act, as an

etiologic agent of enteritis has been debatable. At least two reasons for the controversy are evident. The organism, like other controversial agent of enteritis such as yeast (*Candida albicans*), can be found in individuals without evident pathogenic manifestation or abdominal discomfort. There is also a strong evidence to show that clinical manifestation of illness due to *Blastocystis hominis*, when large numbers of organisms mostly greater than five parasites per high power field (>5/HPF) are seen in the presence of symptoms and in the absence of other well recognised viral, bacterial or parasitic agents (Zierdt, 1988; Telalbasic et.al., 1991).

Infection with *Blastocystis hominis* has gained attention in case of immunocompromised individuals and HIV/AIDS patients (Libre et.al., 1989; Garavelli et.al., 1991; Stagaard et.al., 1996; Escobedo and Nunez, 1997). The association of *Blastocystis 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 (Clark 1997; Kaneda et.al., 2002).

It is generally accepted that *Blastocystis hominis* is transmitted by faecal-oral contamination, in a manner similar to other gastrointestinal protozoa (Keystone, 1995; Stenzel and Boreham, 1996; Leelayoova, et.al., 2004). Other protozoan parasites such as *Cryptosporidium*, *Isospora*, *Cyclospora* and microsporidia, which were previously considered to be non-pathogenic or to have low pathogenicity, are also recognised as causes of human diseases, especially in immunosuppressed individuals and HIV/AIDS patients. Unlike the above mentioned opportunistic intestinal parasites, the possible association of *Blastocystis hominis* infection with HIV/AIDS is not well documented. Thus, during the investigation of the etiology of

gastrointestinal diseases the relationship of *Blastocystis hominis* infection in case of HIV/AIDS patients and immunocompromised individuals has to be considered.

**f) *Strongyloides stercoralis***

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 ( Nucci et.al., 1995; 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 strongyloidiasis in area where the parasite is endemic. The earlier definition of AIDS in fact had included extra – intestinal strongyloidiasis as one of the major clinical staging criteria (WHO, 1986). However, since limited number of cases of disseminated strongyloidiasis has been reported in HIV/AIDS patients (Makis, et.al., 1993, Sing et.al., 1999); it was omitted from the major WHO–staging criteria for definition of AIDS and later cited as a “missing “ infection (Lucas, 1990). However, in the era of HIV pandemic it is one of the enigmas in biomedical research and the anticipated association with *Strongylides stercoralis* infection needs further investigation. The major step in this case should focus on upgrading of the diagnostic techniques in chronic and disseminated strongyloidiasis. Most of the cases of *Strongylides stercoralis* infection among AIDS patients are usually diagnosed during autopsy whereby invasion of different organs of ectopic sites apart from gastrointestinal tract are reported (Grove, 1995; Grove, 1996).

#### **1.4. Immune Activation in Intestinal Parasites and its Possible Relation to HIV/AIDS Co-infection.**

Different investigators have shown that infection by intestinal parasites enhances immune activation and has been suggested to contribute to progression of HIV infection (Bentwich et.al., 1995; Bentwich et.al., 1996). In a study conducted in Ethiopia, there was a significant correlation between the number of excreted worm eggs and plasma viral load (Wolday, et.al., 2002 ). Furthermore, there was a significant reduction of plasma HIV viral load in individuals from whom helminth infections were eradicated, as compared to those in whom helminth infections persisted or were not present at all (Wolday, et.al., 2002 ). These findings indicate that helminth infections may enhance HIV multiplication and increase plasma viral load, thereby contributing to HIV disease progression.

Moreover, other studies have revealed that intestinal parasitic infections have interaction with immunological effectors such as T-cell subsets (CD4+ and CD8+) (Kalinkovich, et.al., 1998). It has been indicated that even healthy Ethiopians (HIV Negatives) have lower CD4+ and higher CD8+ counts as compared to Europeans and other Africans and it is hypothesized that environmental pathogens such as intestinal parasites could play a role. (Messele, et.al., 1999; Tsegaye, et.al., 1999). However, the factors that contribute to these variations are not well defined.

Because of lack of adequate and appropriate techniques available to diagnose some of the opportunistic intestinal parasites, there is no in-depth study on the relationship between HIV/AIDS status and the prevalence of intestinal parasites, especially the opportunistic ones in Ethiopia. Only some limited studies on HIV/AIDS patients and children under five were reported on *Cryptosporidium parvum* and other intestinal parasites (Table 2). An

epidemiological study conducted on intestinal parasites and HIV infection among the Wonji Sugar-Estate residents had indicated limited difference in the overall prevalence of the common intestinal parasites in HIV positive and HIV negative subjects (Fontanet et.al., 2000a). However, the study didn't properly address the problem of opportunistic intestinal parasites and other common "non – pathogenic" intestinal protozoan both in HIV negative and HIV positive subjects. Therefore, there are several questions to be addressed in relation to opportunistic intestinal parasites and HIV infections. These include,

- The immunological spectrum at which opportunistic intestinal parasites infections appear in Ethiopian HIV/AIDS patients.
- The impact of HIV infection on the prevalence of opportunistic and common intestinal parasites fauna.
- The widely application of appropriate diagnostic methods commonly used to identify opportunistic intestinal parasites.

**Table 2. Opportunistic intestinal parasites reported by different studies from Ethiopia**

<b>Parasite identified</b>	<b>Subject type</b>	<b>No. examined</b>	<b>No. Positive(%)</b>	<b>Ref.</b>
<i>Cryptosporidium</i>	Diarrhoea patient	1	1	Assefa & Eshete 1990
<i>Cryptosporidium</i>	Children with acute diarrhoea	100	9(9%)	Mersha and Truneh 1992
<i>Cryptosporidium</i>	AIDS patients	63	25(40%)	Mengesha, 1994
<i>Cryptosporidium</i>	Children under five	214	12(5.6%)	Assefa et.al., 1996
<i>Cryptosporidium</i>	AIDS patients (chronic diarrhoea)	147	38(26%)	Fisseha et.al., 1998
<i>Isospora</i>			2(1.4%)	
<i>Cryptosporidium</i>	HIV patients		6(11%)	Awole et.al., 2003
<i>Isospora</i>	with	54	4(7.4%)	
<i>Cyclospora</i>	Chronic diarrhoea		2(3.7%)	
<i>Cryptosporidium</i>	Diarrhoea patients	442	92(20.8)	Endeshaw et.al., 2004
<i>Isospora</i>			35(7.9)	

## **1.5. Aim of the Study**

### **1.5.1. General Objective**

This study aimed to determine the prevalence of intestinal parasitic infections in HIV/AIDS patients suffering from diarrhoea by using different diagnostic techniques in Ethiopia.

### **1.5.2. Specific Objectives**

- 1.To assess the impact of repeated chemotherapeutic intervention on the prevalence of intestinal parasites by using HIV/AIDS natural history study cohort population in Wonji, Ethiopia.
- 2.To determine the prevalence of opportunistic intestinal parasitic infections in diarrhoeal patients seeking medical care in selected Addis Ababa hospitals.
- 3.To determine the association between opportunistic intestinal parasites and the status of HIV/AIDS patients, with regard to their clinical state, viral load and immunological parameters such as CD4+ cell count.

## **2. MATERIALS AND METHODS**

### **2.1. Study Design**

#### **2.1.1. Cohort site selection**

The cohort study design was described elsewhere (Sahilu et.al 1998; Fontanet and Woldemichael, 1999). In order to identify appropriate site(s) for the cohort study, pilot surveys were conducted in 1995-96 in different areas of Addis Ababa, Akaki and Wonji Sugar Estate factory. Random sampling of the entire population of the Sugar Estate followed by sampling of the entire worker population of each factory was done. Three main criteria were used to select suitable sites for the cohort study (Sahilu et.al., 1998). The criteria were: a sufficient HIV prevalence (around 10% or more), a stable population with median duration of residence longer than 20 years and a high acceptability of a long term research project on HIV/AIDS. Based on these criteria, two sites were selected for the cohort study. A fibre product factory at Akaki 30 Km south east of Addis Ababa and the second, a Sugar Estate at Wonji, 107 Km South east of Addis Ababa. Both sexes with age greater than 15 years were included. Informed consent was obtained from each participant. Clinical and laboratory data were collected at an interval of 6 months.

#### **2.1.2. Hospital based study.**

The Hospital –based study was a cross-sectional study based on hospital settings. The study was more of descriptive and comparative type focused on identifying and describing opportunistic intestinal parasites in diarrhoea patients in relation to HIV-serostatus. Individuals with complaints of diarrhoea and who were not treated with antidiarrhoeal or antiparasitic drugs for the last two to three weeks were included.

## **2.2. The Study Population**

### **2.2.1. Cohort study population**

After informed consent was obtained from each participant, trained counsellors gave pre-test counselling for HIV test. They responded to a standard questionnaire on medical history, sexual behaviour and basic socio-demographic characteristics.

Each participant provided blood and stool for various laboratory analyses. Sample collection was repeated every six months when regular scheduled follow up visits were made. They were asymptomatic individuals visiting the cohort site for providing biological samples during scheduled visits. Patients that visit Wonji hospital could be symptomatic as individuals were seeking medical care. However, the types of clinical symptoms observed in Wonji hospital were not available.

From the two ENARP cohort Sites, the Wonji HIV cohort study site was found appropriate for analysis of treatment effect on intestinal parasites. This is because there is base data that shows the present status of intestinal parasites in the nearby community from the Wonji hospital. In addition, Kato Katz method was done only in Wonji cohort laboratory.

### **2.2.2. Hospital based study**

The study was conducted from March 2002 to December 2003 in the Army, the Police and St. Paul Hospitals in Addis Ababa. Cross-sectional study 330 in-and out-patients were enrolled based on the following criteria: Both sexes of age greater than 14 years; have chronic diarrhoea – (two or more watery or loose stools per day for a period of greater than 28 days) or acute diarrhoea (two or more watery or loose stools per day for less than 4 weeks.)

Informed consent of each study participant was obtained. Pre- and post-test counselling about HIV serological test and its implications was given by trained counsellors to the volunteer study participants. Clinical evaluation of the patients for diarrhoea, basic demographic information registry and HIV/AIDS disease staging according to WHO staging criteria (1993) were done by the attending physicians.

## **2.3. Stool Sample Collection, Processing and Light Microscope Detection**

### **2.3.1. Direct wet mount**

A single stool sample was obtained in labelled caps from all consenting patients selected for the study. A direct saline mount of each sample was checked in the hospitals for motile intestinal parasites microscopically. The samples were brought to the Ethiopian Health and Nutrition Research Institute, Parasitology Laboratory. The direct wet mount with saline was repeated and Lugol's iodine staining was done. The wet mounts were examined under light microscope at 100X and 400X magnification. A small portion of the stool samples were also preserved in SAF (15 gm sodium acetate, 20ml glacial acetic acid, 40ml formaline, 925 distilled water) in a proportion of 1gm of stool in 3ml of SAF for repeating the tests whenever required. This method was also applied for the analysis of stool samples from Wonji hospital patients and cohort population.

### **2.3.2. Concentration methods**

#### **2.3.2.1. Formol ether concentration method**

A portion of each fresh stool sample was taken and processed as described by Ritchie (1948) with some modification. Briefly, one gram of stool sample was mixed with 8ml of 10% formalin and crashed well. It was sieved with double layer cotton gauze into 15ml conical centrifuge test tube. Three ml of diethyl ether was added and hand-shaken for one minute and

then centrifuged for another two minutes at 2000g. The supernatant was discarded and the sediment observed for the presence of ova and/or parasites under the light microscope at a magnification of 100X and 400X. This method was also performed for processing of stool samples from the cohort participants and rarely from Wonji hospital patients.

### **2.3.2.2. Baermann concentration method**

The rhabditiform larvae of *Strongyloides stercoralis* were extracted from fresh stool samples by using the Baermann technique (Lima and Delgado, 1961) with modification. This procedure was applied in the case of Wonji cohort participants. Briefly, about 5 gm of fresh stool was placed on cotton gauze, supported by a tea sieve on a glass funnel filled with hot water (37<sup>0</sup> C). It was allowed to stand for one hour. The water was then decanted and about 10ml retained at the bottom of the funnel. It was then transferred into 15ml conical test tube and centrifuged for 5 minutes at 2000g. After decanting the supernatant, the sediment was observed for motile larvae under the microscope at X100 magnification. A drop of iodine was added to arrest larval motion and for larvae identification.

### **2.3.2.3. Kato-Katz method**

This method was applied for the detection of helminths, especially in stool samples with scanty egg load as in *Schistosoma mansoni* infection. It was done only in the Wonji cohort stool samples. Briefly, about 1 gm of fresh stool was placed on a piece of paper or plastic sheet and a metal sieve was placed on top of it. Then the content scrapped with an applicator stick (or with spatula) onto a 25 mg templet with a hole on a centre of a microscope slide. The templet was filled with the sieved stool using an applicator stick, and the excess faeces was removed from the edge of the hole. Then the scrapped faecal material on the slide was covered with the pre-soaked strip of cellophane tape ( Soaking solution: 1ml 3% aqueous

malachite green, 100ml glycerol, 100ml distilled water). The stool was then pressed with other slide, in order to prepare an evenly distributed smear of approximately 1.5 to 2cm<sup>2</sup>. After removing the slide, the prepared slide was allowed to stand for 15 minutes for the glycerol to clear the faeces. The slides were observed within 30 minutes of preparation. The whole slide was observed and eggs counted in order to determine the number of eggs per gram of stool.

#### **2.3.2.4. Modified Ziehl Neelsen staining method**

A small portion of the fresh stool sample was processed for *Cryptosporidium parvum* and *Isospora belli* oocysts using the Ziehl Neelsen method (Current, 1990) with some modification. Thin smear was prepared directly from fresh stool as well from sediment of concentrated stool and allowed to air dry. The slides were then fixed with methanol for 5 minutes and stained with carbol fuchsin (Merck & Sharp, Diagnostica, Germany) for 30 minutes. After washing the slides in tap-water, they were decolourised with acid alcohol (99ml of 96% ethanol and 1ml HCl) for 1-3 minutes and counterstained in methylene blue for one minute. The slides were then washed in tap water and observed under light microscope with a magnification of X1000. Oocysts in case of *Cryptosporidium* appeared bright orange usually with clear halo against a blue background, measuring usually 4-6µm in size. In case of *Isospora* the oocysts are oval pink-red in colour with 10-19 µm by 20-33 µm in size. Each slide was observed for 10 minutes to decide whether it is negative or positive.

### **2.3.3. Identification of intestinal parasites by fluorescent microscopy**

#### **2.3.3.1. Uvitex-2B staining method**

A portion of each fresh sample was processed by a water-ether sedimentation method and stained with Uvitex-2B for detection of microsporidial infections as described by Van Gool, et.al., (1994). One gm of fresh stool was mixed thoroughly with 8ml of distilled water in a 15ml conical test tube. After sieving with cotton gauze, 3ml of ether was added and the mixture shaken for a minute and centrifuged at 2000g for 2 minutes. From the sediment, thin smears were prepared on a microscope slide and allowed to air-dry. The slides were then fixed with methanol for two minutes and allowed to air-dry and then stained with Uvitex-2B (Ciba, Giegy, Basel, Switzerland) for ten minutes. The slides were then washed in PBS (8.0 gm NaCl; 0.2 KCl; 1.44gm Na<sub>2</sub> HPO<sub>4</sub> 0.24 gm KH<sub>2</sub> PO<sub>4</sub>; pH=7.2) for 5 seconds and counter-stained with Evans blue (Sigma) for 30 seconds; then washed in PBS for 5 seconds and gently rinsed in running tap water. The slides were observed under fluorescent microscope with 50-W mercury high pressure lamp, fitted with excitation filter 355-425 nm and a suppression filter of 460 nm (Leitz, Ploemopak Filter Block D, Germany) at a magnification of X1000. Each slide was examined for about ten minutes to decide whether it is positive or negative.

#### **2.3.3.2. Autofluorescence for *Cyclospora cayetanensis***

Thin smears were prepared from fresh or SAF preserved stool and observed under fluorescent microscope with 50-W mercury high pressure lamp, fitted with excitation filter 355-425 nm and suppression filter of 460 nm (Leitz, Ploemopak Filter Block D, Germany) at a magnification of X 40. Positive slides showed bright green fluorescence measuring 8-10µm in size.

## **2.4.Molecular Diagnosis of Intestinal Microsporidiosis**

As a confirmatory test and to differentiate the species of intestinal microsporidia i.e., *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis*, DNA extraction and nested Polymerase Chain Reaction were performed.

### **2.4.1. DNA extraction from stool for diagnosis of intestinal microsporidiosis**

One gram of fresh stool suspension prepared in 2ml PBS containing 2% Polyvinylpolypyrrolidone (PVPP) (Sigma) was stored at  $-20^{\circ}\text{C}$  until DNA extraction. For DNA extraction an aliquot of 200  $\mu\text{l}$  of the suspension was separated in an Eppendorf tube and was heated for 10 minutes in a heat block at  $100^{\circ}\text{C}$ . An equal volume of Sodium Dodecylsulphate with proteinase K was added and allowed to incubate at  $55^{\circ}\text{C}$  for 2 hours. After incubation, DNA was extracted using the QIAamp Tissue kit spin columns in an Eppendorf microcentrifuge (QIAGEN, Hilden, Germany) as described by Verweij et al.,(2001). After adding 400 $\mu\text{l}$  AL Buffer (tissue lysing buffer), the suspension was mixed thoroughly by vortexing and incubated at  $70^{\circ}\text{C}$  for 10 minutes. After a short spin, the supernatant was transferred to an Eppendorf tube containing 400  $\mu\text{l}$  absolute ethanol and was thoroughly mixed by vortexing. A labelled QIAamp spin column was placed in a 2ml collection tube and the mixture with absolute ethanol was applied to the spin column without moistening the rim. This was followed by centrifugation at 10000 rpm for one minute. The spin column was then placed in a clean 2 ml collection tube and 500  $\mu\text{l}$  of AW1 buffer (washing buffer 1) was added and centrifuged at 10000 rpm for 1 minute. Then, the final washing step was completed after emptying the collection tube, adding 500  $\mu\text{l}$  of AW2 buffer (washing buffer 2) and centrifuging at 10000 rpm for 3 minutes. Finally, the DNA was eluted with 200  $\mu\text{l}$  of AE buffer (elution buffer: Tris 10mM Cl; 0.5mM EDTA; pH: 9.0) after centrifugation at 10,000 rpm for 1 minute.

#### **2.4.2 Polymerase Chain Reaction for detection of intestinal microsporidiosis**

DNA amplification was performed by nested PCR as described by Katzwnikel-Wladarsch et.al. (1996). In the first PCR, three outer Primers (**MSP-1**:TGA ATG KGT CCC TGT, **MSP-2A**:TCA CTC GCC GCT ACT, **MSP-2B**:GTT CAT TCG CAC TAC ) were used. The second (nested) PCR was run by taking 2µl of the first PCR product to the mix containing three inner primers (**MSP-3**:GGA ATT CAC ACC GCC CGT CRY TAT, **MSP-4A**:AA ARG GGT, **MSP-4B**:CAA AGC TTA TGC TTA AGT CCA GGG AG). The Pair of primers MSP-2B and MSP-4B are specific for *Entrocytozoon bieneusi* homologies. Amplification of PCR was performed in a volume of 40µl reaction mixture with 10X PCR buffer (100M Tris-HCl, pH 9.0, 15 mM MgCl<sub>2</sub>, 500mM KCl, 1% Triton X-100, 0.1% (w/v) gelatin (HT Biotechnology, UK), 200 µl of each nucleotide: A, T, G and C; 25 Pmol of each primers, 1U of Taq polymerase ( Super Taq HC, HT Biotechnology) and 2µl of the DNA samples and run using GeneAmp PCR system 9600® (Perkln, Elmer Cerus, USA). PCR cycling parameters were 25 cycles following initial denaturation of DNA at 94<sup>0</sup> C for 4 minutes, followed by denaturation at 94<sup>0</sup> C for 30 seconds, primer annealing at 55<sup>0</sup> C for 45 seconds, elongation at 72<sup>0</sup> C for 45 seconds and 72<sup>0</sup> C for 7 minutes for last elongation.

The PCR mix with Primers MSP3, MSP4a, and MSP4b was added on 2µl of the first PCR product. The nested PCR was performed in the same PCR cycling parameters indicated for the first PCR above. The final PCR product was resolved by 2% agarose gel electrophoresis with TBE buffer (1M Tris base, 0.8M Boric acid, 0.01M ethylenediamine tetraacetic acid, pH 8.0) at 100v. The gel was read under UV illumination using a Polaroid camera.

## **2.5. HIV Determinations**

### **2.5.1. HIV testing**

About 7-10 ml of whole blood was collected in a labelled vacutainer containing EDTA. From the plasma HIV serology was done using Third Generation Sandwich ELISA kit (Vironostika<sup>®</sup>, HIV-Uni-Form II Plus O; BIOMERIEUX, France). Reactive samples were confirmed by Western blot analysis.

### **2.5.2. CD4<sup>+</sup> and CD8<sup>+</sup> cell count**

Lymphocyte subsets, CD4<sup>+</sup>, CD8<sup>+</sup>, lymphocytes, were analysed by FACScan flow cytometry (Becton Dickinson Immunocytometry system, San Jose, CA., USA). Briefly, 100µl of whole blood was mixed with 10µl of each monoclonal combination in separate tubes and incubated at room temperature for 20 minutes. RBC was then lysed by adding 2 ml of fluorescence activated cell sorter lysing solution (Becton Dickinson). After vortexing, tubes were incubated in the dark at room temperature for 10 minutes and centrifuged at 300X g for 5 minutes. The cell pellet was washed once with 2ml of Isoton, resuspended in 500µl of Isoton, and analysed with simulset software (Becton Dickinson) of the FACScan.

### **2.5.3. Viral load determination**

HIV-1 viral load was determined by quantifying the amount of HIV-1 RNA in plasma samples using NUCLISEN kit (NASBA) (Organon Tekinika BV, Boxtel, the Netherlands).

## **2.6. Data Analysis**

Data were analysed using STATA statistical software version 7( Stata Corporation, College Station, Texas,USA). A univariate Mantel-Haenzel analysis was performed to examine at the

association between opportunistic intestinal parasites and CD4+ cell counts, viral load and WHO-staging among HIV positive. Chi Square was employed to test association. Values  $P < 0.05$  were considered as significant.

### **2.7.Ethical Clearance**

Ethical clearance was obtained from the Ethiopian Health and Nutrition Research Institute (EHNRI) and the National Ethical Committee of the Ethiopian Science and Technology Commission (NEC/ESTC).

### **3. RESULTS**

#### **3.1. Assessment of Treatment Intervention for Intestinal Parasites in Wonji Hospital and Wonji Cohort Population.**

The annual routine laboratory results for intestinal parasites from patients examined in Wonji Hospital was observed for consecutive years, 1997-2002 (Figures 1 and 2). A total of 115,115 patients with a mean number of 19,186 per year were examined for intestinal parasites in the Wonji hospital. The prevalence of intestinal parasites among the patients examined in Wonji hospital was found stable with a slight but insignificant increase in 1999-2001 ( $P>0.05$ )(Figure 1).

On the other hand, among the two ENARP cohort sites, Wonji cohort was found to be appropriate to see the impact of treatment intervention on intestinal parasites for three reasons: 1) the base line data was initially described elsewhere (Fontanet, et.al., 2000 a & b), which enables comparison with the present cohort results. 2) Kato method for schistosomiasis and geohelminths was done only in this cohort. 3) There is relevant information from Wonji hospital regarding the status of intestinal parasites in the nearby community. In this study, the impact of treatment effect on intestinal parasites in Wonji cohort population through time in comparison to the Wonji hospital patients was observed. The appearance of the stool, such as loose, watery, mucoid, bloody diarrhoea or formed, were not recorded.

A total of 856 cohort participants in Wonji, enrolled in 1997, were examined for intestinal parasites in the consecutive follow up years (1997-2001). The result is indicated in Figure 3 for intestinal helminths and in Figure 4 for intestinal protozoa. Cohort participants that were found positive for intestinal parasites were successfully treated with appropriate drugs; for

helminths Albendazole (400mg), Mebendazole (100mg) or Lavemisol 40mg, and Praziquantel 40mg/kg for intestinal schistosomiasis. For “*Entamoeba histolytica*” and *Giardia lamblia* infections, Metronidazole (250mg) was given. The treatment was given full dose based on the type of the parasite identified, such as Lavemisol 40mg, 3 tablets stat for *A.lumbricoides*, Mebendazole 100mg 2x2 tablets per day for three days for *Taenia*, *Trichuris* and other helminths. Albendazole and Mebendazole is a broad-spectrum antihelminthic drug effective against many intestinal helminths. Albendazole 400mg, 2tabs/day for 3 days was given for *S.stercoralis* infection. Metronidazole 250mg, 2tablets3X per day for 7 days was prescribed for treatment of “*Entamoeba histolytica*” and *Giardia lamblia* infections.

The effect of repeated treatment intervention was clearly observed among the Wonji cohort participants (Figures 3& 4). The prevalence of intestinal parasites showed a significantly higher reduction with increasing follow up years ( $P<0.05$ ).

When each species of intestinal parasites was considered, *Ascaris lumbricoides* was most common among the helminths in both groups. Among the patients from Wonji hospital, its prevalence was 6.2% and 7.5% in 1997 and 1998, respectively, and 11.8% in 1999 and 10.2% in 2000 (Figure 1). Among the cohort participants, a prevalence of 22.1% in 1997 and 23.1% in 1998 was detected, and from 1999-2001, it has shown a drastic decreasing trend (Figure 3). The observed difference in prevalence in two groups could be a matter of diagnosis and reporting. *Trichuris trichiura* has also shown a similar trend as *Ascaris lumbricoides* (Figures 1 and 3). *Strongyloides stercoralis* was detected among the cohort participants with a prevalence of 16.0%, 10.3% and 4.3 % in 1997, 1998 and 1999, respectively. It showed a drastic decline, below 1% in 2000 and no *Strongyloides stercoralis* infection was detected in 2001, among the cohort participants (Figure 3). On the other hand,

its prevalence was below 1% from 1997-2002 and has shown almost stable infection rate through out the years of record among the patients examined in Wonji hospital (Figure 1). Apart from the treatment effect, the difference in prevalence of *Strongyloides stercoralis* among the two population groups might be due to the difference in the diagnostic techniques; the cohort laboratory technicians have performed Baermann for the detection of strongyloidiasis, whereas the hospital laboratory did not use the Baermann technique. They did conventional methods such as direct and sometimes formol-ether concentration techniques.

*Schistosomiasis mansoni* was detected among the patients examined at Wonji Hospital, the infection rate remained nearly the same throughout the years of record i.e., 4.3% in 1997, 2.3% in 1998 and 1999, 2.4% in 2000 and 3.3% in 2001. Among the cohort participants the prevalence was 14.1% in 1997, 6.1% in 1998, 3.0% in 1999, 2.5% in 2000 and no case was detected in 2001. The difference in percentage prevalence of *Schistosomiasis mansoni* between the two groups was observed during the first years (1997-99) before repeated treatment was initiated among the cohort participants. The difference could be due to differences in diagnostic methods used; the cohort laboratory had applied Kato method whereas the hospital laboratory had performed direct and sometimes concentration methods.

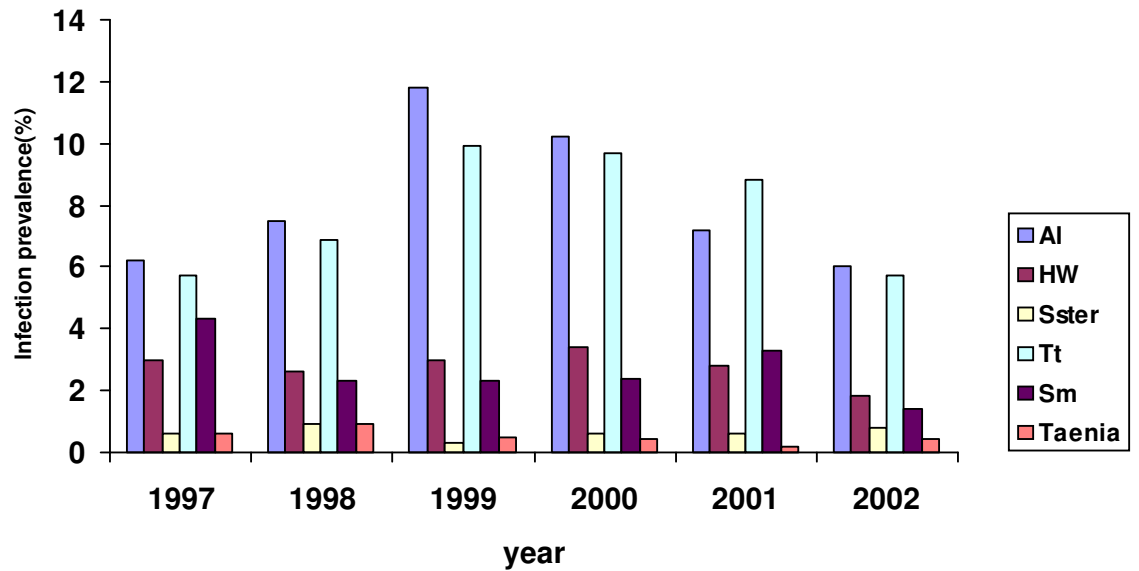


Figure 1. Intestinal helminth parasites recorded in Wonji hospital (1997-2002)

Al=*Ascaris lumbricoides*; Tt=*Trichuris trichiura*; HW= Hookworm Spp.; SM =*Schistosoma mansoni*; Sstr=*Strongyloides stercoralis*; Taenia spp

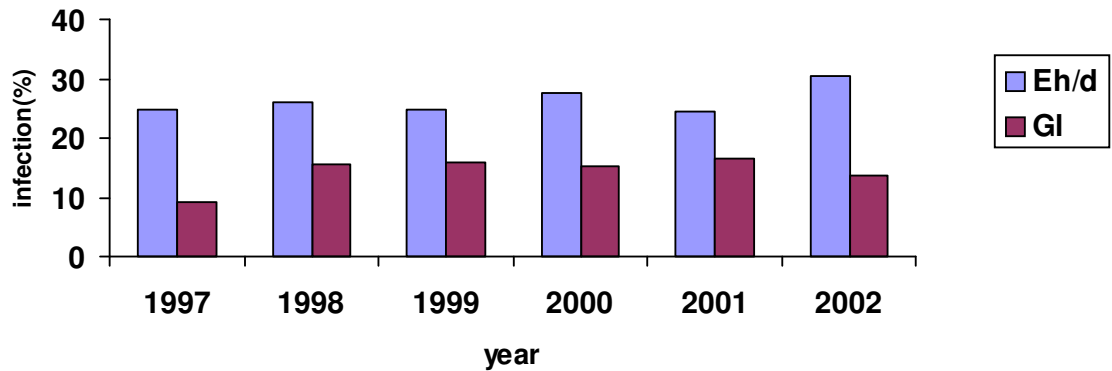


Figure 2. Intestinal protozoan parasites recorded in Wonji hospital (1997-2002)

Eh/d=*Entamoeba histolytica/dispar*; GI= *Giardia lamblia*.

The trends observed in the intestinal protozoa, *Entamoeba histolytica/dispar* and *Giardia lamblia* in the Wonji Hospital patients and in the cohort population were similar to those observed for the helminths. Among the Wonji Hospital patients, the rate of infection by *Entamoeba histolytica/dispar* remained between 25-30% during 1997-2002 (Figure 2). In the cohort participants, the frequency of finding *E.histolytica/dispar* was 46% in 1997, 17.4% in 1998, and dropped below 3% in the consecutive follow up years (Figure 4). The observed difference in prevalence among the two groups might be either due to the effect of treatment intervention or differences in reporting system of the two laboratories. Almost the same trend was recorded for *Giardia lamblia*; in 1997 it was reported as 8.9% among the cohort and since then it dropped below 1%. In the Wonji Hospital, *Giardia lamblia* was recorded as 9.2% in 1997, 15.6% in 1998, 15.8% in 1999, 15.1% in 2000 and 16.4% in 2001. It showed almost stable trend during the years of report. The composition of study population in the cohort was mainly adult group. In Wonji Hospital more children might have been involved (data not available), who in turn are more susceptible to *Giardia lamblia* infection.

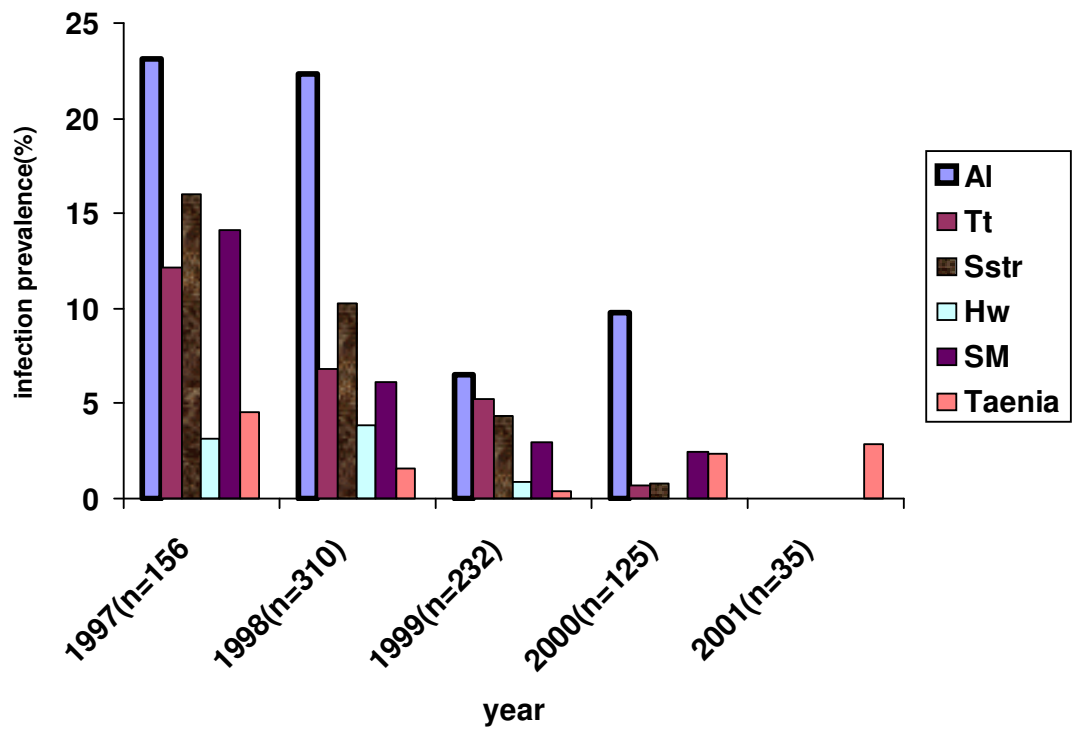


Figure3. Intestinal helminth parasites in Wonji cohort ( 1997-2001)

AI=*Ascaris lumbricoides*; Tt=*Trichuris trichiura*; HW= Hookworm Spp.; SM =*Schistosoma mansoni*; Sstr=*Strongyloides stercoralis*; *Taenia* spp.

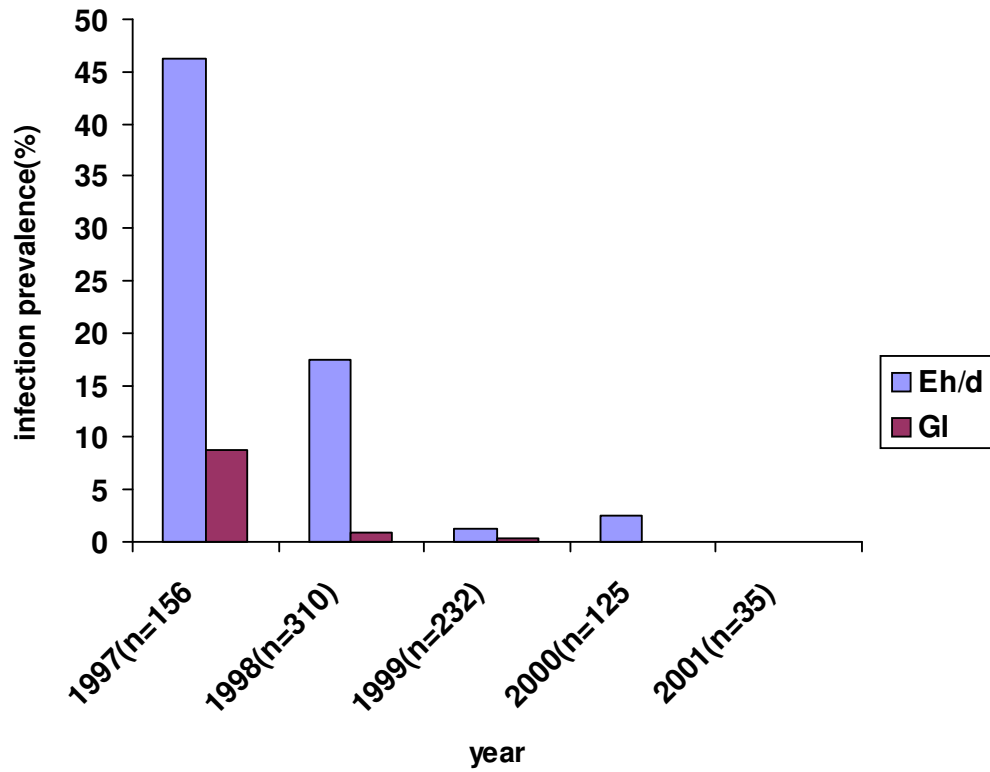
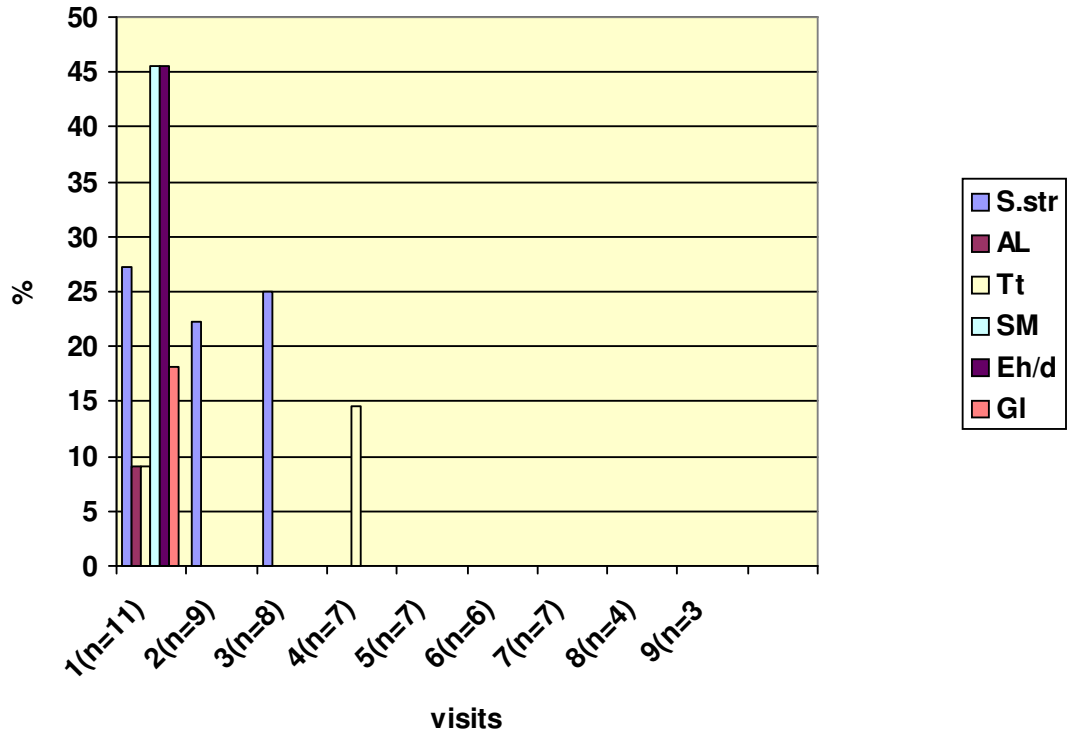


Figure4. Rate of infection by intestinal protozoa parasites in wonji cohort ( 1997-2001)

Eh/d=*Entamoeba histolytica/dispar*; GI= *Giardia lamblia*



**Figure 5. Intestinal parasites in HIV positive individuals in Wonji cohort by scheduled visit at intervals of 6 months (1997-2001) n=62.**

Low prevalence of intestinal parasites was detected in HIV positive individuals enrolled in 1997 in Wonji cohort (Figure 5) with consecutive scheduled visits at the interval of six months. During the first visit most of the common intestinal parasites were detected, with highest percentage of *S. mansoni* 5/11 (45.5%) among the helminths and *E. histolytica/dispar* 5/11(45.5%) among the protozoan parasites. The second most common helminth was followed by *S. stercoralis* and *Ascaris lumbricoides* and *Trichuris trichiura* in that order. In general through out the study HIV positives were very few and the proportion of infected individuals by intestinal parasites was very limited.

**Table 3. Study on 273 Wonji cohort participants for intestinal helminths and protozoan parasites (February 2001-July 2001)**

<b>Parasite identified</b>	<b>No. Observed (%)</b> <b>(N=273)</b>
<b>Helminths</b>	
<i>Ascaris lumbricoides</i>	2(0.7)
<i>Trichuris trichiura</i>	4(1.5)
<i>Hookworm Spp.</i>	3(1.1)
<i>Strongylides stercoralis</i>	0(0.0)
<i>Taenia Spp.</i>	2(0.7)
<i>Schistosoma mansoni</i>	2(0.7)
<b>Protozoa</b>	
<i>Giardia lamblia</i>	14(5.1)
<i>Entamoeba histolytica/dispar</i>	32(11.7)
<i>Entamoeba trophozoite</i>	28(10.3)
<i>Entamoeba coli</i> cyst	136(49.8)
<i>Entamoeba hartmanni</i> cyst	40(11.7)
<i>Endolimax nana</i> cyst	47(17.2)
<i>Iodoamoeba butschilii</i>	29(10.9)
<i>Blasocystis hominis</i>	162(59.3)
<i>Chilomastix mesnelli</i>	30(10.9)
<i>Sarcocystis spp.</i>	7(2.6)
Mononucleated cyst	27(9.9)
<i>Cryptosporidium parvum</i>	3(1.1)
<i>Isospora belli</i>	0(0.0)

In addition to the cohort data, which is based on routine laboratory diagnosis at the cohort site level, 273 subjects were randomly selected among the scheduled visitors of Wonji cohort participants and examined for intestinal parasites by using additional diagnostic methods such as modified Ziehl Neelsen and ocular micrometer for measuring cysts of intestinal protozoa at the EHNRI Parasitology Laboratory. Intestinal helminths were very rare, below 2% (Table 3). No *Strongyloides stercoralis* was detected. The application of broad spectrum

antihelminthic drug has reduced helminthic infection among the cohort participants. *E.histolytica/dispar* (11.7%) and *G.lamblia* (5.1%) were detected. The observed difference in prevalence could be due to repeated examination of the sample and experience of the technicians at EHNRI, Parasitology Laboratory as compared to the cohort result (Figure 4). On the other hand, non-pathogenic intestinal protozoa such as *Entamoeba coli*, *E. hartmanni*, *Endolimax nana* and others were identified only in EHNRI parasitology laboratory. Among the intestinal protozoa, *Blastocystis hominis* (59.3%) was the most common. Non – pathogenic intestinal protozoa were not reported in the cohort data. In this study coccidian intestinal parasites were also detected; *Cryptosporidium parvum* (1.1%), and no *Isospora belli* and *Cyclospora cayetanensis* were identified. Examination for coccidian intestinal parasites was not performed at the cohort site laboratory. The cohort participants were repeatedly treated with Co-trimoxazole for bacterial infections and other illnesses.

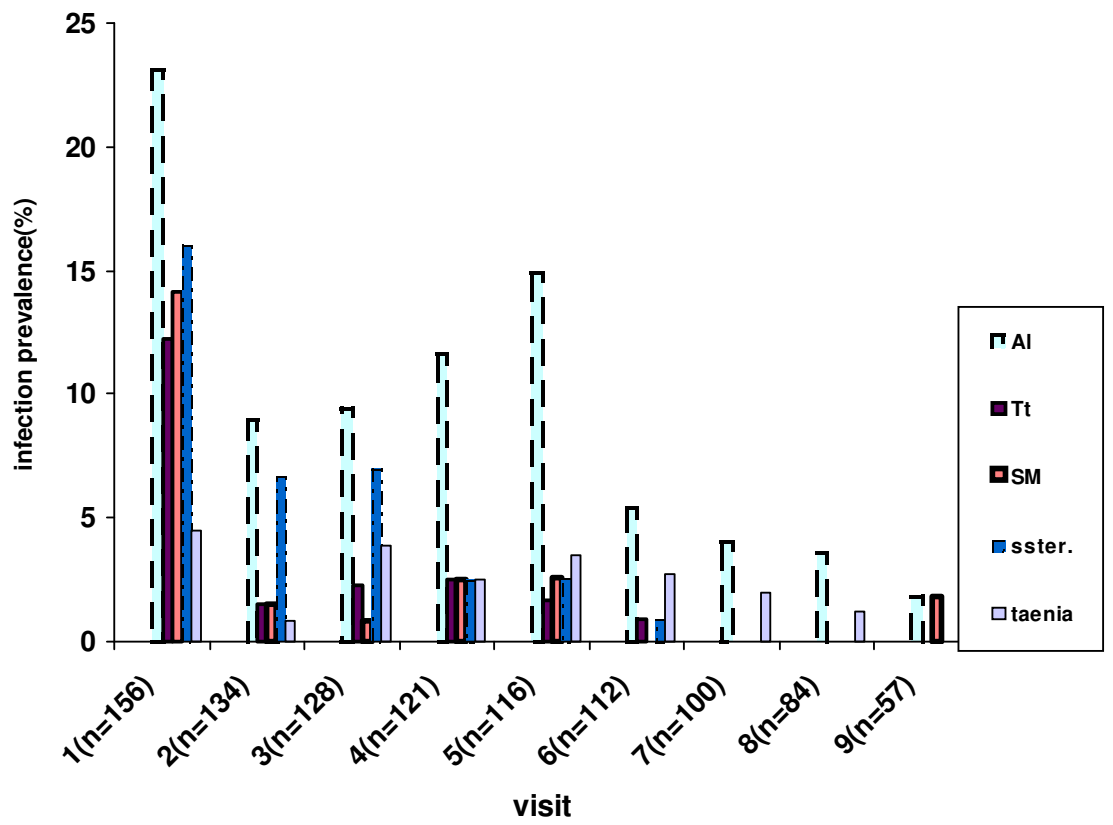
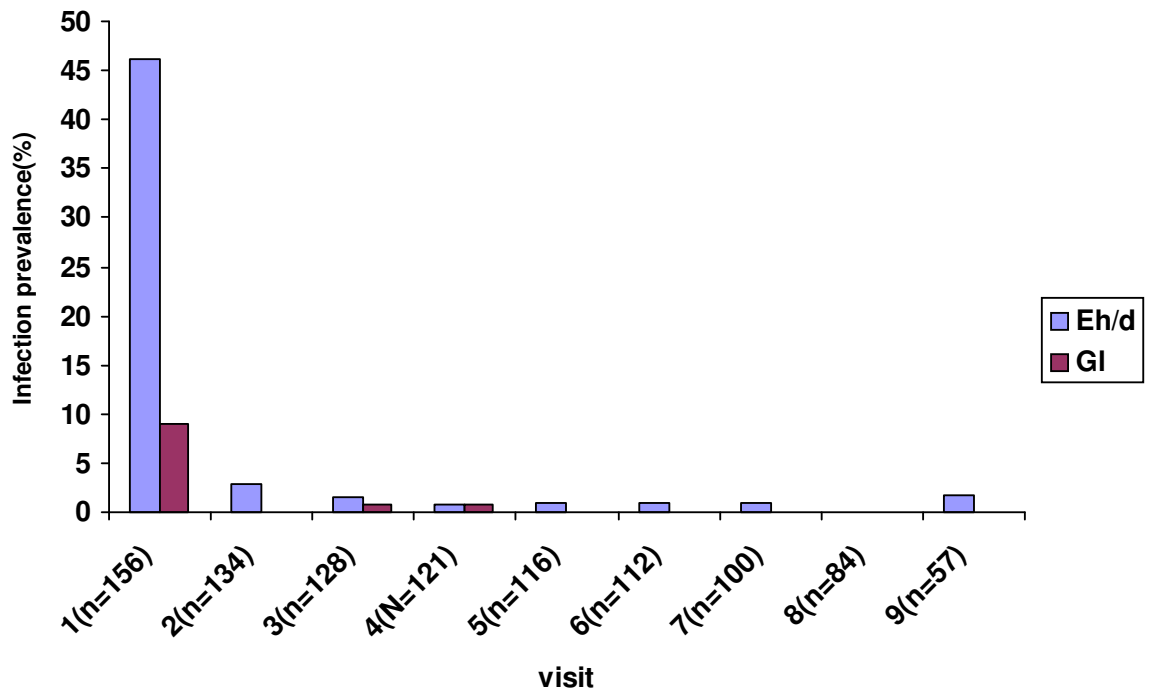


Figure 6. Intestinal helminth parasites in Wonji cohort by scheduled visits (1997-2001).

Al=*Ascaris lumbricoides*; Tt=*Trichuris trichiura*; SM =*Schistosoma mansoni*;  
 Sster=*Strongyloides stercoralis*; Taenia spp.



**Figure 7. Intestinal protozoa in wonji cohort by scheduled visits(1997-2001)**

Eh/d=*Entamoeba histolytica/dispar*; GI= *Giardia lamblia*.

Based on scheduled visits at the interval of six months, the prevalences of intestinal helminths and protozoa have also significantly decreased ( $P=0.001$ ) among the cohort participants (Figures 6 and 7). *Ascaris lumbricoides* was the highest (23.1%) during the first visit and was lower (9.2%) in the 2<sup>nd</sup> and 3<sup>rd</sup> visits. But in the 5<sup>th</sup> visit it had increased to 15% and that might have been due to re infection. From the 6<sup>th</sup> visit onwards, highly significant ( $P<0.05$ ,  $P=0.001$ ) reduction was observed. With increasing numbers of visits, a similar decreasing trend was documented in other helminths (Figure 6). During the first visit, high prevalence of *Trichuris trichiura* (12.3%), *Strongyloides stercoralis* (16.0%) and *Schistosoma mansoni*

(14.1%) was recorded; in the 2<sup>nd</sup> visit the prevalence declined to 1.5%, 6.7%, and 1.5%, respectively. After the 3<sup>rd</sup> visit the prevalence of each one of the helminth parasites showed gradual decrease during subsequent visits.

The same decreasing trend was observed among *Entamoeba histolytica/dispar* and *Giardia lamblia* (Figure 7) *E.histolytica/dispar* (46%) and *Giardia lamblia* (9%) showed significant reduction ( $P<0.05$ ,  $P=0.001$ ) with increasing number of visits.

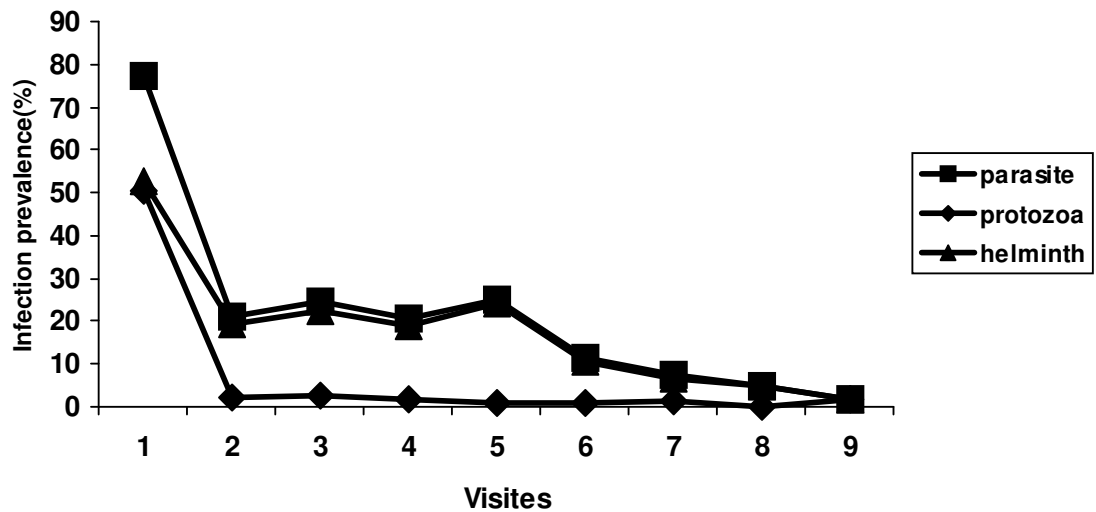


Figure 8. Effect of chemotherapy on total intestinal parasites in wonji cohort by scheduled visit (1997-2001) n=856

The decreasing trend in the prevalence of intestinal parasites as a group based on scheduled visits in Wonji cohort is summarised in Figure 8. It was observed that there was significantly high reduction of intestinal parasites with follow up visits. The prevalence of the parasite infection rate as a group was 78.2% during the first visit, then drastic decline followed and showed significantly high reduction in the consecutive visits ( $P<0.001$ ). Helminths with a prevalence of 52% during the first visit had dramatically reduced to 1.8% in last visit. The protozoan parasite group have also shown a decreasing trend, 51% in the first visit to below 1% during the last visit. At every scheduled visit, the trend of intestinal parasites as a whole showed significantly high reduction ( $P<0.001$ ).

### **3.2. Hospital-based Study**

Between March 2002 and December 2003 a total of 330 diarrhoeal patients were examined for intestinal parasites and HIV-status. Two hundred twenty (66.7%) of the patients were males and 110(33.3%) females; their mean age was 34 years (range 16 to 70 years); 245 (74.2%) were HIV positive and 85(25.8%) HIV negative. In this study, 190 (57.6%) in-patients and 140(42.4%) out-patients complaining of diarrhoea were included. Out of the 190 in-patients, 181 (95.3%) and from the 140 out-patients 64 (45.7%) were HIV positives indicating that most of the hospitalised cases due to chronic diarrhoea and its complications were HIV/AIDS patients. In terms of profession, the majority 85 (25.8%) belong to the police force and 81 (24.5%) were the members of the army (Table 4).

The age and sex category of 330 diarrhoeal patients in relation to HIV status is shown in Table 5. The highest percentage with diarrhoea was recorded in age group 25-34 years in HIV positive (40.0%) and HIV negative (38.8%). In general, the total HIV negative diarrhoea patients were small as compared to HIV positives, the ratio being 1:4. In age group

35-44 years, HIV positives (38.4%) were more than HIV negatives (22.8%). Overall, 192/245 (78.4%) HIV positives were within the age group 25-44 years old as compared to HIV negatives 52/85(61.6%). The sex distribution of HIV status showed that males (68.6%) were more victimized than (31.0%) females.

The clinical presentations of the diarrhoea patients in relation to HIV sero-status is described (Table 7). Over all chronic diarrhoea that lasted more than four weeks was recorded in 72.1% (238 out of 330) of the patients. Of 245 HIV positives, 224 (91.4%) had chronic diarrhoea that lasted for more than four weeks which is significantly much higher ( $P<0.05$ ) than a similar record for HIV negatives 14(16.5%). In 27.6% (91/330) of the diarrhoeal cases, the duration of diarrhoea lasted from 1 to 4 weeks; more in HIV negative (83.5%) than in HIV positive (8.2%). Only a single HIV positive (0.3%) individual had less than one week. Unlike HIV positives (16.5%), most HIV negative diarrhoea patients, 71/85(83.5%), had diarrhoea that lasted 1 to 4 weeks. The majority of patients with chronic diarrhoea, 66.9% (221 of 330), had 2 to 6 times bowel motion per day; of these 208/245(84.9) were HIV positive, much higher compared to HIV negatives 15/85 (15.3%). Only 2 (0.8%) HIV positive patients had greater than 6 times bowel motion per day. Most of the patients had loss of appetite and had also abstained from different kinds of solid foods that limited their bowel motion during diarrhoeal episodes. The major clinical symptoms observed in diarrhoeal patients were abdominal pain with cramping, weight loss of more than 10% of the body weight and anorexia that is more frequently observed in HIV positives than HIV negative diarrhoeal patients (Table 7). The minor clinical presentations noted as low grade fever, nausea, vomiting frequently observed in HIV positive diarrhoeal patients, whereas tenesmus and flatulence were more common in HIV negative than HIV positive diarrhoeal patients.

**Table 4. Demographic characteristics of 330 diarrhoeal patients enrolled in the study from 3 hospitals in Addis Ababa ( March 2002-December 2003)**

Characters	No. Examined(%) (N=330)
Patient	
In-patients	190(57.6)
Out-patients	140(42.4)
Sex	
Male	220(66.7)
Female	110(33.3)
Age	
	mean34yrs (range16-70 yrs)
Occupation	
Police force	85(25.8)
Army	81(24.5)
Factory workers	33(10.0)
House wives	69(20.9)
Administration/office workers	31(9.4)
Students	7(2.1)
Traders	17(5.2)
Drivers	7(2.1)
Marital Status	
Married	234(70.9)
Single	67(20.3)
Divorced	15(4.5)
Widowed	14(4.2)
HIV status	
HIV positive	245(74.2)
HIV negative	85(25.8)

**Table 5. Distribution of 330 diarrhoeal patients in relation to HIV-serostatus by age and sex among 3 hospitals in Addis Ababa ( March 2002-December 2003)**

	HIV-positive (%)	HIV-negative	Total
Age (years)	(n=245)	(n=85)	N=330)
15-24	23 (9.4)	12 (14.1)	35(10.6)
25-34	98 (40.0)	33 (38.8)	131(39.7)
35-44	94 (38.4)	19(22.4)	113(34.2)
≥ 45	30 (12.3)	21(24.7)	51(15.5)
<b>Sex</b>			
Male	169(68.6)	51(60.0)	220(67.7)
Female	76(31.0)	34(40.0)	110(33.3)

**Table 6. Distribution of CD4+ (n=209), Viral load (n=107) and WHO-staging of HIV-disease (n=245) on diarrhoeal patients with HIV/AIDS from 3 hospitals in Addis Ababa ( March 2002-December 2003).**

<b>CD4+cell count</b>	<b>Observed( %)</b>
<50cell/mm <sup>3</sup>	88(42.1)
>or =50 and<100cell/mm <sup>3</sup>	51(24.4)
>or=100 and <200cell/mm <sup>3</sup>	44(21.1)
>or=200cell/mm <sup>3</sup>	26(12.4)
<b>HIV-1 RNA Viral load</b>	
<10000copies/ml	13(12.2)
>or=10000 <100000	37(34.6)
>or = 100000 copies/ml	57(53.3)
<b>WHO-Staging</b>	
I	13(5.3)
II	27(11.0)
III	74(30.2)
IV	131(53.5)

Table 6 shows HIV positive patients in relation to CD4+ cell counts, viral load and clinical staging. The mean CD4+ cell count of HIV/AIDS diarrhoea patients was 95 cells/mm<sup>3</sup> ranging from 1 to 756 cells/mm<sup>3</sup>. The majority of HIV positive patients 88/209 (42.1%) had a CD4+ cell count below 50 cells/mm<sup>3</sup> with a mean 22.8 cells/mm<sup>3</sup> ranging (1-48 cells/mm<sup>3</sup>). A few HIV positive individuals 26/209 (12.4%) had CD4+ cell count greater or equal to 200 cells/mm<sup>3</sup>. Over all, the patients with CD4+ cell count less than or equal to 100 cells/mm<sup>3</sup> were 139/209 (66.5%) or below 200 cells/mm<sup>3</sup> were 183/209(87.6%). This indicates that the majority of HIV/AIDS diarrhoea patients were severely immunosuppressed and highly susceptible to various opportunistic infections including opportunistic intestinal parasites.

HIV-1 RNA viral load was analysed for 107 diarrhoeal patients positive for opportunistic intestinal parasites (Table 6). More than half of the patients had a viral load greater or equal to 100000 copies/ml and 13/107(12.2%) had less than 10000 copies/ml. The majority, 94/107(87.8) were greater than or equal to 10000 copies/ml. The mean viral load for the study subjects was 293391.6 copies/ml. The detection limit for the test was 80 copies/ml.

As shown in Table 6, the majority of HIV positive cases, 131/245(53.5%) were categorized in the late stage IV, whereas 74/245(30.2%) were in stage III and the rest were in stage II(11%) and I (5.3%), respectively. This is based on the WHO-staging criteria for HIV-disease progression (annex III) (WHO, 1993). The result showed that there is severe depletion of immunity among diarrhoeal HIV/AIDS patients, and the majority were at similar spectrum of immunosuppression.

Out of 330 diarrhoeal patients examined for intestinal parasites, 268 (81.2%) were positive for one or more parasites. Based on HIV status of diarrhoea patients, 203/245 (82.9%) of HIV positive and 65/85 (76.4%) of HIV negatives were positive for at least one intestinal parasite.

As shown in Table 8, conventional intestinal parasites particularly helminths as compared to the intestinal protozoa were very rare. *Ascaris lumbricoides*, *Trichuris trichiura*, Hookworm Spp., *Taenia* Spp, *Schistosoma mansoni*, *Fasciola* spp. were documented in a prevalence of less than 2% in HIV positive diarrhoea patients. Among the helminths, *Strongyloides stercoralis* was detected in 23 (9.4%) in HIV positives and in 4(4.7%) HIV negatives; with no significant difference ( $P>0.05$ ) observed between HIV positive and HIV negative diarrhoea patients. *Ascaris lumbricoides* and *Taenia* Spp. were more common in HIV negatives than HIV positives, with high significant difference between the groups ( $P<0.001$ ).

*Entameaba histolytica/dispar* was 2(0.8%) in HIV positives and 10(11.8%) HIV negatives with significant difference ( $P<0.05$ ) between the groups. However, *Giardia lamblia* 14 (5.7%) in HIV positives and 9(10.6%) HIV negatives were detected with no significant difference among the group (Table 8). Non-pathogenic protozoa such as *Entamoeba coli* in 48 (14.6%), *Entamoebna hartmanni* in 9 (2.7%), *Endolimax nana* in 14 (4.7%), *Iodoamoeba butschilii* in 5(1.5%), *Chilomastix mesnelli* in 13 (3.9%) and *Sarcocystis* Sp. in 10(3.0%) were identified. They were more frequent in HIV negative than HIV positive diarrhoeal patients but no difference was observed ( $P>0.05$ ) (Table 8). *Blastocystis hominis* was more frequently detected, 89(36.3%) in HIV positives than 27 (31.8%) in HIV negatives, but no significant difference ( $P>0.05$ ) among the group was again observed. *Entamoeba* trophozoite was commonly identified in HIV negatives 16(18.8%) than HIV positives 34(13.9%). *Entamoeba coli* was more prevalent 17(20.0%) in HIV negatives than 31(12.4%) in HIV positives and showed no significant difference among the groups ( $P>0.05$ ).

**Table 7. Clinical presentations of 330 diarrhoea patients from 3 hospitals in Addis Ababa (March2002-December 2003)**

Features		HIV positive(%) (n=245)	HIV-negatives(%) (n=85)	Total(%) (n=330)
Duration of diarrhoea				
<1week		1(0.4)	0	1(0.3)
1-4weeks		20(8.2)	71(83.5)	91(27.6)
>4weeks		224(91.4)	14(16.5)	238(72.1)
Diarrhoea	type/Bowel			
Motion/Day				
Acute(2-6BM/D)		16(6.5)	65(76.5)	81(24.6)
(2-6BM/D)		3(1.2)	5(5.9)	8(2.4)
(>6BM/D)		208(84.9) *	13(15.3)	221(66.9)
Chronic(2-6BM/D)				
(2-6BM/D)		2(0.8)	0(0.0)	2(0.6)
(>6BM/D)		16(6.5)	2(2.4)	18(5.5)
Intermittent				
Appearance				
Loose		62(25.3)	27(31.6)	89(26.9)
Watery		154(62.9) *	34(40.0)	188(56.9)
Mucoïd		22(8.9)	20(21.5)	42(2.7)
Bloody		7(2.9)	4(4.7)	11(3.3)
Clinical symptoms				
Abdominal pain		178(72.2)	59(69.4)	237(70.9)
Weight loss		216(86.5) *	10(11.8)	226(68.5)
Vomiting		106(43.3) *	13(15.3)	119(36.1)
Nausea		115(46.9) *	22(25.9)	137(41.5)
Anorexia		153(62.5) *	29(34.1)	182(55.2)
Fever (low grade)		119(48.6) *	11(12.9)	130(39.4)
Tenesmus		64(26.1)	36(42.4)	100(30.3)
Flatulence		49(20.0)	20(23.5)	69(20.9)

\*P<0.005

**Table 8. Prevalence of common intestinal parasites among 330 patients, HIV positive and HIV negative with diarrhoea from 3 hospitals in Addis Ababa using direct and formol ether concentration methods (March 2002-December 2003).**

<b>Parasite identified</b>	<b>HIV-Positive(%)</b> (n=245)	<b>HIV-Negative(%)</b> (n=85)	<b>Total (%)</b> (N=330)
<b>Helminths</b>			
<i>Ascaris lumbricoides</i>	3 (1.2)	8 (9.4) *	11(3.3)
<i>Trichuris trichiura</i>	4 (1.6)	1 (1.2)	5 (1.5)
<i>Hookworm Spp.</i>	2 (0.8)	3(3.5)	5 (1.5)
<i>Strongyloides stercoralis</i>	23 (9.4)	4 (4.7)	27 (8.2)
<i>Taenia Spp.</i>	2 (0.8)	6 (7.1) *	8(2.4)
<i>Schistosoma mansoni</i>	3 (1.2)	1 (1.2)	4 (1.5)
<i>Fasciola Spp.</i>	1 (0.4)	0 (0)	1 (0.3)
<b>Protozoans (non-opportunistic)</b>			
<i>Entamoeba histolytica/dispar</i>	2 (0.8)	10 (11.8) *	12(3.6)
<i>Giardia lamblia</i>	14 (5.7)	9(10.6)	23 (6.9)
<i>Entamoeba trophozoite</i>	34 (13.9)	16 (18.8)	50 (15.2)
<i>Entamoeba coli</i>	31 (12.4)	17 (20.0)	48 (14.6)
<i>Entamoeba hartmanni</i>	7 (2.9)	4 (4.7)	11 (3.3)
<i>Endolimax nana</i>	8 (3.3)	6 (7.1)	14 (4.2)
<i>Iodoamoeba butscillii</i>	4 (1.6)	1 (1.2)	5 (1.5)
<i>Blastocystis hominis</i>	89 (36.3)	27 (31.8)	116 (35.2)
<i>Sarcocystis Spp.</i>	8 (3.3)	2 (2.4)	10 (3.0)
<i>Chilomastix mesnelli</i>	9 (3.7)	4 (4.7)	13 (3.9)

**\*P=0.000, P<0.05**

The more frequently detected intestinal parasites in diarrhoeal patients with HIV infection were opportunistic intestinal parasites (Table 9); 67% (164 out of 245) well-known opportunistic intestinal parasites (i.e. *Cryptosporidium*, *Isospora* and intestinal microsporidia)

were detected in HIV positive diarrhoeal patients (Figures 13 and 14). No *Cyclospora cayetanensis* was identified among the diarrhoea patient be they HIV positive or HIV negative.

When *Strongyloides stercoralis* was considered the infection rate of “opportunistic” intestinal parasites had increased to 76.3 % (187 out of 245) indicating that gut associated opportunistic intestinal parasites were major causes of diarrhoea in late stage of HIV infection in Ethiopia. Whereas in HIV negatives 7.1% (6 out of 85) of opportunistic intestinal parasites were detected.

**Table 9. Prevalence of opportunistic intestinal parasites among 245 HIV positive and 85 HIV negative diarrhoeal patients among 3 hospitals in Addis Ababa using concentration, modified Ziehl Neelsen, Uvitex-2B and PCR ( March 2002-December 2003).**

	HIV+ve(%)	HIV-ve(%)	Total	P-value
Parasite identified	(n=245/214)	(n=85/68)	(n=330/282)	
<i>Cryptosporidium parvum</i>	70 (28.6)*	6(7.1)	76(23.0)	(P=0.000)
<i>Isospora belli</i> (MZN)	55 (22.5) *	0	55 (20.1)	(P=0.000)
<i>Isospora belli</i> (D & Conc) <sup>©</sup>	30 (12.3)	0	30 (12.2)	
<i>Enterocytozoon bienesui</i>	30/214(14.0)	0/68	30/282 (10.6)	(P=0.004)
<i>Encephalitozoon intestinalis</i>	6/214(2.8)	0/68	6/282(2.1)	
Double infection	3/214(1.4)	0/68	3/282(1.1)	
Intestinal microsporidia¶	39/214(18.2)	0/68	39/282(13.8)	(P=0.004)
Total OIP	164	6	170	

© *Isospora belli* in direct and concentration was also observed by Modified Ziehl Neelsen

OIP=opportunistic intestinal parasites

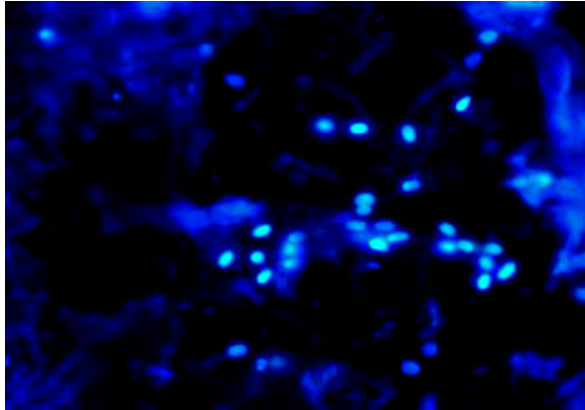
¶ Intestinal microsporidia= *E.bienesui* +*E.intestinalis* +double infections

An important opportunistic intestinal parasite identified in this study was intestinal microsporidia detected by Polymerase Chain Reaction (PCR) and Uvitex- 2B methods. Out of 282 diarrhoeal stool samples, 39( 13.8%) cases were positive for intestinal microsporidia by either of the two methods, 18.2%(39/214) were in HIV positives diarrhoeal patients. (Table 9). However, of the 39 microsporidial positive cases 18 positive by microscopy were also positive by PCR; and 21/39 were positive only by Polymerase Chain Reaction (Table 10). It showed a sensitivity of 47% and specificity of 100%, taking PCR as a gold standard. The spores of *Enterocytozoon bieneusi* are small in size (Figure 9). The spores of *Encephalitozoon intestinalis* are bigger and considerably variable in shape and size, usually being broad and kidney shape (Figure 10). Based on both PCR and microscopy analysis, the microsporidial parasites species involved were identified as 30 (77%) *Enterocytozoon bieneusi*, 6 ( 15.4%) *Encephalitozoon intestinalis* and double infection both *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis* in 3 (7.7%). All patients positive for intestinal microsporidiosis were HIV positives. No case was observed among HIV negative diarrhoeal patients. Hence this could be one of the possible causes of chronic diarrhoea in HIV/AIDS diarrhoea patients.

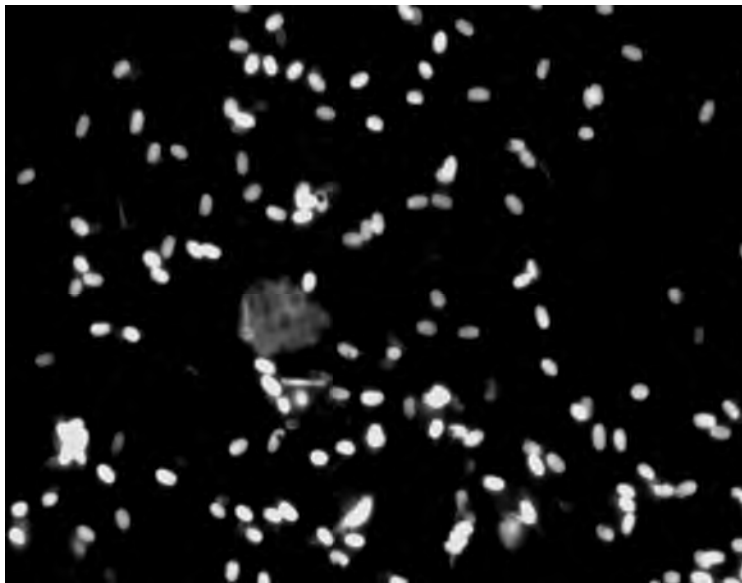
The clinical symptoms due to intestinal microsporidiosis is documented (Table 11). The result showed that among the microsporidial positive cases, majority of the patients 36 (92.3%) had diarrhoea for more than four weeks. Most of the patients 80.0% (31 out of 39) had watery diarrhoea and 7 patients (17.9%) had loose type of diarrhoea. Only a single individual with intestinal microsporidiosis (2.7% ) had mucoid stools.

A weight loss of more than 10% was recorded in the majority 94.9% (37/39) of the cases with intestinal microsporidial infections.

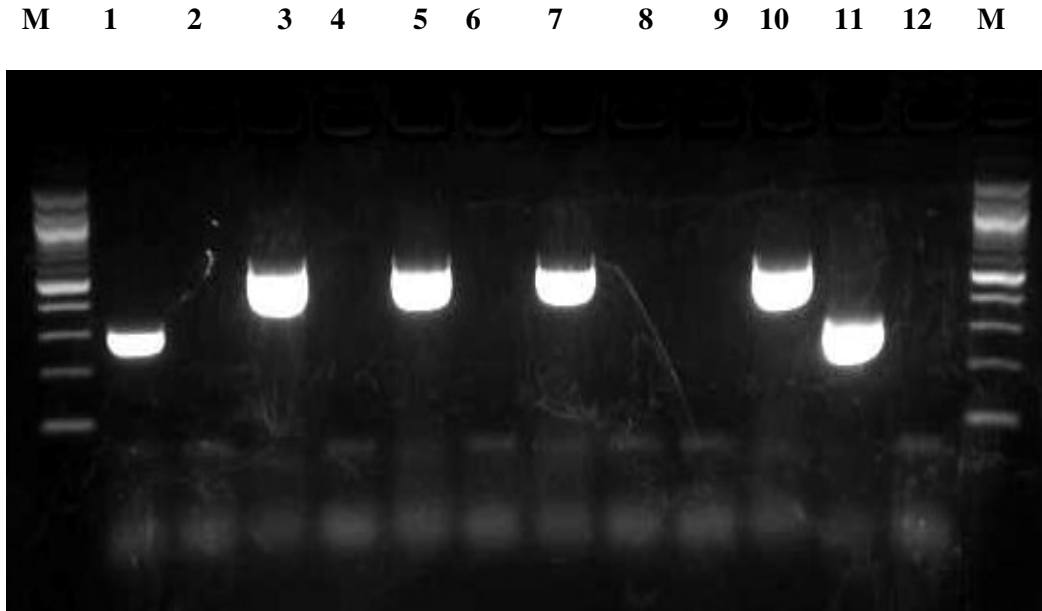
Further analysis of CD4+ cell counts into different categories showed that 26.8% (19/71) with intestinal microsporidiosis were under category of CD4+ cell counts below 50 cells/mm<sup>3</sup>. Eight cases(17.8%) were at CD4+ cell count between 51-100cells/mm<sup>3</sup> (Table 12). Five cases (12.8%) were at CD4+ cell count between 101-200cells/mm<sup>3</sup>.



**Figure 9.** *Enterocytozoon bieneusi* spores from stool sample stained with Uvitex-2B under fluorescent microscope (magnification 1000X).



**Figure 10.** *Encephalitozoon intestinalis* spores from stool sample stained with Uvitex-2B under fluorescent microscope (magnification 1000X)

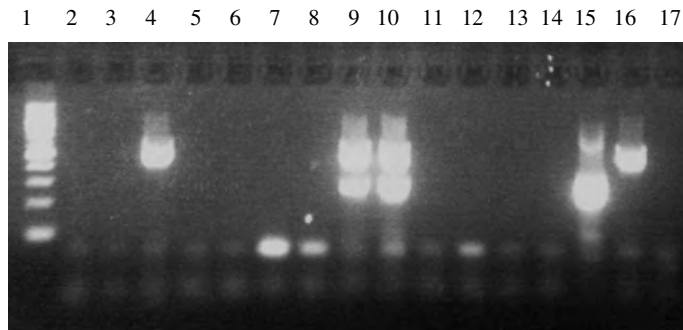


**Figure 11. An agarose gel showing PCR products amplified from human faecal samples by nested microsporidium PCR . *Enterocytozoon bienesui* specific products(500bp) detected in lanes 3, 5 and 7, and non *Enterocytozoon bienesui* microsporidium specific product (300bp) in lane1. Positive controls with *Enterocytozoon bienesui* (lane 10) and with *Encephalitozoon intestinalis* (lane 11). Negative control (lane 12). M represents a 100bp ladder.**

All patients with intestinal microsporidiosis were HIV positives and their CD4+ cell count was below 200 cells/mm<sup>3</sup> (Table 13). In general, 19 out of 32 (59.4%) were under CD4+cell count below 50 cells/mm<sup>3</sup> or 84.4% (27/32) were under CD4+cell count below 100 cells/mm<sup>3</sup>, suggesting that the patients were severely immunosuppressed.

The majority of the patients (25 out of 28) with intestinal microsporidiosis were found under the category of high HIV-1 RNA viral load count, above 10000 copies/ml. Only few cases (3 out of 28) were at lower viral load count, below 10000copies/ml (Table 14), but no significant difference ( $P>0.05$ ) among the group was observed.

Based on the WHO clinical staging of HIV disease (1993), the majority of the patients with intestinal microsporidiosis were in stage III 27.5% (18/65) and in stage IV 15.8% (18/114). No case was identified in stage I (Table 15). Half of the patients with intestinal microsporidiosis were at stage IV and the rest were apparently at stage III, which implies that the patients involved in the present study were at late stages of HIV infections. Double infection with *E.bieneusi* and *E.intestinalis* is a rare occurrence in diarrhoea HIV/AIDS patients. Three of the patients with double infection were CD4+ cell count below 50 cells/mm<sup>3</sup> and at stage IV of clinical AIDS that implies severe immunosuppression.



**Figure 12. An agarose gel showing PCR products amplified from human faecal samples by nested microsporidium PCR. Lane 1 (100bp) marker, lane 17 negative control, lane 16 *E.bieneusi* positive control, lane 15 *E.intestinalis* positive control, lane 4 *E.bieneusi* positive product, lane 9&10 double infection**

**Table 10. Comparison of microscopy and PCR for intestinal microsporidiosis on diarrhoea stool samples from 3 hospitals in Addis Ababa (n=282 )**  
**PCR**

	<b>Positive</b>	<b>Negative</b>	<b>Total</b>
<b>Microscopy</b>			
<b>Positive</b>	18	0	18
<b>Negative</b>	21	243	264
<b>Total</b>	39	243	282

*Cryptosporidium parvum* was another opportunistic intestinal parasite frequently observed with a prevalence rate of 23.0% (76/330) in diarrhoeal patients (Table 9). Cryptosporidial infection was detected with the prevalence of 28.6% (70/245) in HIV positives and 7.1% (6/85) in HIV negatives, with highly significant difference (P<0.001) observed among the group.

HIV positive patients with cryptosporidiosis, 60/70 (85.5%) had diarrhoea that lasted for more than four weeks and in 10 (14.3%) the diarrhoea lasted for less than four weeks (Table 11). The majority of cryptosporidiosis cases with HIV infection 54/70(77.1%) had 2-6 bowel movement per day and in 5 (7.1%) the diarrhoea was intermittent. A weight loss of 10% was recorded in 61/70(87.3%) in cryptosporidiosis cases with HIV infection. The other clinical presentations noted in HIV positives with cryptosporidiosis were: abdominal cramp, vomiting, nausea, anorexia, low grade fever and flatulence. The appearance of diarrhoea in HIV Positives with cryptosporidiosis was watery in 49/70 (70.0%), loose in 14/70 (20.0%), mucoid in 5/70 (7.1%) and was bloody in 2/70 (2.9%) that could be due to other concurrent infections.

The highest category in cryptosporidial infection 39.8% (35/88) in relation to CD4+ cell was observed in patient with cell count below 50 cells/mm<sup>3</sup> (Table 12). Among cryptosporidiosis patients, 7/44(15.9%) were grouped in CD4+ cell counts above and equal to 100 and below 200 cells/mm<sup>3</sup>. Overall diarrhoeal patients with cryptosporidial infection, 56 out of 59 (89.8%) had CD4+ cell counts below 200 cells/mm<sup>3</sup> which is typical of this parasite in late stage of HIV infection. Only 3(11.5%) had CD4+ cell count greater than 200 cells/mm<sup>3</sup> (Table 13).

Based on the viral load analysis, the majority of *cryptosporidium* infected HIV positive diarrhoeal patients, 90.0% (45 out of 50) were in the range greater or equal to 10000 copies/ml. Whereas 10% (5/50) were in below 10000 copies/ml. Viral load count has shown no significant association (P>0.05) with *Cryptosporidium* infection (Table 14).

Clinical staging of *Cryptosporidium* infected HIV positive diarrhoeal patients is described in Table 15. Large number of patients with cryptosporidiosis, 60 out of 131, were grouped in stage IV category of AIDS (P=0.001) that is highly associated with the late stage of HIV infection. The rest were categorized as 5 (18.5%) in stage II and 4/74 (5.4%) in stage III. Only a single case, 7.7% was ranked in stage I. Over all, 60 out of 70 (85.7%) cases of cryptosporidial infection were in stage IV.

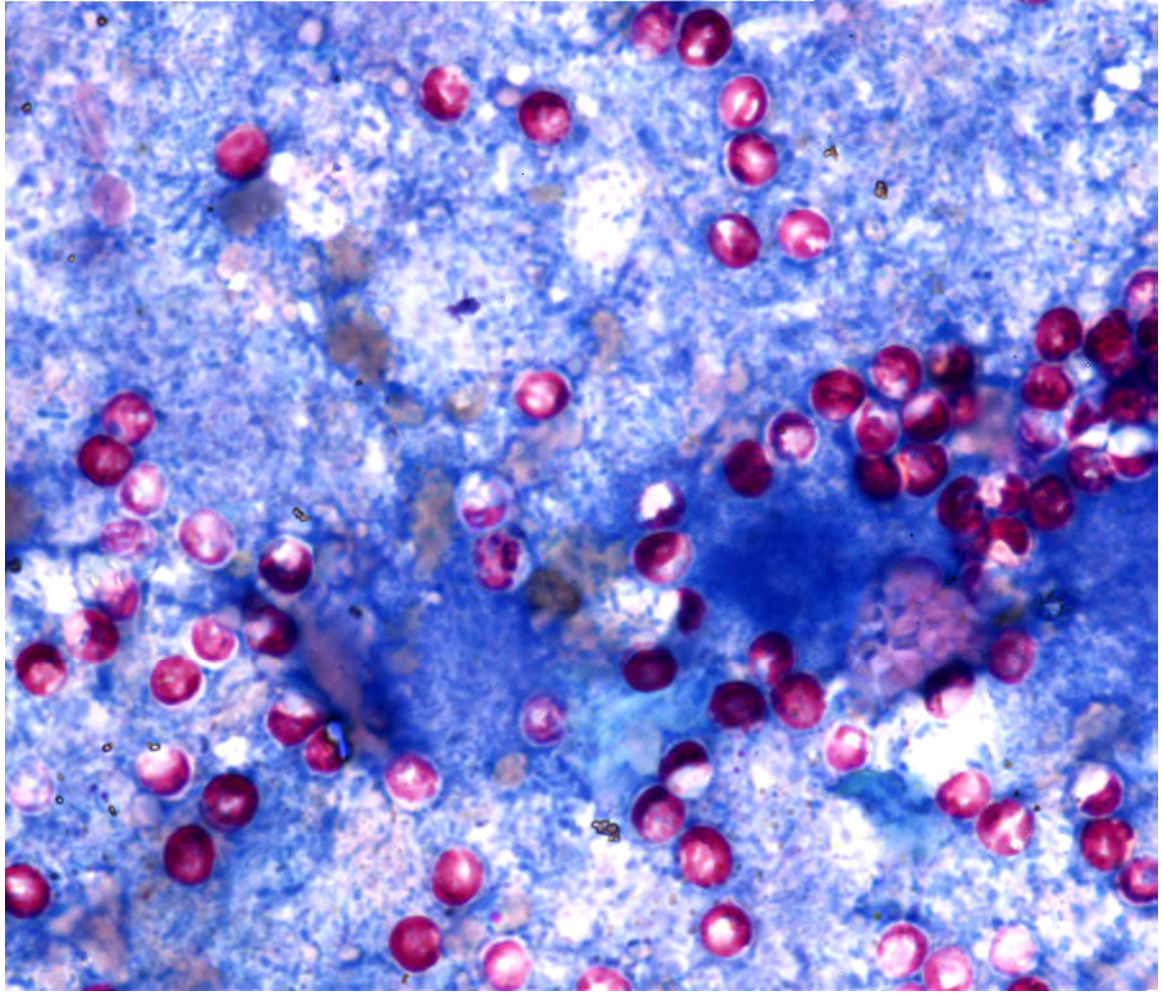


Figure 13. Oocyst of *Cryptosporidium parvum* from HIV/AIDS diarrhoea patient stained with modified Ziehl Neelsen, magnification (1000X)

**Table 11. Main clinical features of diarrhoea patients associated with opportunistic intestinal parasites (n=164) among HIV/AIDS patients among 3 hospitals in Addis Ababa (March 2002-December 2003)**

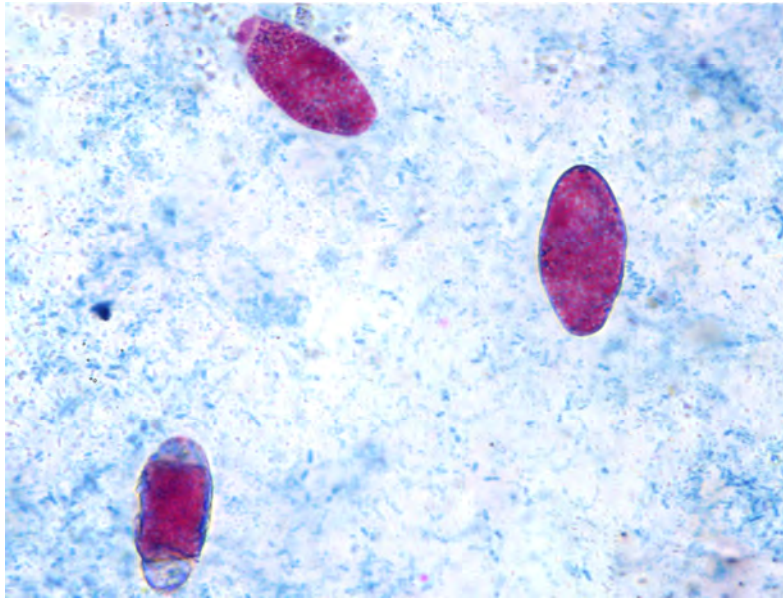
Clinical Features	<i>C. parvum</i> (n=70)	<i>I. belli</i> (n=55)	<i>E. bienersi</i> (n=30)	<i>E. intestinalis</i> (n=6)	Double Infection (n=3)
1. Diarrhoea duration					
<1 week	0	0	0	0	0
1-4 weeks	10(14.3)	2(3.6)	2(6.7)	0	1(33.3)
>4 weeks	60(85.7)	53(96.4)	28(93.3)	6(100.0)	2(66.7)
2. Type of Diarrhoea (Bowel motion/day)*					
Acute(2-6BM/D)	8(11.4)	2(3.6)	1(3.3)	0	0
(>6BM/D)	2(2.9)	0	0	0	0
Chronic(2-6BM/D)	54(77.1)	49(89.1)	27(90.0)	5(83.3)	2(66.7)
(>6BM/D)	1(1.4)	0	0	0	0
Intermittent	5(7.1)	4(7.3)	2(6.7)	1(16.7)	1(33.3)
3. Nature of Diarrhoea					
Watery	49(70.0)	45(81.8)	25(83.3)	4(66.7)	2(66.7)
Loose	14(20.0)	8(14.6)	5(16.7)	2(33.3)	0
Mucoid	5(7.1)	1(1.8)	0	0	1(33.7)
Bloody	2(2.9)	1(1.8)	0	0	0
4. Abdominal cramp	50(71.4)	40(72.7)	17(56.7)	5(83.3)	3(100.0)
5. Weight Loss(>10%)	61(87.1)	47(85.5)	28(93.3)	6(100.0)	3(100.0)
6. Nausea	33(47.1)	27(49.1)	17(56.7)	2(33.3)	3(100.0)
7 Vomiting	33(47.1)	31(56.4)	12(40.0)	4(66.7)	2(66.7)
8. Anorexia	42(60.0)	36(65.5)	23(76.7)	5(83.3)	1(33.3)
9. Fever (low grade)	36(51.4)	25 (45.5)	13(43.3)	4(66.7)	2(66.7)
10. Tenesmus	17(24.5)	8(14.6)	7(23.3)	1(16.7)	1(33.3)
11. Flatulence	15(21.4)	14(25.5)	10(33.3)	1(16.7)	1(33.3)

\*BM/D=Bowel motion per day

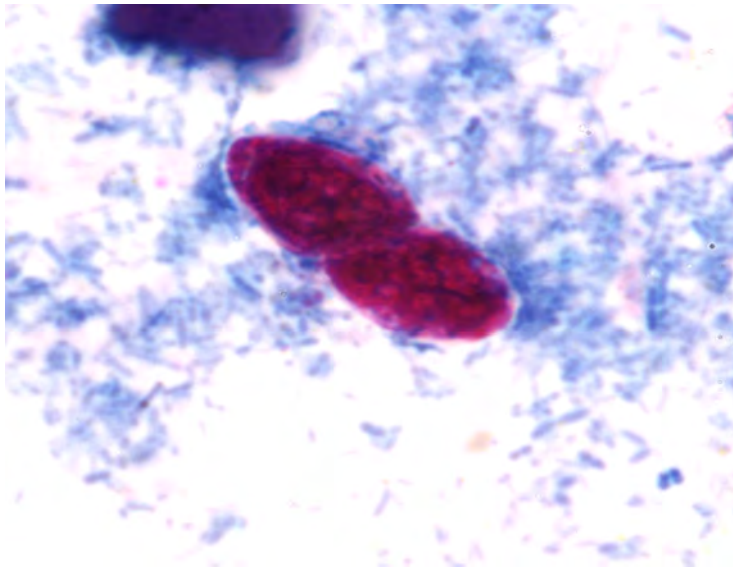
*Isospora belli* was another commonly encountered opportunistic intestinal parasite in this study with overall prevalence of 16.7% (55/330) recorded using modified Ziehl Neelsen method. All patient with *Isospora belli* infection were HIV positives with a prevalence of 22.5% (55/245). In HIV positive patients with diarrhoea a significant association ( $P<0.05$ ,  $P=0.004$ ) was observed with *Isospora belli* infection compared to HIV negative diarrhoea

patients. Out of 55 *Isospora belli* positive cases, direct microscopy (by direct and concentration methods) only 11.0% (30/274) were detected (Table 9). No parasite was isolated from HIV negative patients with diarrhoea. As shown in Table 11, diarrhoeal patients with *Isospora belli* infection, 96.4% (53/55) had diarrhoea duration of more than four weeks and only two cases (3.6%) had diarrhoea lasting for less than four weeks. The majority, 89.1% (49/55) with chronic diarrhoea had 2-6 bowel motions per day and 4 (7.3%) had intermittent type of diarrhoea. Only two cases (3.6%) with acute diarrhoea had 2-6 bowel motions per day. In the majority of the cases with isosporiasis, the appearance of diarrhoea was watery in 45/55 (81.8%), loose in 8(14.6%), mucoid in 1(1.8%) and bloody in 1(1.8%). Severe weight loss was observed in 47/55 (85.5%) of diarrhoeal patients with *Isospora belli* infections, which is a typical manifestation in the late stage of HIV infection with diarrhoea. The other clinical presentations noted in patients with *Isospora* infections were: abdominal pain, vomiting, anorexia, fever (low grade), nausea and flatulence.

The distribution of *Isospora belli* infection in relation to CD4+ cell count is presented in Table 12 and Table 13. Diarrhoeal patients with isosporiasis that had CD4+ cells below 50 cells/mm<sup>3</sup> were 21.6%(19 out of 88). While 14/51 (27.5%) were grouped in CD4+ cell counts greater or equal to 50 and below 100 cells/mm<sup>3</sup>. The rest 10 (22.7%) were in greater or equal to 100 and below 200 cells/mm<sup>3</sup>. Overall, 33 out of 45 (73.3%) were below 100 cells/mm<sup>3</sup> and 43 out of 45 (95.5%) were categorized below 200 cells/mm<sup>3</sup>, a characteristic feature of late stage of HIV infection. Only 2 cases (7.7%) were observed to have CD4+ above 200 cells/mm<sup>3</sup>.



a)



b)

Figure 14. Oocyst of *Isospora belli* stained with modified Ziehl Neelsen magnification (1000X).a) Un sporulated oocyst b) Sporulated oocyst

Determination of HIV-1 RNA viral load with *Isospora belli* infections is shown in Table 14; that 82.4% (28 out of 34) were categorized in viral load count greater or equal to 10,000 copies/ml. But no significant association was ( $P>0.05$ ) observed with viral load greater or equal to 10000 copies/ml. However, 6/34 (17.6%) was in viral load count below 10,000 copies/ml.

Another important feature was that, 40.8% (53/ 131) of *Isospora* infections in HIV positives was noted in stage four patients indicating high association ( $P= 0.001$ ) with the late stage of HIV infection. Only one case each in stage I (7.7%) and in stage III (1.4%) were detected. No case was detected in stage II (Table 15). Overall, 96.4% (53/55) HIV positive patients with *Isospora belli* infection were at the latest stage of HIV infection.

### 12.Spectrum of CD4+ cell count and opportunistic intestinal parasites in 209 HIV positive patients from 3 hospitals in Addis Ababa (March 2002-December 2003).

Parasite identified	CD4+ cell counts (cell/mm <sup>3</sup> )				Total (n=209)
	<50 CD4+ cell/mm <sup>3</sup> (n=88)	51-100 cell/mm <sup>3</sup> (n=51)	101-200 cell/mm <sup>3</sup> (n=44)	>200 cell/mm <sup>3</sup> (n=26)	
<i>Cryptosporidium parvum</i>	35 (39.8)	14(28.6)	7(15.9)	3(11.5)	59(28.2)
<i>Isospora belli</i>	19(21.6)	14(27.5)	10(22.7)	2(7.6)	45(21.5)
Intestinal microsporidia*	19/71(26.8)	8/45(17.8)	5/39(12.8)	0/22	32/177(18.1)
<i>Enterocytozoon bieneusi</i>	15/71(21.1)	6/45( 2.2)	5/39(12.8)	0/22	26/177(14.7)
<i>Encephalitozoon intestinalis</i>	3/71(4.2)	1/45(2.2)	0/39	0/22	4/177(5.2)
Double infection¶	1/71(1.4)	1/45(2.2)	0/39	0/22	2/177(1.1)
Total OIP	73	32	22	5	136

OIP= Opportunistic Intestinal Parasites

¶ Double infection= *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis*

**\*Intestinal Microsporidia= *E. bieneusi* + *E. intestinalis* +double infection**

Based on the result shown in Table 12, each opportunistic intestinal parasite in relation to CD4+ cell count was described under the result indicated for each opportunistic intestinal parasite in the text.

**Table 13. Summary of CD4+ cell count and opportunistic intestinal parasites (n=136) in HIV positive diarrhoea patients from 3 hospitals in Addis Ababa (March 2002-December 2003)**

CD4+ cell count	Opportunistic intestinal parasites			Total (n=136)
	<i>C.parvum</i> (%) (n=59)	<i>I.belli</i> (%) (n=45)	Intestinal microsporidia(%) (n=32)	
Less than 200 cell/mm <sup>3</sup> (n=183)	56(95.6)	43(94.9)	32(100.0)	131(96.3%)
Greater or equal to 200 cell/mm <sup>3</sup> (n=26)	3(4.4)	2(5.1)	0(0.0)	5 (3.7%)

The summarised value of CD4+ cell count in relation to known opportunistic intestinal parasites of 136 patients is presented in Table 13. The majority of patients were infected with *Cryptosporidium parvum* (95.6%) and *Isospora belli* (94.9%) had CD4+cell count below 200 cell/mm<sup>3</sup>. The patients with intestinal microsporidiosis all had CD4+ cell counts below 200 cell/mm<sup>3</sup>. The majority of diarrhoeal patients with HIV/AIDS included in this study were in one spectrum of immunosuppression. (i.e. their CD4+ cell count was below200 cell/mm<sup>3</sup>). And they were severely immunosuppressed. A few patients were in the threshold that the CD4+ cell counts (greater or equal to200 cell/mm<sup>3</sup> ) likely protects diarrhoea illness caused by opportunistic intestinal parasites.

The relationship of opportunistic intestinal parasites has shown with increasing CD4 cell counts shown in Figure 15; at CD4+ less than 50 cells/mm<sup>3</sup> the prevalence of opportunistic intestinal parasites had increased significantly.

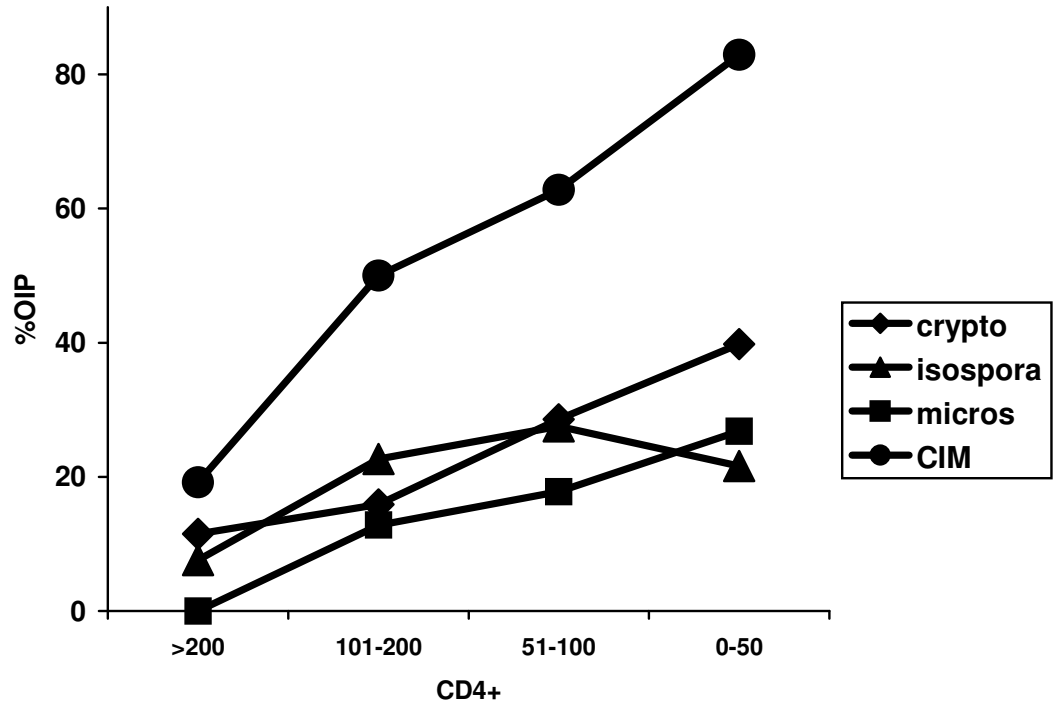


Figure 15. CD4+ and opportunistic intestinal parasites in 164 diarrhoea HIV/AIDS patients

Note: Microsporidia = *E. bieneusi* and *E.intestinalis*; CIM= *Cryptosporidium-Isospora* and *Microsporidia*

CD4+ cell count in relation to each opportunistic intestinal parasites has shown that with higher CD4+ cell count the trend in percentage of opportunistic intestinal parasites was low. However, in those with CD4+ cell count 51-200 cells/mm<sup>3</sup> *Isospra belli* was almost at stable order where as in these with two parasites showed decreasing trend. Very Low percentage

of *Cryptosporidium parvum* (11.5%) and *Isospora belli* (7.6%) have identified in those with CD4+ cell count above 200 cells/mm<sup>3</sup>.

**14. HIV-1 RNA viral load and opportunistic intestinal parasites in HIV positive diarrhoeal patients among 3 hospitals in Addis Ababa (March 2002-December 2003)**

<b>Opportunistic intestinal parasites</b>				
<b>Viral load</b>	<b><i>C. parvum</i> (%) (n=50)</b>	<b><i>Isospora belli</i> (%) (n=34)</b>	<b>Intestinal microsporidia (%) (n=28)</b>	<b>Total (n=112)</b>
Less than 10000copies/ml (n=13)	5(10.0)	6(17.6)	3(10.7)	14
Greater or equal to 10000 copies/ml (n=94)	45(90.0)	28(82.4)	25(89.3)	98

**Table 15. Distribution of opportunistic intestinal parasites among 245 HIV positives based on the WHO-staging criteria from 3 hospitals in Addis Ababa (March 2002-December 2003)**

<b>Parasite identified</b>	<b>Stage I (n=13)</b>	<b>stage II (n=27/22)</b>	<b>stage III (n=74/65)</b>	<b>Stage IV (n=131/114)</b>	<b>Total (n=245/214)</b>
<i>Cryptosporidium parvum</i>	1(7.7)	5(18.5)	4(5.4)	60(45.8)	70
<i>Isospora belli</i>	1(7.7)	0	1(1.4)	53(40.5)	55
Intestinal microsporidia	0/13	3/22(13.6)	18/65(27.7)	18/114(15.8)	39/214
<i>Enterocytozoon bieneusi</i>	0/13	2/22(9.1)	14/65(21.5)	14/114(9.7)	30/214
<i>Encephalitozoon intestinalis</i>	0/13	1/22(4.6)	1/65(1.5)	4/114(2.8)	4/214
Double ¶ infection	0/13	0/22	3/65(4.6)	0/114	2/214
Total OIP	2	8	23	131	164

© PCR and Uvitex-2B for intestinal microsporidiosis was done for 214 HIV positive cases.

// Double infection= *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis*

As shown in Table 15, almost diarrhoeal patients in stage IV had one opportunistic intestinal parasite. Two cases were identified in stage I. In stage II no *Isospora belli* was identified but 5 cases of *Cryptosporidium parvum*, 3 cases of intestinal microsporidia were detected. Relatively much less opportunistic intestinal parasites were identified in stage I and II. More than half of the diarrhoeal HIV/AIDS patients were at late stage of HIV infection.

The pattern of multiple parasitism in HIV positives and in HIV negatives in diarrhoeal patients is shown in Table 16. Of the total examined, 35.7% (117 out of 330) had only one parasite. One parasite was more common in HIV positive than HIV negative individuals. HIV positive patients (44.9%) had two or more parasites less compared to HIV negatives (48%), but no significant difference ( $P > 0.05$ ) was observed among HIV positive and HIV negative diarrhoea patients.

**Table 16. Pattern of multiple parasitic infection among 330 HIV positive and HIV negative diarrhoeal patients among 3 hospitals in Addis Ababa using wet mount, formol ether concentration, modified Ziehl Neelsen, Uvitex-2B and PCR .**

Parasite identified	HIV-positive (%) (n=245)	HIV-negative (%) (n=85)	Total (%) (N=330)
none	42 (17.1)	20 (23.0)	62 (18.8)
1 species	93 (37.9)	24 (28.2)	117 (35.5%)
2 species	63 (25.7)	29 (34.0)	92 (27.9)
3 species	38 (15.5)	8 (9.4)	46 (13.9)
≥4 species	9 (3.7)	4 (4.7)	13 (3.9)

The prevalence of opportunistic parasites in HIV/AIDS diarrhoea patients in relation to sex and age is depicted in Table 17. The highest prevalence of *Cryptosporidium* infection was detected (43.5%) in age group 15 to 24 years followed by age group 25-34 years (32.7%). It was lower although not significant ( $P>0.05$ ) in older age group ( $>35$  years) as compared to the younger age group (15-34 years). *Isospora* infection was low in younger age group 15-34 years than older age group of the study population. The least infection rate was noted in age group 15-24 years. Intestinal microsporidial infections were more common in age group 35-44 years followed by 25-34 years. The same trend was observed in HIV status in these age groups (Table 5). Male had more infections with opportunistic intestinal parasites than female, even though no significant difference was observed ( $P>0.05$ ), which also showed similar pattern to HIV infection in both sexes.

**Table 17. Prevalence of opportunistic intestinal parasites by age and sex among 245/208 HIV/AIDS diarrhoeal patients from 3 hospitals in Addis Ababa (March 2002-December 2003)**

		<i>C. parvum</i> (%)	<i>I. belli</i>	<i>E. bieneusi</i>	<i>E. intestinalis</i>	Double Infection	Total
<b>Age (N)</b>							
15-24	23/19	10(43.5)	3 (13.0)	1/19 (5.3)	3/19 (15.8)	0/19(0.0)	17
25-34	98/86	32 (32.7)	18 (18.4)	12/86 (13.9)	2/86(2.3)	0/86(0.0)	64
35-44	94/78	20 (21.3)	25 (26.6)	14/78(17.9)	0/78 (0.0)	2/78 (2.6)	61
≥45	30/25	8 (26.7)	9(30.0)	3/25(12.0)	1/25(4.0)	1/25 (4.0)	22
<b>Sex</b>							
Male		52 (31)	39 (23)	25/147 (17)	5/147 (4)	2/147 (1.4)	123
	n=169/147						
Female		18 (24)	16 (21.1)	5/61(8.2)	1/61 (1.6)	1/61 (1.6)	41
	n=76/61						

**Eb= *E. bieneusi*, Ei= *E. intestinalis*, OIP=opportunistic intestinal parasites**  
**NB:In HIV negative diarrhoea patients *Cryptosporidium* was detected in 3 cases at age group 15-24 years and 3 cases at age group of 25-34 years.**

Among the helminths, *Strongyloides stercoralis* was the most common among HIV positive diarrhoeal patients. Major clinical presentations observed with this parasite in HIV/AIDS patients is described in Table 18. The majority of the patients with strongyloidiasis, 95.7% (22/23), had diarrhoea that lasted more than four weeks, 86.9%(20/23) had chronic diarrhoea with 2-6 bowel motions per day while 13.0%(3/23) developed intermittent diarrhoea. In most of the cases, 73.9%(17/23) had watery diarrhoea, 13.0% with loose diarrhoea. Main clinical

manifestations observed were abdominal pain in 73.9%(17/23), weight loss in 95.7%(22/23), anorexia in 73.9%, nausea in 52.2%, fever in 60.9% and vomiting in 43.5%(10/23).

**Table 18. Some clinical manifestations in 23 HIV positive diarrhoeal patients with *Strongyloides stercoralis* infection from 3 hospitals in Addis Ababa detected using wet mount and formol ether concentration methods (March 2002-December2003).**

<b><u>Features</u></b>	<b><u>observed(%)</u></b> <b><u>(n=23)</u></b>
<b>Diarrhoea duration</b>	
<1week	0
1-4weeks	1(4.4)
>4weeks	22(95.6)
<b>Diarrhoea type&amp;BMD</b>	
Acute(2-6BM/D)	0
(>6BM/D)	0
chronic(2-6BM/D)	20( 87.0)
(>6BM/D)	0
Intermittent	3(13.0 )
<b>Appearance of Diarrhoea</b>	
Watery	17(73.9)
Loose	3(13.0)
Mucoid	2(8.7)
Bloody	1(4.4)
<b>Clinical Symptoms</b>	
Abdominal pain	17(73.9)
Weight loss	22(95.7)
Vomiting	10(43.5)
Nausea	12(52.2)
Anorexia	17(73.9)
Fever low grade	14(60.9)
Tenesmus	9(39.1)
Flatulence	1(4.4)

Out of 23 cases (9.4%) of *Strongyloides stercoralis* identified in HIV positive diarrhoeal patients, the majority 14/23 (60.9%) were in stage IV. The remaining 7/23 (30.4%) were in stage three (Table 19). A few were in stage I and stage II of HIV infection. The CD4+ cell count of 20 HIV positives with *Strongyloides stercoralis* infection was determined (Table 19). The majority 8/20 (40%), were grouped within CD4+ cell counts below 50cells/mm<sup>3</sup> and 5/20 (25%) were at CD4+ cell counts between 51-100 cells/mm<sup>3</sup>. Five cases (25%)



Figure 16. *Strongyloides stercoralis* rhabditiform larvae from HIV/AIDS diarrhoea patient magnification (400X).

were at CD4+ cell counts between 101-200 cells/mm<sup>3</sup> and only a single case was identified at CD4+ cell counts above 200cells/mm<sup>3</sup>.

**Table 19. Clinical staging (n=245) and CD4+ cell counts (n=209) of HIV positive diarrhoeal patients infected with *Strongyloides stercoralis* using direct and formol-ether concentration methods from 3 hospitals in Addis Ababa (March 2002-December2003)**

<u>WHO-staging*</u>	<u>No(%)</u>
Stage I	1/13(7.7)
Stage II	1/27(3.7)
Stage III	7/74 (9.5)
Stage IV	14/131(10.7)
<b>CD4+cells count*</b>	
<50cells/mm <sup>3</sup>	8/88 (9.1)
50-100cells/mm <sup>3</sup>	6/51 (11.8)
101-200cells/mm <sup>3</sup>	5/44 (11.4)
>200cells/mm <sup>3</sup>	1/26 (3.9)

\* Proportion was rated base on total CD4+cell count(n=209) and staging (n=245) on table 6.

Among the intestinal protozoan, *Blastocystis hominis* was more frequently detected than other common intestinal protozoa in diarrhoeal patients. Some of the clinical features in patients with *Blastocystis hominis* alone, co-infections with other intestinal parasites and other intestinal parasites without *Blastocystis hominis* among the diarrhoeal patients is presented on Table 20. Diarrhoeal patients with *Blastocystis hominis* alone (72.7%), *Blastocystis hominis* with other intestinal parasites in (74.5%) and other intestinal parasites

without *Blastocystis hominis* in 71.1% had diarrhoea duration of greater than four weeks. But watery diarrhoea was more common in those *Blastocystis hominis* with other intestinal parasites than *Blastocystis hominis* alone and other parasites without *Blastocystis hominis* , but no significant difference was observed among the group (P>0.05).

**Table 20. Some clinical manifestations in 268 diarrhoeal patients with *B. hominis* alone, *Blastocystis* with other parasites and other parasites without *B. hominis* from 3 hospitals in Addis Ababa (March 2002-December2003).**

<b>Features</b>	<b><i>Blastocystis</i> with others(%) (n=94)</b>	<b><i>Blastocystis</i> alone(%) (n=22)</b>	<b>Others without <i>Blastocystis</i>(%) (n=152)</b>
<b>Diarrhoea duration</b>			
<1week	0	0	1(0.7)
1-4weeks	24(25.5)	6(27.3)	42(27.6)
>4weeks	70(74.5)	16(72.7)	109(71.1)
<b>Nature of Diarrhoea</b>			
Watery	67(71.2)	9(40.9)	89(58.6)
Loose	16(17.4)	9(40.9)	35(22.0)
Mucoid	9(9.8)	4(18.2)	20(13.2)
Bloody	2(2.2)	0(0.0)	8(5.3)
<b>Clinical Symptoms</b>			
Abdominal pain	65(69.2)	15(68.2)	110(72.8)
Weight loss	70(79.5)	13(59.4)	104(68.4)
Vomiting	38(40.2)	5(22.7)	54(35.7)
Nausea	38(40.2)	7(31.8)	65(42.8)
Anorexia	56(59.6)	13(59.4)	78(51.3)
Fever low grade	38(40.2)	7(31.8)	65(42.8)
Tenesmus	31(32.2)	11(50.0)	39(25.7)
Flatulence	30(31.9)	6(27.9)	24(15.8)

But bloody diarrhoea was observed in 4.3% of *Blastocystis hominis* with others while no case was identified in *Blastocystis hominis* infection alone and with other intestinal parasite

infection without *Blastocystis hominis* in 5.3%. Major clinical presentations such as abdominal pain, weight loss, nausea, anorexia observed among the groups is almost comparable and with no significant difference. From this result, the pathogenic potential of *Blastocystis hominis* infection among the diarrhoeal patients is apparently acceptable. However, in this study, due to lack of bacteriological and virological results its true status of pathogenicity cannot be confirmed.

#### 4. DISCUSSION

Different studies had indicated that intestinal parasites are common causes of public health problems in Wonji area (Tedla and Yimam, 1987; Woldemichael et, al, 1990; Assefa et.al., 1991). Fontanet et.al (2000a and 2000b) had reported high prevalence of intestinal parasites in HIV positive and HIV negative individuals among Wonji Sugar Estate residents. However, with the establishment of the HIV/AIDS natural history study cohort in 1997 at Wonji, through which all positive cases got treated, the prevalence of intestinal parasites among the cohort participants had substantially decreased. Another privilege of the cohort participants was that whenever they had any complaint outside the scheduled follow up program, they could visit cohort physicians for medical care, including treatment for intestinal parasites. The drastic reduction in the prevalence of intestinal parasites following systematic treatment shows that drug based control measures that were reported from other countries (Albonico, et.al., 1996; Olsen, 2003; De Siliva, 2003) may also be feasible in Ethiopia. The fact that the Wonji cohort study population and patients that reported to Wonji hospital were living in the same area and shared the same drinking water, etc and yet showed differences in the prevalence of infection by intestinal parasites was an indication that the difference was not a matter of personal hygiene or safe way of living but the chemotherapeutic intervention in the cohort study population. In areas where there is no regular and targeted mass chemotherapy, the chance of re- infection by intestinal helminths is very high (Bundy et.al., 1992; Albonico et.al., 1999), but in the case of cohort population, there was repeated treatment for scheduled as well as un scheduled participants.

Previous reports had revealed that Wonji was a known endemic place for intestinal helminths especially for intestinal schistosomiasis (Tedla and Yimam,1987; Tedla, 1989; Birrie, et.al., 1989). However, recently there was a drastic reduction of the prevalence of *Schistosoma*

*mansoni* in the area. This was observed not only among the cohort participants but also among the patients that visited the Wonji hospital for medical care. This could be attributed to focally applied snail control measures and chemotherapy using praziquantel.

One major uncertainty in the interpretation of Wonji cohort data is the diagnosis and reporting of *E.histolytica/dispar*. In the 2001 report, the Wonji cohort study showed no *E.histolytica/dispar* positive cases whereas analysis of 273 stool samples collected from the cohort population at EHNRI, Parasitology Laboratory, in the same year, indicated 11.7% *E.histolytica/dispar* cyst and 10.3% *Entamoeba* trophozoites. Kedede et.al. (2003) had reported 21 and 91 cases of *E.dispar* out of 232 stool samples examined by PCR-SHELIA and by microscopy, respectively from the same cohort population of scheduled and unscheduled visitors. This indicates that the reported absence of *E.histolytica/dispar* among the cohort population by the routine diagnostic method might be a problem of diagnostic efficiency and not the effect of treatment.

The prevalence of *Giardia lamblia* was much lower in the cohort population. This might be because the cohort population comprised all adults, in whom *Giardia lamblia* prevalence is much lower compared to the children (Tatichef et.al., 1981; Birrie and Eriko, 1995).

A major conclusion that can be derived from the follow up examinations of the cohort population in Wonji is that regular visits of the population to the health facility and lowering the barriers that may prevent people from doing so, together with the provision of appropriate care in terms of diagnosis and treatment resulted in a sustainable control of intestinal parasitic infections. Thus, the cohort study experience should encourage the design of selected and

targeted mass chemotherapy in a community where intestinal parasites are causes of major public health problems in Ethiopia.

As a consequence of control of intestinal parasitic infections through repeated chemotherapy further studies on the possible interaction between intestinal parasites and HIV/AIDS could not be done in the cohort study population in Wonji. Thus, the HIV/AIDS cohort study in Wonji was not ideal for the study on the possible interaction between HIV-infection and the multitude of common and more rare intestinal parasitic infections in Ethiopia. Instead, a hospital based study focusing on diarrhoeal HIV/AIDS patients that are not under chemotherapeutic cover for intestinal parasites would be an ideal setting.

Even though the enrolment criterion in the hospital based study was diarrhoea and no chemotherapy for intestinal parasites, the finding that 74.2% of all diarrhoeal patients and 91.4% with chronic diarrhoea were HIV positives, indicated a strong association of HIV infections with diarrhoea in Ethiopian AIDS patients. This finding is in agreement with the report from other African countries where 60-90% of AIDS patients have diarrhoea at one stage of disease progression (Bartlett et.al., 1992; Germani et.al., 1998). In addition, a possible causal association of opportunistic intestinal parasites with diarrhoea in HIV/AIDS patients is indicated by the 67% prevalence of one or more opportunistic intestinal parasites in the HIV/AIDS patients.

In most of the investigations related to HIV/AIDS and chronic diarrhoea the opportunistic intestinal parasites, play an important role (Tarimo, et.al., 1996; Gumbo, et.al., 1999; Clavero, et.al., 1999). Such relationship of opportunistic intestinal parasites and HIV/AIDS is not well documented in Ethiopia.

The present cross-sectional study aimed at filling this gap. It focused on intestinal parasites with an emphasis on opportunistic intestinal parasites in diarrhoeal HIV/AIDS patients. So bacteriological and virological analysis of stool samples for determination of etiological agent of diarrhoea were not considered.

The rate of the occurrence of non-opportunistic extracellular intestinal parasites such as *Ascaris lumbricoides*, *Taenia* spp. and *Entamoeba histolytica/dispar* in the HIV negative diarrhoeal patients was significantly higher ( $P < 0.001$ ) than in HIV positive diarrhoeal patients. Still other conventional intestinal parasites such as *Giardia lamblia*, *Entamoeba trophozoite*, *Entamoeba coli*, and *Endolimax nana* were more common in HIV negative than HIV positive individuals with no significant difference between the groups.

The high prevalence of opportunistic intestinal parasites (*Cryptosporidium parvum*, *Isospora belli*, *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis*) that are consistently found in HIV/AIDS patients with chronic diarrhoea has been reported as the most likely causes of diarrhoea from elsewhere (Tarimo, et.al. 1996; Clavero, et.al., 1999; Gumbo, et.al., 1999; Wiwanitket, 2001). In HIV negatives, these opportunistic intestinal parasites were very rare which is in agreement with what was reported by others (Gomez Morales, et.al., 1995; Lindo, et.al, 1998; Mohandas et.al., 2002) and appears to be predominantly infected by the common intestinal parasites such as *Ascaris lumbricoides*, *Taenia* spp. and *Entamoeba histolytica/dispar*. It has been suggested that HIV infection may have caused some change in the gut structure that may not be suitable for the common intestinal parasites (Gomez Morales, et.al., 1995; Sharpstone and Gazzard, 1996; Lindo, et.al; 1998)

It is for the first time that intestinal microsporidia (*Enterocytozoon bieneusi* and *Encephalitozoon intestinalis*) were identified in diarrhoea HIV/AIDS patients in Ethiopia. In Africa in general, information regarding intestinal microsporidiosis is very limited, mainly due to lack of appropriate diagnostic methods and experienced skilled manpower (Drobnieruski et.al., 1995). The application of Uvitex –2B staining detected less number of microsporidia cases as compared to PCR. Previous work by Katzwnikel-Wladarsch et.al., (1996), had indicated that the PCR applied in this study can detect 3-100 spores in 0.1gram of stool. Such low number of spores cannot be reliably detected by light microscope. Furthermore, the sensitivity of the microscopy test most likely depends on the expertise of the examiner. In addition, chitin of the spores may sometimes be shaded and the spores do not stain with Uvitex-2B stain (Van Gool et.al., 1994). There can be also false positive cases with microscopy due to staining of some species of bacteria and fungi. Similar study done by Gumbo et.al (1999) in Zimbabwe, detected 18% with microscopy and 51% by using PCR. The present study confirmed that PCR is more sensitive in detecting low numbers of spores based on microsporidial DNA in stool samples. The other significance of PCR is that it facilitates species identification (Fedorko, and Hijazi, 1996). This is important, because *Encephalitozoon intestinalis* can be effectively treated with albendazole (Molina, et.al., 1995; Conteas, et.al 2000), while no established treatment exists so far for *Enterocytozoon bieneusi*.

Even though intestinal microsporidial infections have been sporadically reported in HIV negative individuals with diarrhoea (Sobottka et.al., 1995; Desports, et.al., 1998), this study revealed its association only with chronic diarrhoea in HIV/AIDS patients. In this study, the majority of intestinal microsporidial infections (84.6%) were due to *Enterocytozoon bieneusi*, which is more associated with chronic diarrhoea. This finding is almost in

agreement with previous findings whereby more than 90% *Enterocytozoon bieneusi* cases was incriminated as a cause of the diarrhoea caused by intestinal microsporidiosis (Dallabetta and Miotti, 1992; Van Gool et.al., 1995; Gumbo; 1999). The present study has also demonstrated a relatively more proportion of *Encephalitozoon intestinalis* associated with chronic diarrhoea than has been reported for intestinal microsporidiosis (Van Gool et.al., 1994). This indicated that in Ethiopia, both of intestinal microsporidial agents of chronic diarrhoea are prevalent in HIV/AIDS patients.

The CD4+ cell count is one of the most widely used prognostic markers for the management of HIV infected individuals and is helpful in the decision on when to intervene with the antiretroviral treatment or with prophylaxis for opportunistic infections (Flanigan, 1994; Gross, et.al, 1995; Navin et.al., 1999). Reports show that AIDS patients at highest risk of intestinal microsporidiosis are those with the CD4+ cell counts below 100 cells/mm<sup>3</sup> (Rabeneck, et.al., 1993; Asmuth, et.al., 1994). However, in the present study, the category of diarrhoeal patients with the highest frequency of intestinal microsporidiosis were those with a CD4+ cell count below 50 cells/mm<sup>3</sup> and as the CD4+ cell count increases, the rate of infection with intestinal microsporidiosis decreases. This implies that infection with intestinal microsporidiosis is manifested when the immunity of HIV infected individuals was severely compromised. In general, the majority of HIV/AIDS patients with intestinal microsporidiosis in this study were at CD4+ below 100 cells/mm<sup>3</sup>, which is in agreement with previous studies (Asmuth, et.al., 1994). Thus, initiation of intervention with highly active antiretroviral therapy with protease inhibitors at an earlier stage (at around CD4+ 200 cells/mm<sup>3</sup>) may protect HIV infected individual from developing diarrhoea illness due to intestinal microsporidial infection.

The association between increased viral load and microsporidial infection, reported by other investigators elsewhere (Goodgame, 1996; Dascomb, et.al., 1999) was apparently observed in this study.

A large majority of diarrhoeal HIV/AIDS patients infected with intestinal microsporidia were in stage III and stage IV, based on the WHO clinical staging criteria (1993) showing that intestinal microsporidiosis is the major problem in HIV/AIDS patients during the late stages of the disease. Similar studies in Thailand (Punpoowong, et.al, 1998; Waywa, et.al, 2001) had shown that HIV infected patients with chronic diarrhoea; at the late stage of the HIV infection commonly had intestinal microsporidial infections.

All the major clinical symptoms reported from patients with intestinal microsporidiosis such as severe weight loss of >10% of the body weight, chronic watery diarrhoea, anorexia, abdominal pain, vomiting, nausea and low-grade fever were also evident in microsporidial infected patients in this study (Bryan, 1995; Chukwuma, 1996; Kotler and Orenstein, 1998).

Although aggravation of diarrhoeal illness due to intestinal microsporidiosis by co-infection with diarrhoeerogenic coccidian and other intestinal parasites (Van Gool, 1995; Tarimo, et.al. 1996), in this study this was not evident with the few cases of *Cryptosporidium parvum*, *Isospora belli*, and *Strongyloides stercoralis* co-infection with intestinal microsporidial.

In developing countries, the risk of getting infected with intestinal microsporidiosis is high due to lack of safe and well-protected drinking water. Once infected with intestinal microsporidiosis, HIV/AIDS patients that do not have access to antiretroviral therapy will be prone to severe complication. According to the USPHS/IDSA guideline (1995) in such

conditions the only available protection for HIV infected individuals is to keep personal hygiene and have safe handling of food and drinking water.

The role of *Isoospora belli* as an opportunistic intestinal parasite was clear in this study as it was detected only among HIV positive diarrhoeal patients. Contrary to the very low prevalence of *Isoospora belli* infection among HIV/AIDS patients reported from Ethiopia (Fisseha et. al., 1998; Awol, et.al, 2003), in the present study has recorded a much higher prevalence. All of these reports were among HIV/AIDS patients restricted to hospitalised condition. The high prevalence of isosporiasis in the present study may be due to the more sensitive diagnostic methods used for detecting the oocyst. It may also reflect a high degree of immunosuppression of the patients. In the present study, the application of the autofluorescence technique may have provided additional advantage in the diagnosis of *Isoospora belli* infection (Curry and Smith, 1998; Bialek, et.al, 2002). The Uvitex-2B stain method is useful for the diagnosis of three opportunistic intestinal parasites including *Isoospora belli* infection in HIV/AIDS patients (Franzen et.al., 1996; Bialek, et.al, 2002). The Modified Ziehl Neelsen method was effective in detecting the unsporulated oocysts of *Isoospora belli* that are highly transparent, refractile and may be unrecognised in wet mount preparations (Sorvillo, et.al., 1995). Therefore, its application in health institutions and peripheral laboratories would enable the early detection of *Isoospora belli* infection in HIV/AIDS patients.

Compared to other tropical and subtropical countries, the prevalence of *Isoospora belli* infection observed in this study was high. It was identified in approximately 15-20% patients with chronic diarrhoea and AIDS; low prevalence was recorded in other African countries including Zambia (16%), Uganda (1.1%), Zaire (12%) and Tanzania (11.6%) (Henry et.al.,

1986; Colebunders et.al, 1988; Conlon et. al., 1990; Hunter et.al., 1992; Gomez Morales, et.al., 1995; Brink et.al., 2002). In USA, less than 0.2% of the patients with AIDS have been recognised to have *Isospora belli* infection (Cimerman et.al., 1999; Sears and Kirkpartrick, 2001). This may be because that the patients observed in the present study were free from antiretroviral treatment and took no prophylaxis for *Pneumocystis carinii* infection showed high infection rate, which are measure known to reduce infection rate in HIV/AIDS patients (Sorvillo et.al., 1995; Cimerman, et.al., 1999).

*Isospora belli* infection in HIV/AIDS patients has been shown to appear when the patient is immunospressed, usually when the CD4+ cell count is below 200 cells/mm<sup>3</sup> (Goodgame, 1996; Certad, et.al; 2003). The same was true for the majority of isosporiasis cases, which were observed at CD4+ cell counts below 200 cells/mm<sup>3</sup>. However, few cases were observed at CD4+ cell counts above 200 cells/mm<sup>3</sup> which is in agreement with the study conducted by Certad, et.al (2003) in Venezuela where 6 cases (18.8%) were found at CD4+ cell counts above 200 cells/mm<sup>3</sup> among HIV/AIDS patients.

In the present study, higher percentage of patients with *Isospora belli* infection were found under the category of higher HIV-1 RNA viral load count. In the lowest viral load the infection rate with *Isospora belli* had declined. Even though not statistically significant, (P>0.05), a high percentage of *Isospora belli* infection was observed at higher viral load. This was associated with decrease in CD4+ cell counts.

The majority of *Isospora belli* infected patients (95.6%) were at stage IV of clinical stage of AIDS indicating that the infection mostly occurs at the latest stage of HIV infection (P=0.001). It was reported by others that in early stages, especially in stage I and II, infection

with *Isoospora belli* was rare (Hunter, et.al., 1992; Brink, et.al., 2002). Thus, *Isoospora belli* infection in HIV/AIDS patients is usually expected at the latest stage of HIV infection. This parasite is treatable with Trimethoprim-Sulfamethoxazole and Pyrimethmine Sufonamide in combination or alone (Weiss et.al., 1988; Sorvillo, et.al, 1995; Verdier, et.al., 2000). However, after treatment of *Isoospora belli* infection in HIV/AIDS patients, recrudescences of infection are commonly reported (DeHovitz et.al., 1986; Curry and Smith, 1998; Verdier, et.al., 2000) for which maintenance dose treatment at daily or weekly bases is recommendable (Lindsay, et.al., 1997; Curry and Smith, 1998).

In addition, in this study most of the patients (96.4%) with *Isoospora belli* infections had chronic diarrhoea that lasted more than four weeks. This is in agreement with WHO-staging criteria of HIV disease (1993), which categorized patients with *Isoospora belli* infection and had chronic diarrhoea more than a month at stage IV. The majority of clinical symptoms are similar to *Cryptosporidium parvum* infections (Wittner, 1993). However, co-infection with both coccidian intestinal parasites was not detected in the present study.

Consistent with earlier reports ( Sorvillo, et.al.,1994; Clark and Sears, 1996; Hunter and Nichols, 2002) cryptosporidiosis is an AIDS defining disease and infection with *Cryptosporidium parvum* is considered an important cause of chronic diarrhoea in immunocompromised individuals and HIV/AIDS patients. The present study showed that the majority of cryptosporidial infections are associated with chronic diarrhoea in HIV/AIDS patients. Furthermore, the few cases of cryptosporidiosis infections detected in this study are the first report of its kind in Ethiopia among adult HIV negative diarrhoea patients. However, earlier studies in Ethiopia have shown different prevalences of cryptosporidial infections among children under five and HIV/AIDS patients (Mersha and Tiruneh, 1992; Assefa et.al.,

1996; Mengesha, 1994; Fisseha et.al., 1998; Awol et.al., 2003). The prevalence of *Cryptosporidium parvum* infection in diarrhoeal HIV/AIDS patients in the present study was within the same range of reported cases from different parts of the world, particularly from African countries (Colebunders, et.al., 1988; Gomze Morales, et.al., 1995; Kelly, 1998). Generally the variation in the prevalence of cryptosporidial infections in diarrhoeal HIV/AIDS patients could be related to the immune status of the HIV/AIDS patients examined, the sensitivity of the diagnostic techniques used and the experience of the technicians. Moreover, oocyst excretion is usually variable (Ungar, 1990; Blackman, et.al., 1997). In addition, single stool specimen processing might underestimate the prevalence of cryptosporidial infection (Blackman, et.al., 1997).

*Cryptosporidium parvum* infection among HIV/AIDS patients has been reported to appear when the immune status of the patient is severely suppressed, with the CD4+ cell count below 200cells/mm<sup>3</sup> (Goodgame 1996; Hunter and Nichols, 2002). Similarly, in this study the majority of patients with cryptosporidial infections had CD4+ cell count within the same range indicating that the immunity among the diarrhoea patients with HIV/AIDS was severely depleted. Different studies have indicated that individuals with CD4+ Cell counts of less than 50 cells/mm<sup>3</sup> develop a fulminant cryptosporidial disease (Flannigan et.al 1992; Theodos, 1998; Sterling, 2000). Flannigan (1994), demonstrated that some of HIV infected patients with CD4+ cell counts higher than 180 cells/mm<sup>3</sup> had displayed ceasing of diarrhoea and clinical healing over a period of four weeks, while 87% of the patients presenting more severe immunosuppression, with CD4+cell counts below 140 cells/mm<sup>3</sup> presented persistent and hard-to-control diarrhoea. Thus, the present study has provided additional evidence for the close association of the severity and duration of diarrhoea in cryptosporidial infections with CD4+ cell counts. As a whole, in this study, in the majority of diarrhoeal HIV/AIDS patients,

most of cryptosporidial infection appeared in the very low CD4+ cell counts as expected (Cimerman et.al., 1999; Streling, 2000). Since there is no established treatment for cryptosporidial infection, initiation of antiretroviral therapy for HIV/AIDS patients is the available treatment (Mitra et.al., 2001) to reduce the risk of diarrhoeal illness as well as preventing the development of severe chronic cryptosporidiosis.

Even though, no sufficient information is available on viral load and cryptosporidiosis, the present study revealed infection with *Cryptosporidium parvum* in diarrhoeal patients with high HIV-1 RNA viral load was higher compared to the lower viral load. Since the majority of the patients were at similar level of immunosuppression that is at latest stage of HIV infection, no significant difference ( $P>0.05$ ) was observed.

WHO staging criteria on HIV disease progression (1993), indicates that patients with chronic diarrhoea lasted for more than a month and with cryptosporidiosis are categorized in stage IV. Similarly, the present study confirms that the majority of diarrhoeal HIV/AIDS patients with cryptosporidial infections are in stage IV clinical stages of AIDS.

As indication of similar route of transmission, faecal–oral contamination of food and drinking water, in the present study, *Cryptosporidium parvum* occurred in co-infection with other intestinal protozoan parasites, both non pathogenic such as *Entamoeba coli*, *Endolimax mana*, *Chilomastix mesnelli* and *Sarcocystis* spp. and the pathogenic such as *Girardia lamblia*. Different studies have indicated that *Cryptosporidium* species are highly resistant to standard dose of chemical disinfectants such as chlorine that is used in the treatment of drinking water (Juranck, 1995; Hunter and Nichols, 2002). So, in cryptosporidiosis infection, with no effective treatment and no standard and cheap way of clearing drinking water is available, it is

important to advise HIV/AIDS patients on how to avoid risk of infection (USPHS/IDSA, 1995), including the potential use of unboiled water for consumption and protect themselves from contact with young domestic animals and swimming in public pools. Because young domestic animals such as calves are more susceptible to cryptosporidial infection than the older ones (Ungar, 1990; Tzipori and Griffiths, 1998).

With regard to helminths, *Strongyloides stercoralis* was a frequently detected intestinal parasite in HIV positive than in HIV negative diarrhoeal patients, with no significant difference observed between the groups ( $P>0.05$ ). The difference observed in infection prevalence determinations between the present study and earlier reports among HIV/AIDS patients from Ethiopia (Fisseha, et.al., 1998; Fontanet et.al., 2000; Awol et.al., 2003; Hailemariam et.al., 2004) may be a reflection of the difference in the diagnostic method used, the endemicity and variation in infective dose of the parasite in certain locality and possibly the immune status of the patients.

Few reports of significantly higher prevalence of *Strongyloides stercoralis* infection in HIV-positive patients have been published (Gomez Morales et.al., 1995). However, its status as an opportunistic intestinal parasite is not established (Grove, 1996; Lindo, et.al., 1998) although it is known that the parasite multiplies in the human host by means of autoinfection. On other hand in the majority of high HIV prevalence countries, the prevalence of *Strongyloides stercoralis* is also high (Grove, 1996; Lindo and Lee, 2001). But, the association with AIDS patients leading to disseminated strongyloidiasis has been rarely reported (Conlon et.al., 1990; Grove, 1996). That was why infection by this parasite was withdrawn from WHO- AIDS-defining criteria (Lucas, et.al.1990).

*Strongyloides stercoralis* is one of the most difficult intestinal parasitic infections to diagnose with conventional stool examinations having low sensitivity even when examined several times. This is because the parasite load is low in the majority of the infected individuals and the larval output is minimal and irregular (Conway et.al., 1995; Mohmoud, 1996). Serological methods have also their own limitations in endemic areas (Siddiqui and Berk, 2001). In the present study, direct and concentration methods were applied whereas the Baermann method could not be used, since the majority of the stool samples were watery diarrhoea and the debris could not be retained by sieving gauze. However, as described by others, the direct and concentration methods used in this study were the easiest and quite specific but extremely insensitive (Grove, 1996; Lindo and Lee, 2001). In rare occasion of hyperinfection leading to disseminated cases, the rhabditi from larvae (L1) may be easily detected by these methods as lots of swarming larvae are known to occur, which was evident in some of the stools in this study (fig. 16).

It is known that strongyloidiasis causes a chronic diarrhoea, abdominal pain and malabsorption in immunocompromised and HIV/AIDS patients (Grove, 1996; Lindo and Lee, 2001). Similar clinical presentations were observed in this study. However, co-infection with *Strongyloides stercoralis* and other opportunistic intestinal parasites (*Enterocytozoon bieneusi*, *Isoospora belli* and *Cryptosporidium parvum*) was frequent and this made it difficult to conclude the clinical manifestations observed to be associated with *Strongyloides stercoralis* infection.

In rare clinical reports, strongyloidiasis was noted at lower CD4+ cell counts; Sing et.al. (1999) had reported at a CD4+ cell count of 26/ $\mu$ l from a patient with terminal stage of HIV infection. Another report by Hong et.al. (2004) indicated that AIDS patient had a CD4+ cell

counts of 59 cells/mm<sup>3</sup> and diarrhoea and ascitis. In this study in the majority of the patients with *Strongyloides stercoralis* infection, CD4+ cell counts were below 100 cells/mm<sup>3</sup> and at stage IV. This was similar to what was observed with other opportunistic intestinal parasites. However, due to co-infection with other opportunistic intestinal parasites the decrease in CD4+ cell counts could be unequivocally assigned to it.

Although the opportunistic nature of *Strongyloides stercoralis* in HIV/AIDS patients is still debatable, its pathogenic complications are well established (Makis et.al, 1993; Cahill, 1996; Sing, et.al., 1999;). So, prevention and control of *Strongyloides stercoralis* infection has to be addressed in HIV infected individuals. However, this was limited by the fact that like diagnosis, treatment of *Strongyloides stercoralis* infection remains very difficult. However, recent studies have shown that Ivermectin is the best drug for the treatment of uncomplicated *Strongyloides stercoralis* infection and more effective than Thiabendazole especially for HIV/AIDS patients (Adenusi, 1997; Orem et.al, 2003). Orem et.al, (2003) have shown that *Strongyloides stercoralis* hyperinfection in patients with AIDS in Uganda was successfully treated with Ivermectin. Thus, most of the fatal infections in immunocompromised and HIV/AIDS patients by *Strongyloides stercoralis* can be prevented by early detection and treatment.

There are many reports that show the possibility of *Blastocystis hominis* infection causing gastrointestinal disorders in patients with HIV infection (Garavelli, et.al., 1988; Libre, et.al., 1989; Garavelli,et.al., 1990; Albrecht, et.al., 1995; Stargaard, et.al., 1996; Escobedo and Nunez, 1997). It has been reported that patients with full-blown AIDS and positive for *Blastocystis hominis* presented with diarrhoea, nausea, flatulence, tenesmus, itching, abdominal pain and peripheral blood eosinophilia (Garavelli, et.al., 1990; Albrecht, et.al.,

1995). However, unlike other newly emerging opportunistic intestinal parasites, *Blastocystis hominis* is not well studied and recognised as an important potential pathogen in immunocompromised and HIV/AIDS patients. Fisseha et.al., (1998) identified a single case out of 143 HIV/AIDS patients with diarrhoea and Hailemariam, et.al., (2004) detected 5 cases among 234 HIV infected patients in Ethiopia. A study conducted on randomly selected cohort participants at Wonji showed about 59% of *Blastocystis hominis* infection. This is in agreement with the rate recorded in developing countries which showed more than 50% (Stenzel and Boreham, 1996). However, in this study, 36.3% of HIV/AIDS patients with diarrhoea harbouring *Blastocystis hominis* were too high a prevalence to dismiss as incidental compared to other intestinal protozoa parasites. The observed difference among HIV/AIDS patients reported so far from Ethiopia could be due to difference in the experience of the technicians or/and the clinical condition of the patients examined.

*Blastocystis hominis* is now gaining acceptance as human intestinal parasitic agent showing different clinical symptoms (Telalbasic et.al., 1991; Keystone, 1995; Morgan et.al., 1996; Stenzel and Boreham, 1996). The symptoms commonly attributed to infection with *Blastocystis hominis* are non-specific and include diarrhoea, abdominal pain, discomfort and nausea. Profuse diarrhoea without blood or leukocytes has been reported in acute infection. Anorexia, bloating; flatulence with other non-specific gastroenteritis effects are also associated with *Blastocystis hominis* (Zierdt, 1989; Udknow and Markell, 1993; Boreham, et.al., 1996; Stenzel and Boreham, 1996). Similarly, diarrhoeal patients with *Blastocystis hominis* alone, *Blastocystis hominis* with other parasites and other parasites with out *Blastocystis hominis* had shown clinical symptoms such as chronic diarrhoea which lasted more than four weeks, abdominal pain, anorexia with no significant difference among the groups ( $P>0.05$ ). Based on the present study, it seems not sufficient to conclude that

*Blastocystis hominis* is a potential cause of diarrhoea, because it lacks the result of bacteriological and virological analysis of diarrhoeal stools in order to identify the etiological agent.

However, based on the clinical and epidemiological information that is available so far (Vannatta et.al., 1985; Zierdt, 1988; Zierdt, 1989; Telalbasic et.al., 1991; Keystone, 1995; Stenzel and Boreham, 1996) it is reasonable to assume that faecal oral route of contamination of food and drinking water is the most likely way of *Blastocystis hominis* transmission. Leelayoova et.al., (2004) has indicated that waterborne transmission of *Blastocystis hominis* is evident although it needs further study on other route of transmission. Therefore, control measures would include better personal hygiene, improvement of community sanitary facilities and prevention of faecal contamination of the environment.

Due to different outlooks on the pathogenicity, the issue of treating *Blastocystis hominis* infection has remained controversial. However, treating *Blastocystis hominis* infection when defined symptoms are present with large number of parasites in the stool and in the absence of other cause of the disease is obviously recommendable ( Vennatta, et.al., 1985; Markell, 1995; Stenzel and Boreham, 1996). As a result, Metronidazole (2 tablets 3X/day for 10 days) has become the acceptable treatment of choice for *Blastocystis hominis* infection (Garavelli, et.al., 1988; Doyle, et.al., 1990).

## 5. CONCLUSIONS AND RECOMMENDATIONS

The study has shown scheduled follow up and examination of the cohort population in Wonji with provision of appropriate care in terms of diagnosis and treatment resulted in a sustainable control of intestinal parasites. Thus, information from the cohort study could be a good example to design targeted mass chemotherapy in a community where intestinal parasites are causes of major public health problem in Ethiopia.

The general trend in prevalence and species of intestinal parasites fauna is dramatically changed with the HIV/AIDS epidemic in Ethiopia. In this study, intestinal protozoan parasites were found to be dominant as compared to helminthic infections. This calls for establishment of specific diagnostic tests in all health laboratories and train health professionals, with special attention to intestinal protozoan parasites.

The majority of HIV/AIDS patients with diarrhoea had opportunistic intestinal parasites. Therefore, for proper management and treatment of HIV/AIDS patients with chronic and intermittent diarrhoea, adequate diagnosis of opportunistic intestinal parasites must be carried out.

This study has established that opportunistic intestinal parasites (*Cryptosporidium parvum*, *Isospora belli*, *Enterocytozoon bienersi* and *Encephalitozoon intestinalis*) are associated with disease severity in diarrhoeal HIV/AIDS patients in Ethiopia. The present study showed that 67% of the HIV/AIDS diarrhoeal patients had at least one opportunistic intestinal parasite, which is associated with uncontrollable, life threatening diarrhoea. Majority of opportunistic intestinal parasites (80%) were detected at lowest CD4+ cell count (<50 cells/mm<sup>3</sup>), at late stage of HIV/AIDS. Thus, it is necessary to diagnose diarrhoeal patients with HIV infection

for opportunistic intestinal parasites, since some of them, such as *Isospora belli* are treatable. In addition, early initiation of antiretroviral therapy could decrease the risk of developing illness due to opportunistic intestinal parasites. Suppression of replication of HIV-1 virus by antiretroviral treatment, which is known to lead to an increase in circulating CD4+ T-lymphocytes, may enhance repopulation of the intestinal mucosa with CD4+ T-cells. This would effectively restore mucosal immunity leading to eradication of opportunistic intestinal parasites such as (*Cryptosporidium parvum* and *Enterocytozoon bieneusi*) resulting in cessation of diarrhoea. Thus, for those opportunistic intestinal parasite infections for which no proven therapy exists, easy access to ART is the only option, and the availability of ART for all with CD4+ cell count most likely at or below 200 cells/mm<sup>3</sup>, is urgently needed.

The prevalence of *Blastocystis hominis* was high in diarrhoeal patients be they HIV positive or HIV negative. Therefore, further in-depth studies must be conducted before concluding about its pathogenicity among such population.

*Strongyloides stercoralis* was the only helminth parasite diagnosed predominantly in diarrhoeal HIV positive and HIV negative patients. This study indicated that a combination of different diagnostic methods may have to be applied for diagnosis of this parasite, especially in HIV/AIDS patients. The opportunistic characteristic of *Strongyloides stercoralis* is unclear and thus, needs further study to confirm its actual status in HIV/AIDS patients.

The study indicated that the predominance of non-pathogenic intestinal protozoa such as *Entamoeba coli*, *Entamoeba hartmanni*, *Endolimax nana* serve as an index of faecal contamination of drinking water sources with opportunistic intestinal parasites which are hazardous to HIV/AIDS patients. It is well known that opportunistic intestinal parasitic

infections among HIV/AIDS patients are high in developing countries compared with industrialized countries. Because of unhygienic conditions transmission of these parasites is more frequent in developing countries. Infact development of diarrhoeal illness due to opportunistic intestinal parasites and its severity depends on level of immunity (CD4+ cell count) of the individuals. Thus, HIV infected individuals must be advised about the need for avoiding faecally contaminated drinking water, unsafe handling of food and have to be aware of the major ways of transmission of opportunistic intestinal parasites.

This study showed different opportunistic intestinal parasites were more frequently detected from diarrhoea patients with HIV/AIDS. This was true by application of conventional diagnostic methods and advanced techniques such as PCR and fluorescence methods. Diagnosis of intestinal microsporidiosis was also established for the first time in Ethiopia. Since HIV/AIDS is a major problem in Ethiopia, it is recommended to extend the application of these diagnostic methods to hospital level and to referral peripheral laboratories for early detection of opportunistic intestinal parasites for better understanding and management of diarrhoeal illness in HIV/AIDS patients.

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**Annex: I Clinical data format**

**Enrolment Criteria:**

1. Diarrhoea
    - Chronic (3 or more loose or watery stool per day for more than 4 weeks) Or
    - Acute (3 or more loose or watery stool per day for more than a week & less than 4 weeks)
  2. Patient s should be free from any anti parasitic drug during 4 weeks before enrolment and anti bacterial treatment under remarks (type of drug and duration).
  3. Either outpatient or patients admitted to the Hospital
  4. Age greater than or equal to 14 years
- 

**ANNEX I-A**

**1. Study Code**

For example

	H			
A	H	0	8	9

Use AH: Army; PH: Police; WH: Wonji; SH: St.Paul

2. Hospita

l

- Army
- Police
- Wonji
- St. Paul

3. Physician

4 Date

of

Enrolment

		<i>dd</i>		<i>mm</i>		<i>yy</i>
--	--	-----------	--	-----------	--	-----------

**PERSONAL DATA**

5. Hosp. ID. Of patient

6. Patient

- Out patient
- In patient

<input type="checkbox"/>	
<input type="checkbox"/>	

7 Date

Hosp.

Admission

		<i>dd</i>		<i>mm</i>		<i>yy</i>
--	--	-----------	--	-----------	--	-----------

8. Address

- 8.a Woreda
- 8.b Kebele
- 8.c House No.
- 8.d Other


9. Sex

- Male
- Female

<input type="checkbox"/>	
<input type="checkbox"/>	

10. Age

11. Occupation

- Factory Worker
- Field (seasonal) worker
- House wife
- Medical
- Administrative /office
- Student
- Farmer
- Police
- Military
- Other

12 Marital Status

- Married
- Single
- Divorced
- Widowed

**Diarrhoea Condition /any individual complaining of Diarrh/**

- 13.1 Acute  *BM/Day (1-6, > 6)*
- 13.2 Chronic  *BM/Day (1-6 > 6)*
- 13.3 Intermittent

- Acute diarrhoea: 3 or more loose or watery stool per day for one week
- Chronic diarrhoea: 3 or more loose or watery stool per day for 4 weeks

14 Duration

- 1-4 Week
- >4 Week

**HISTORY**

15. Clinical Manifestation (more options can be marked)

- 15.a Loose Diarrhoea
- 15.b Watery Diarrhoea

- 15.c Mucoïd Diarrhoea
- 15.d Bloody Diarrhoea
- 15.e Abdominal Pain
- 15.f Malabsorption
- 15.g Weight Loss
- 15.h Fever (Low Grade)
- 15.i Vomiting
- 15.j Nausea
- 15.k Anorexia
- 15.l Tenesmus
- 15.m Gas (Flatulence)
- 15.n Others

**16 Sputum for cases that are suspected for disseminated strongyloides stercoralis**

**17. HIV/AIDS Status (with Diarrhoea) Date if HIV test done**

		dd	mm	yy
Positive	<input type="checkbox"/>	<input type="text"/>	<input type="text"/>	
Negative	<input type="checkbox"/>	<input type="text"/>	<input type="text"/>	
Unknown	<input type="checkbox"/>	<input type="text"/>	<input type="text"/>	
AIDS case	<input type="checkbox"/>			

**18 Clinical stage WHO-staging HIV/AIDS disease (Please see attached WHO stage criteria)**

- Stage I
- Stage II
- Stage III
- Stage IV

**19 WHO stage code of diagnosed events**

19.a Stage I	<input type="text"/>	<input type="text"/>	<input type="text"/>
19.b Stage II	<input type="text"/>	<input type="text"/>	<input type="text"/>
19.c Stage III	<input type="text"/>	<input type="text"/>	<input type="text"/>
19.d Stage IV	<input type="text"/>	<input type="text"/>	<input type="text"/>



**Annex: II Sample collection format**

**Notes**

1. Stool samples must be full Cap, because the tests that will be performed are many.
  2. Parasite identified (Special data sheet will be used)
- 

<b>ANNEX I-B LABORATORY TECHNICIAN FORM</b>
---

Date

2. Study Code 

	H			
--	---	--	--	--

For example 

A	H	0	8	9
---	---	---	---	---

1 Technicians  
an

**Use AH: Army; PH: Police; WH: Wonji; SH. St. Paul**

3. Hospital

1

Army	<input type="checkbox"/>
Police	<input type="checkbox"/>
Wonji	<input type="checkbox"/>
St. Paul	<input type="checkbox"/>

4. Sample Type

4.a Stool	<input type="checkbox"/>
4.b Blood	<input type="checkbox"/>
4.c Sputum	<input type="checkbox"/>

5. Stool Appearance

5.a Loose	<input type="checkbox"/>
5.b Watery	<input type="checkbox"/>
5.c Mucoid	<input type="checkbox"/>
5.d Bloody	<input type="checkbox"/>

6. Parasite Identified by Direct method

6.a Ascaris	<input type="checkbox"/>
6.b Trichuris	<input type="checkbox"/>
6.c Strongyloides	<input type="checkbox"/>
6.d Hook worm	<input type="checkbox"/>
6.e S.mansonia	<input type="checkbox"/>
6.f E.vermicularis	<input type="checkbox"/>
6.g Taenia sp.	<input type="checkbox"/>
6.h H. nana	<input type="checkbox"/>
6.i E.histolytica/dispar cyst	<input type="checkbox"/>
6.j E.histolytica/dispar troph.	<input type="checkbox"/>
6.k E.coli troph.	<input type="checkbox"/>
6.l E.coli cyst	<input type="checkbox"/>
6.m Chilomasti	<input type="checkbox"/>
6.n Giardia cyst	<input type="checkbox"/>
6.o Giardia troph.	<input type="checkbox"/>

6.p Blastocyst  
6.q Other

