ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES

Evaluation of Techniques for the Diagnosis of *Strongyloides stercoralis* in HIV positive and HIV negative individuals in selected health institutions in Addis Ababa

By
Tamirat Hailegebriel

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Approved by Examining Board:

Dr Amha Kebede (Examiner) _____

W/o Tsehai Assefa (Examiner) _____

Prof. Beyene Petros (Advisor) _____

Dr Tekola Endeshaw (Advisor) _____

Dr Dawit Abate (Chairman) _____

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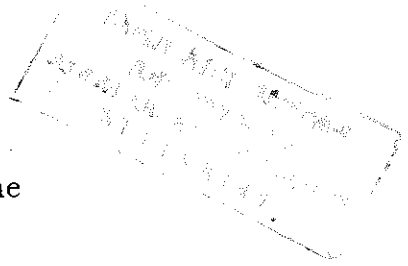
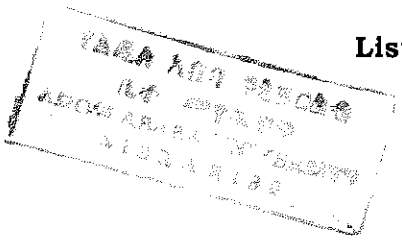


AIDS	Acquired Immunodeficiency Syndrome
CDC	Center for Disease Control
EHNRI	Ethiopian Health and Nutrition Research Institute
ELISA	Enzyme Linked Immunosorbent Assay
HIV	Human Immunodeficiency Virus
HMFP	Harada Mori Filter paper
HTLV-1	Human T-Lymphotrophic Virus-1
OR	Odd Ratio
Rpm	Revolution per minute
SPSS	Statistical Package for Social Science
VCT	Voluntarily Counseling and Testing
WHO	World Health Organization



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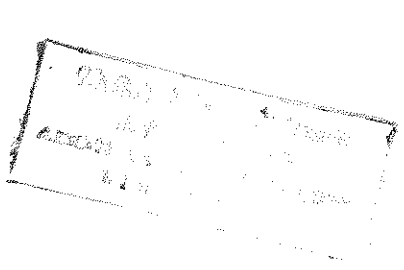
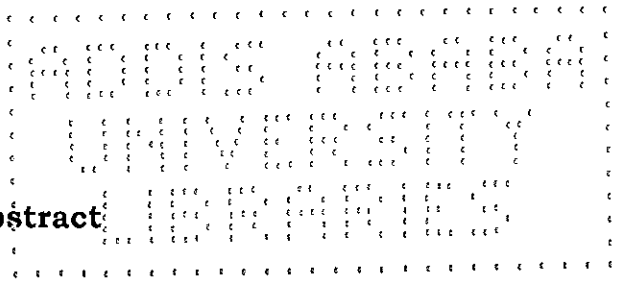
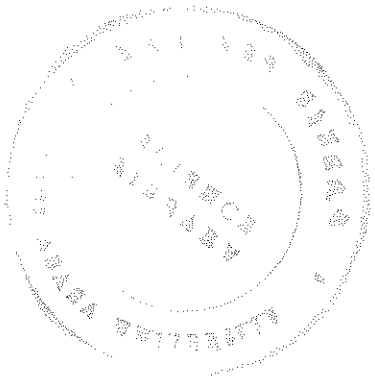
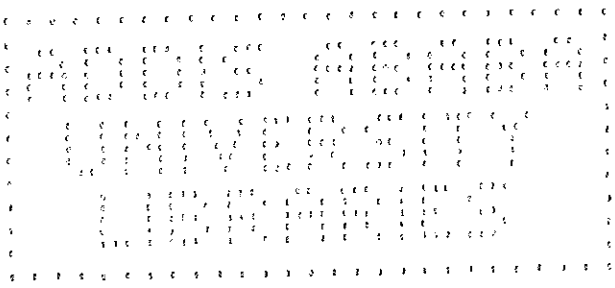




Abstract

A total of 351 individuals, 226 HIV positive and 125 HIV negative, were examined by nutrient agar plate, Baermann method, direct fecal smear, concentration method, Harada Mori filter paper and charcoal culture in this study. Among these 139(39.6%) were positive for different type of intestinal parasites. The common intestinal parasites detected in this study were S. stercoralis, E. histolytica/dispar, A. lumbricoides, B. hominis, G. lamblia, *hookworm*, C. parvum, *and* I. belli. *Out of these parasites, more than 90% of* S. stercoralis *and all cases of* C. parvum *and* I. belli *were detected from HIV positive subjects. Although different intestinal parasites were detected from HIV patients,* S. stercoralis *was the predominant infection detected by nutrient agar plate and was associated with chronic diarrhea in the HIV/AIDS patients included in the study. Since* S. stercoralis *is known to exist as an asymptomatic chronic infection, more classical parasitological methods such as direct and concentration methods were not as sensitive as the agar plate culture and the Baermann method. The present study showed that the nutrient agar plate culture method was the most sensitive method for the detection of* S. stercoralis *infection. It detected 97.7% of the infection among the total study subjects. It furthermore detected 90.7% of* S. stercoralis *infection in HIV positive patients with and without diarrhea. By using this method, it was possible to show that 76.9% of* S. stercoralis *infection to be associated with diarrhea in HIV positive patients (P<0.001). The agar plate culture method has shown a strong association between HIV/AIDS and* S. stercoralis *infections (P<0.001). Thus, this sensitive and specific diagnostic method can be used to detect early and latent infections of* S. stercoralis *in HIV/AIDS patients.*





1. Introduction

Parasitic diseases are distributed worldwide, with a higher prevalence in developing countries. The main reason for high prevalence of parasitic disease in developing countries are due to poor living standard, deficiency of sanitary facilities, unsafe human waste disposal systems, inadequacy and lack of safe water supply, and low socioeconomic status in general (Dinleyici *et al.*, 2003). Parasitic diseases represent one of the most common types of human infection throughout the world. Intestinal parasites are amongst the most common parasitic infections in the world, being responsible for considerable morbidity and mortality. According to the World Health Organization (WHO) estimates, there are one billion cases of ascariasis, 500 million hookworm infections, 500-600 million trichuriasis and 50–100 million strongyloidiasis (Hayes, 2004), 200 million giardiasis and 500 million amoebiasis through out the world (Ali *et al.*, 1999).

According to Hayes (2004), 25% of the populations of developing countries are infected with at least one species of nematodes. About 200-500 million peoples of Sub-Saharan Africa populations are infected at least one or more species of nematodes. Similar situations are observed in Ethiopia, due to low level of living standards, poor environmental sanitation, and ignorance of simple health promoting factors (Ali *et al.*, 1999). The prevalence of different intestinal parasites in Ethiopia is varying considerably based on altitudinal ranges and difference in a living standard of the population. The most common intestinal protozoa are *Giardia lamblia, Entamoeba histolytica/dispar,* and *Blastocystis hominis.* As indicated by Fisseha *et al.* (1999) and Endeshaw *et al.* (2004) *Cryptosporidium parvum* is common in HIV /AIDS patients. Among the known helminths, *Ascaris lumbricoides* is the most prevalent helminthic parasite, followed by *Trichuris trichuria,* hookworm,

and *Strongyloides stercoralis* in Ethiopia (Taticheff *et al.,* 1981). In general, the most common intestinal parasites detected from HIV/AIDS patients are *C. parvum, I. belli,* and *S. stercoralis* (Endeshaw *et al.,* 2004). These parasites are responsible for the majority of chronic diarrhea in AIDS patients and associated with higher mortality.

Strongyloidiasis is one of the intestinal parasitosis of humans and other vertebrates caused by members of the genus strongyloides. This genus has two important species that affect humans (Zaha *et al.,* 2000). The most common and important human pathogen is *Strongyloides stercoralis,* which affects 50 to 100 million people worldwide (Ferriera, 2003; Marty *et al.,* 2005). The other species that infects humans is *Strongyloides fuelleborni* having sporadic distribution in Africa (South Africa and Namibia) and Papua New Guinea (Genta, 1989; Stephenson *et al.,* 2000: Ferriera, 2003).

Strongyloides stercoralis was first reported in 1876 in the stool of French soldiers returned from Vietnam who had chronic and severe diarrhea (Mahmoud, 1996; Siddique and Berk, 2001). Initially named as *Anguillula stercoralis,* but eventually renamed as *S. stercoralis* after subsequent elucidation of its unique lifecycle (Tsai *et al.,* 2002) and the larvae were identified as those of the intestinal nematode *S. stercoralis* (Mahmoud, 1996). Elucidation of the complete lifecycle, clinical manifestation, and syndromes has a recent history. For example, complete life cycle of *S. stercoralis* was elucidated after 50 years of discovery (Siddique and Berk, 2001).

Strongyloides stercoralis is one of the smallest intracellular nematode that infects humans, whose body size is estimated about 2 mm length and 5 micrometer diameter (Mahmoud, 1996). The body of the worm contains an esophagus occupying approximately one third of the anterior portion while the posterior contains ovaries, oviduct, and uteri. The parasitic females are

usually embedded in the intestinal mucosa and laid an embryonated eggs that hatches immediately in to rhabditiform larvae (Siddique and Berk, 2001).

Strongyloides stercoralis is commonly considered as parasites of human. Sometimes non-human primates, dogs, cats, and Mongolian gerbils are also infected and serve as reservoir host for the parasite (Stephenson *et al.*, 2000; Herbert *et al.*, 2002). The parasites complete the whole life cycle inside a single host and remains for long period within the infected host.

Strongyloides stercoralis is unique among helminthic parasites in the following features. First, the larvae instead of eggs are passed in the feces of infected individuals (Neva, 1986). Second, there is no parasitic male in the life cycle, only parasitic females multiplied parthenogenetically within infected host (Mahmoud, 1986). Third, *S. stercoralis* is the only nematode with an endogenous cycle (autoinfection) that infects humans up to 50 years without requiring further exposure to contaminated soil (Hammad and Lenox, 1999; Roman-Sanchez *et al.*, 2003) a situation characterized by few or no symptoms in most cases, but it becomes serious during immunosuppression. Fourth, this nematode is definitely one of the most versatile of all human parasites in that it may exist indefinitely as a free living parasite with both sexes present, or as a self perpetuating parasitic female population (Egido *et al.*, 2001). Fifth, the infective larvae are capable of carrying bacteria and fungi from the bowel and evading any organ of the body including the central nervous system during dissemination (Chiu and Lia, 2005).

Human acquire *S. stercoralis* infection through penetration of the skin with infective filariform larvae (L3) from contaminated soil or through autoinfection. Recently, other ways of infection are also reported. For example, infections can be induced experimentally by oral administration of water contaminated with filariform larvae (Keiser and Nutman, 2004), the infective larvae can be transmitted via breast milk (Stephenson *et al.*, 2000)

but there is no evidence for congenital transmission (Shoop *et al.*, 2002). Moreover, infections can also be acquired rarely from intimate skin contact or from inadvertent coprophagy, such as ingestion of contaminated food scavenged from garbage (http: //aapredbook).

Human infections of *S. stercoralis* usually results in asymptomatic chronic disease of the gastrointestinal tract that can remain undetected for many decades in most "health infected" individual (Chieffi *et al.*, 2000; Dad-Adabola and Bakate, 2004; Satoh *et al.*, 2004) or may develop a wide variety of complaints during immunosuppression (Nonaka *et al.*, 1998). According to Stephenson *et al.* (2000), *S. stercoralis* infection is asymptomatic in around 50% of cases.

The chronic pathway of continuous autoinfection can lead to a massive and life threatening infection in AIDS patients and patients who are receiving ongoing steroids therapy. Mortality rate exceed 70% among immunosuppressed individuals (Martinez *et al.*, 2005) and almost all deaths caused by helminths in the United State are caused by *S. stercoralis* hyperinfection (Siddique and Berk, 2001). During immunosuppression, especially suppression of T-cell function, multiplication of the parasites become uncontrolled and leading to an overwhelming parasite load (Dryer *et al.*, 1996; Mahmoud, 1996; Ferriera, 2003) thus, the larvae disseminated throughout the body (Nonaka *et al.*, 1998; Satoh *et al.*, 2004), which may result in serious illness.

The most common immunosuppressive conditions that predispose to hyperinfective or disseminated disease includes hematologic malignancy, Human T-Lymphotrophic Virus-1 (HTLV-1) infection, HIV/AIDS, immunosuppressive treatment in transplant recipients (Paula *et al.*, 2000; Adedayo *et al.*, 2002; Dinleyici *et al.*, 2003) malnutrition, diabetes mellitus,

chronic renal failure, and alcohols liver cirrhosis (Adedayo *et al.*, 2002; Oliveira *et al.*, 2002).

1.1. Life Cycle of *S. stercoralis*

The life cycle of *S. stercoralis* is complex and involves two stages: one as a free-living stage and the other as a parasitic stage. The parasitic adult females are usually embedded in the intestinal mucosa (Siddique and Berk, 2001) or lamina propria of the duodenum (Genta, 1992) and lay approximately 40 embryonated eggs per day (Nonaka *et al.*, 1998). These eggs are hatched within few hours inside the submucosa or during passage through the gut into first stage (rhabditiform) larvae (Cox, 2002; Lia *et al.*, 2002; Siddique and Berk, 2003) and escape to the lumen and then to the external environment with feces. These rhabditiform larvae molt either into infective stages or free-living stages (Schmidt and Roberts, 2000; Cox, 2002).

In some patients, however, the larvae may have enough time to molt twice into infective filariform larvae (L3) before leaving the infected host and may penetrate the lower part of the intestine or the per-anal area. This particular route of infection is called autoinfection (Lai *et al.*, 2002), which is responsible for the perpetuation of the parasite even after a long period without infestation (Welch and Gill, 2003; Carvalho and Porto, 2004). According to Hammad and Lenox (1999) the infection can persist up to 50 years without external exposure. Autoinfections can lead to not only persistence strongyloidiasis but also leads to hyperinfection syndrome and disseminated disease during impaired T-cell function (Lim *et al.*, 2004). This route of the life cycle is very important and need a special attention particularly during HIV/AIDS, because the virus is known to impair cellular immunity.

Autoinfection present little problem in the immunocompetent host, in most cases the impact is balanced and asymptomatic coexistence between host and

parasite. If diseases or drugs upset this equilibrium, they allow the parasite to proliferate rapidly (Scowden *et al.*, 1978) and may result in hyperinfection and disseminated disease, often with fatal consequences (Atkins *et al.*, 1999; Reiman *et al.*, 2002). Autoinfection is usually enhanced in the immunosuppressed individuals, larval dissemination occurs, and the patient develops severe strongyloidiasis.

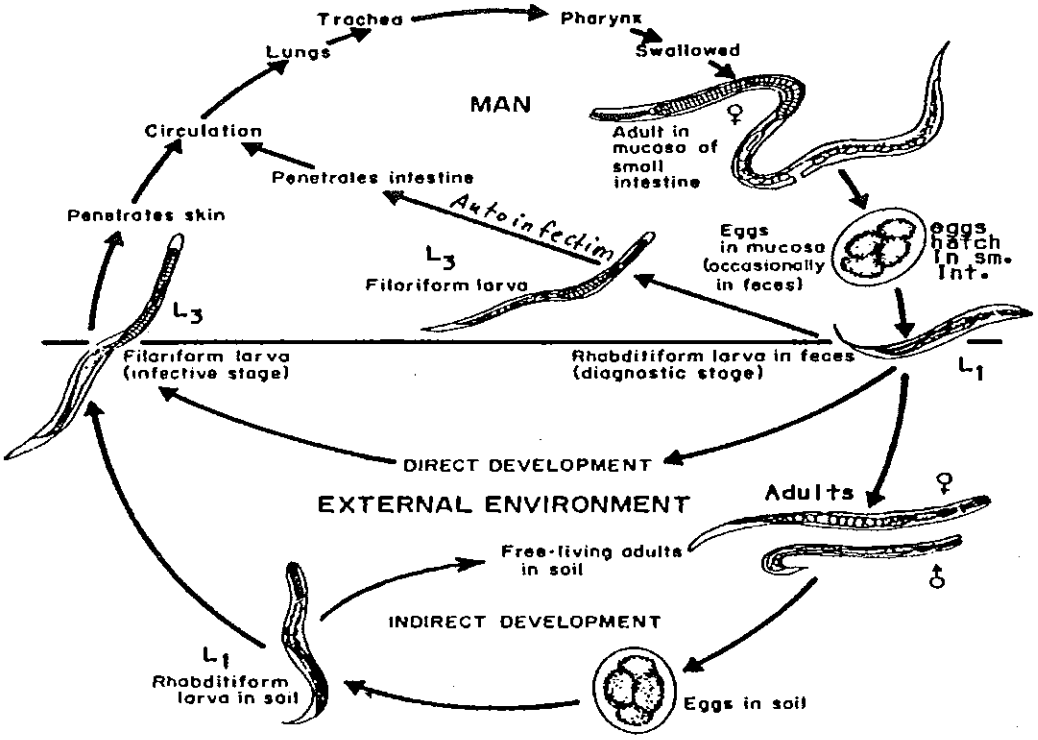
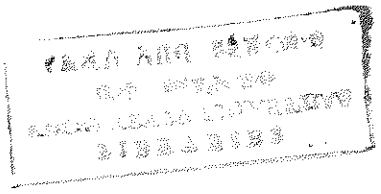


Figure 1: Life cycle of *S. stercoralis* (adopted from:http://www.uwec.edu)

The developmental pathways can be influenced by environmental and host factors such as temperature, humidity, food, and immune status of the host (Genta, 1992; Sing, 1999). For example, external temperatures above 34°C (Nolan *et al.*, 2004) or harsh environmental conditions (Keiser and Nutman, 2004) favor the development into infective larvae whereas temperatures lower than 34°C and higher humidity supports the free-living phase (Nolan *et al.*,

2004). In general, the rabiditiform larvae can follow any of the three (direct, indirect or autoinfective) developmental pathways to complete its life cycle (Figure 1).



1.2. Epidemiology of *S. stercoralis*

Strongyloides stercoralis is an intestinal nematode with worldwide distribution, especially endemic in tropical and sub tropical countries (Sing *et al.*, 1999; Lia *et al.*, 2002) with hot and humid climate (Adedayo *et al.*, 2002). It is more common in South East Asia, Sub-Saharan Africa and Latin America particularly Brazil and Argentina (Siddique and Berk, 2003; Namisato *et al.*, 2004). As stated by Adedayo *et al.* (2001), *S. stercoralis* infect 35% of some tropical population. Several pockets of low endemicity were observed in parts of the United State, Italy, France, Spain, Portugal, Baltic countries, Rumania, Russia, and Australia (Ferriera, 2003; Siddique and Berk, 2003). In general, strongyloidiasis has a hetrogenic worldwide distribution based on the predominance of the infection rate and classified as, sporadic (<1%), endemic (1-5%), and hyper endemic (>5%) (Machado and Costa-Cruz, 1998).

The prevalence of strongyloidiasis is mostly dependant on the geographical area, environmental safety conditions, physical and chemical characteristics of the soil, temperature, humidity, vegetation, socio-economical status such as quality of housing, standard of hygiene of the community and crowding (Egido *et al.*, 2001). A variety of occupations that increase contact with soils also account for the higher infection rate within endemic countries. For example, higher prevalence was observed among populations working in coal mining (Keiser and Nutman, 2004), gold extraction in riverbeds (Egido *et al.*, 2001) and farm workers (Roman-Sanchez *et al.*, 2003).

According to Siddique and Berk (2003), the prevalence of *S. stercoralis* infections may be as high as 25% in high endemic countries and ranges from

1% to 4% in low endemicity. However, these values can be reached up to 83% in some rural areas of Argentina and up to 85% among less privileged social class in Brazil (Ferriera, 2003). Moreover, higher prevalence of *S. stercoralis* infections were observed among HIV positive patients in Brazil, which ranges from 4% to 15% compared to 1% to 4% in the general population (Cimerman *et al.*, 1999).

In most non-endemic countries, immigrants, veterans, returned travelers from endemic areas and institutionalized individuals are at higher risk of acquiring the infection (Nair, 2001; Adedayo *et al.*, 2002; Sudarshi *et al.*, 2003; Johnston *et al.*, 2005). For example, 11% of pediatrics migrants to Australia from East Africa (Somalia, Ethiopia, Kenya, and Eritrea) were positive for *S. stercoralis* and 18% harbored other organism capable of causing serious disease (Rice *et al.*, 2003). *S. stercoralis* infections were reported among World War Second prisoners and refugee in Southeast Asia 40 years after leaving the endemic areas (Chieffi *et al.*, 2000). In addition, strongyloidiasis has the highest impact on health among nematodes in Switzerland due to imported from endemic countries (Nuesch *et al.*, 2005). Mahmoud (1996) has indicated that the prevalence of *S. stercoralis* infection was very high among residents of mental institutions (1.7-40%), Veterans or prisoners of war (0.5-37%), and refuges and immigrants (0.6-38%).

S. stercoralis infection is still a public health problem in many parts of the world and poses serious hazards for individual patients even outside endemic areas due to the possibility of hyperinfection and disseminated disease (Graeff-Teixeira *et al.*, 1997). Moreover, it is considered as emerging public health problem due advanced cases of immunocompromised conditions particularly with HIV/AIDS pandemic, HTLV-1 infection and immunosuppressive drug therapy (Chieffi *et al.*, 2000).

In addition to immunosuppression, high temperature, humidity, and environmental contamination with fecal matter also contribute to high prevalence of infection in endemic areas. Patients in mental institution, chronic alcoholic consumption, and prisoners of war are also among the risk groups (Carvalho and Porto, 2004).

Ethiopia is one of the endemic countries for *S. stercoralis* infection. The rate of infections varies from locality to locality and in most cases lower infections reported might be due to low sensitivity of detection methods. For example, studies carried out at community level indicated that the prevalence of *S. stercoralis* infection is within the ranges of 3% to 44% in the central plateau (McConnell and Armstrong, 1976). Other studies also confirmed that the prevalence of this infection is within this range. For instance, studies carried out on HIV/AIDS patients showed that high prevalence of the infections in the country, which is 9% in Jimma town of which 3.3% had disseminated disease (Endris, 2001), 11.1% in Southwestern Ethiopia (Awole *et al.,* 2003), 13% Wonji Sugar Estate (Fontanet *et al.*, 2000) and 3.4% in Addis Ababa (Fisseha *et al.,* 1999). Other studies carried out at community level also show differing infection rate: among school children (5.8%) around Lake Langano (Legesse and Erico, 2004), among diarrheal patients (8.6%) in Addis Ababa (Endeshaw *et al.*, 2004) and among randomly examined stool specimen (17.4%) in Wonji-Shewa Sugar Estate (Assefa *et al.*, 1991).

The diagnostic methods used in these studies were direct fecal smear and concentration techniques with a single visit of study participants with the exceptions of Fontanet's and Assefa's work, which were done by Baermann and modified Baermann method, respectively. The sensitivity of direct fecal smear and concentration methods is very low (Nonaka *et al.*, 1998; Koosha *et al.*, 2004; Lim *et al.*, 2004). This is an indication that the reported infection of this helminthic parasite in Ethiopia might be lower than the actual infection rate particularly in HIV/AIDS patients. Moreover, less emphasis was given to

this infection due to poor diagnostic methods that might result in loss of life among chronic diarrheal patients associated with HIV/AIDS.

1.3. Clinical Presentations of *S. stercoralis*

Strongyloides stercoralis is a common parasitic infection in tropical areas and is associated with a wide spectrum of clinical manifestations ranging from asymptomatic to disseminated hyperinfection (Uparanukraw *et al.*, 1999) or multi-organ failure (Lim *et al.*, 2004). The symptoms may occur decades after the primary infection consequently resulting in fatal outcome in immunocompromised patients (Uparanukraw *et al.*, 1999). The worm colonizes the duodenum, upper jejunum, and usually affects the mucosal layer of the intestine; in severe cases the whole intestinal wall may be involved (Werneck-Silva *et al.*, 2001). As stated by Genta (1992), no other human parasitic nematodes have been associated with a broad spectrum of manifestation and clinical syndromes as *S. stercoralis*.

S. stercoralis usually causes chronic asymptomatic infections of the gastrointestinal tract in immunocompetent human hosts that may remain undetected for several decades. However, during immunosuppression, particularly during impaired T-cell function, the silent infection becomes disseminated through out the body due to accelerated reproduction of the parasite because of internal autoinfection (Siddiqui *et al.*, 1997; Wong *et al.*, 2005), which may result in fatal outcome. The most common reported clinical presentations of *S. stercoralis* infections are gastrointestinal, cutaneous, respiratory, disseminated and hyperinfection (Siddiqui and Berk, 2003; Johnston *et al.*, 2005).

Gastrointestinal symptoms include diarrhea, nausea, anorexia, and abdominal blotting and discomfort (Aparecida *et al.*, 2001; Helderman and Goral, 2002; Aure *et al.*, 2005; Johnston *et al.*, 2005). Other symptoms such

as dysentery, protein-losing enteropathy and malabsorption, progressive weight loss (Dinleyici *et al.*, 2003), vomiting and elevated eosinophilia (Lim *et al.*, 2004), hemorrhage, edema or erosion of the mucossa are also observed during endoscopic examination of the upper gastrointestinal tract (Nonaka *et al.*, 1998). According to Kim *et al.* (2005), gastrointestinal problems dominate strongyloidiasis infection clinically.

Dermatologic manifestation includes a migratory rash called larva currens (snake-like track) and recurrent urticaria (Johnstone *et al.*, 2005), which can appear and disappear around the trunk in a few hours or days, which distinguishes it from the similar, long lasting cutaneous larva migrans rash of hookworm (Welch and Gill, 2003). Intense itching and erythemateous papule can also develop at the site of the infection (Dinleyici *et al.*, 2003; Speare and Durrheim, 2004; Reddy and Swarnalata, 2005). The larvae most often affect the lower parts of the body particularly around the buttocks, groin and trunk regions.

Respiratory symptoms include dyspnea, bronchospasm, pneumonia and lung abscess (Johnston *et al.*, 2005), asthma (Welch and Gill, 2003), cough, wheezing and hemoptysis develop as pulmonary complications during transpulmonary migration of the larvae (Dinleyici *et al.*, 2003). Acute respiratory distress syndrome, pulmonary infiltrates on chest radiograph, or both is the setting of rapid clinical deterioration that may represent hyperinfection (Lim *et al.*, 2004).

The clinical presentation of hyperinfection can be non-specific but includes exacerbation of gastro-intestinal and pulmonary symptoms together with the detection of increased numbers of larvae in stool and/or sputum (Wong *et al.*, 2005). In addition to the direct consequences of massive larval migration in tissues, hyperinfections are frequently complicated by infections caused by gut flora that gain access to intestinal sites; most probably through ulcers

induced by the moving larvae or failure of mucosal integrity (Orlent *et al.*, 2003; Seet *et al.*, 2005) or directly carried by the moving larvae. The most common gut floras that frequently associated with *S. stercoralis* hyperinfections are bacteria and fungi. These agents assumed to be the major cause of morbidity and mortality during hyperinfection. As stated by Siddique and Berk (2001), massive secondary bacterial infections are the immediate cause of death in patients with hyperinfection. For example, secondary bacterial invasions are responsible for 87% mortality associated with hyperinfection (Link and Orenstein, 1999). Studies carried out in the United State showed that one-third of the patients with hyperinfection syndrome develop secondary bacterial infection, of which 82% died by associated complication (Igra-Siegmen *et al.*, 1981).

Disseminated hyperinfection is the most serious manifestation of *S. stercoralis* infection and has a reported case fatality rate of up to 87%(Johnston *et al.*, 2005). Symptoms like diffuse pulmonary infiltrate, septicemia, bactermia, pneumonia, or meningitis from enteric gram-negative bacilli are also observed during disseminated hyperinfection (Genta, 1992; Mahmoud, 1996; Siddique and Berk, 2001; Ferriera, 2003; Satoh *et al.*, 2003). Central nervous system manifestation like brain abscess and neurological dysfunctions are the most frequent during disseminated hyperinfection (Chiu and Lai, 2005).

1.4. *S. stercoralis* infection and HIV/AIDS

It is known that, T cell dependent immune systems are responsible for controlling nematode infections (Dunne and Riley, 2004; Porto *et al.*, 2005). For example, the interaction of the parasite with IgE-mast cell complex result in mast cell degranulation and parasite killing. However, in individuals infected by HIV/AIDS there might be impairment of this immune system that predisposes to disseminated strongyloidiasis. Patients who present immunocompromised cellular response have qualitative and/or quantitative

alterations that impede them from acting efficiently against the infections manifested a deterioration of their general conditions. Intestinal helminths usually cause immuneactvation; the high prevalence of intestinal parasites could be a factor involved in AIDS progression (Fietosa *et al.*, 2001) or chronic helminths infection facilitates the progression of HIV infection to clinical AIDS (Bentwich *at al.*, 1995). For example, chronic infection with helminths illustrate best some of the elements of chronic immune activation that may also be found in HIV infection (Bentwich *at al.*, 1998). This type of immune activation is suggested to be a major factor for increasing susceptibility and progression of HIV/AIDS in Africa and other developing countries (Bentwich *at al.*, 1995). Most of the clinical manifestation of HIV/AIDS results either from the reactivation of preexisting latent infection or exposure to locally predominant pathogens (Bentiwich *et al.*, 1998)

Infections of the gastrointestinal tract play a critical role in AIDS pathogenesis, diarrheal disease is assumed to play a prominent role, reaching up to 50% in developed countries and up to 95% in developing countries (Cimerman *et al.*, 1999; Gassama, 2001). HIV/AIDS is a major threat to people particularly in Sub-Saharan Africa and it has been associated with chronic diarrhea in a considerable number of infected patients. The main source of this chronic diarrheal illness could be intestinal parasitic infections, which are a leading cause of morbidity and mortality in patients with the virus (Hailemariam *et al.*, 2004; Zali *et al.*, 2004). Infections that cause diarrhea have been found in 30% to 80% of patients living with the virus in Southwestern Ethiopia (Awole *et al.*, 2004). Moreover, parasitic infections that cause self-limiting diarrhea in immunocompitent patients may cause profuse diarrhea in immunocompromised individuals such as AIDS patients (Botero *et al.*, 2003). In tropical countries, chronic diarrhea associated with weight loss is often the presenting illness of HIV infected individuals. According to WHO classification, diarrhea-wasting syndrome in association with a positive HIV-1 serology test is an AIDS defining illness (Fisseha *et al.*, 1999). Most of the time

chronic diarrhea is associated with the presence of one or more opportunistic intestinal parasites.

Opportunistic agents are parasites that consistently cause severe, chronic, or frequent gastrointestinal disease whereas non-opportunistic agents are usually causes acute and treatable diarrheal illness. The emergence of HIV/AIDS has heightened the spectrum of opportunistic parasites by enabling more aggressive and disseminated parasitic infections in human such as cryptosporidiosis, isosporiasis, toxoplasmosis, and strongyloidiasis (Kaminsky *et al.*, 2004). The etiologic agents of diarrhea could be parasitic, bacterial, fungal, enteric virus or HIV itself (Awole *et al.*, 2004). In addition to microbes, other factors such as medication, immune dsregulation, autonomic dysfunction, and nutritional supplementation play substantial role in diarrhea of HIV/AIDS patients (Harris and Beeching, 1991).

Amongst the intestinal parasites that cause diarrhea, protozoa and helminths are the predominant infections particularly in HIV/AIDS patients. Several species of protozoa have been associated with acute and chronic diarrhea in HIV/AIDS patients in Ethiopia. These include: *Cryptosporidium parvum, Isospora belli, Giardia lamblia, Entamoeba histolytica/dispar,* and *Cyclospora cayetanenis* (Awole *et al.*, 2003; Endeshaw *et al.*, 2004). Among these, *C. parvum* was exclusively associated with AIDS patients in Addis Ababa (Fisseha *et al.*, 1999). In addition to protozoan parasites, the nematodes including *Strongyloides stercoralis, Trichuris trichiura, Ascaris lumbricoides* and hookworms are detected from HIV/AIDS patients in Addis Ababa (Fisseha *et al.*, 1999). Among the helminths *S. stercoralis* is ubiquitous in tropical and subtropical areas causing chronic diarrhea and overwhelming infestation in patients with HIV/AIDS (Simon, 2002; Awole *et al.*, 2003; Sathiyasekaran and Shivbalan, 2005). According to Cimerman *et al.* (1999), among the helminths in association with AIDS, there is no doubt that the most important pathogen is *S. stercoralis.*

HIV/AIDS causes countless immunodeficiency that creates suitable conditions for the multiplication and dissemination of *S. stercoralis* larvae as well as associated bacterial and fungal infection. At the beginning of the AIDS epidemic, Center for Disease Control and prevention (CDC) includes severe form of strongyloidiasis in the list of opportunistic disease that define a suspected case of ADIS. However, in 1987, CDC removed this infection from the list of AIDS defining illness (Ferriera, 2003; Siddique and Berk, 2003) because only small cases of disseminated infection reported in the literature. Although vast number of people coinfected, the relative rarity of disseminated infection in reports is striking (Keiser and Nutman, 2004).

In African countries, for example, infection with *S. stercoralis* has been found in 2% to 5% of patients infected with this retrovirus, but disseminated strongyloidiasis or extra-intestinal strongyloidiasis was apparently rare (Grove, 1996; 1999; Ferriera, 2003). Based on these, *S. stercoralis* is not considered to be an important opportunistic infection associated with AIDS; the infection should still be searched for and promptly treated among HIV-infected patients who have a history of residence in and/or travel to areas of endemicity (Siddique and Berk, 2001).

In some cases, strong associations between HIV/AIDS and *S. stercoralis* infection were reported. For example, strong correlation was observed between HIV/AIDS and parasitological proven *S. stercoralis* infection in Jamaica (Robinson, 1990; Lindo *et al.*, 1998). Similarly, severe clinical forms of strongyloidiasis among AIDS patients were observed in Brazil (Ferriera, 2003). Moreover, the prevalence of strongyloidiasis in HIV-positive patients (4% to 15%) compared to 1.4% in the general population in Southern Brazil (Cimerman *et al.*, 1999), and higher larval load in HIV patients than HIV negative individuals in Southwestern Ethiopia (Awole *et al.*, 2003) is an indication of association. In addition, the detection of *S. stercoralis* infections

is about four times in HIV/AIDS patients compared to the prevalence of this parasite in HIV non-infected individuals from case study (Awole *et al.*, 2003).

Several reports indicate the occurrence of interaction between HIV/AIDS and enteric parasites; the association between HIV/AIDS and *S. stercoralis* infection are consistent and are the common conclusion of the majority of the studies (Cirioni *et al.*, 1989; Nomura and Rekrut, 1996; Feitosa *et al.*, 2001). Severe form of strongyloidiasis in patients with HIV/AIDS was observed strongly associated with bacterial infection in the form of bacteremia and meningitis (Siddique and Berk, 2001). According to Genta (1992) it has been specifically mentioned as one of the "missing infection" in AIDS and has been removed from the list of HIV associated opportunistic infection. Mortality is as high as 70% during hyperinfection syndrome, increase up to 80% in the presence of secondary bacterial infection and may reach upto 90% among HIV/AIDS patients (Hammad and Lenox, 1999).

Ethiopia is one of the endemic countries for *S. stercoralis* infection, in which the infection occurs in a situation of poverty, with deficient in sanitary infrastructure, lack of latrines or poor use of them, overcrowding, high temperature, humidity, and soil characteristics favorable for the proliferation of parasites provide conditions for the maintenance and transmission of such infections. Moreover, the emergence of HIV/AIDS pandemic increases the prevalence of this infection due to countless immunodeficiency caused by the virus, which may result in chronic diarrheal illness.

In addition to the well-known opportunistic intestinal parasites, such as *C. parvum, I. belli* and Microsporidium species*: S. stercoralis* is also considered as an important intestinal parasite (Wiwanitkit, 2001). It is very important to target *S. stercoralis* infections while treating HIV patients for opportunistic infections in developing countries like Ethiopia. In general, the opportunistic

nature of *S. stercoralis* infection in HIV-positive populations is still unclear and needs further investigation.

1.5. Diagnosis of *S. stercoralis*

Strongyloides stercoralis is one of the most difficult parasitic infections to diagnosis (Siddique *et al.*, 1997). The definitive diagnosis usually depends on the demonstration of *S. stercoralis* first stage larvae (L1) in the feces, duodenal fluid, or sputum (Scowden *et al.*, 1978; Koosha *et al.*, 2004). Common parasitological methods such as direct fecal smear and concentration methods have low sensitivity (Welch and Gill, 2003) even repeated for several times, especially in chronic cases with minimal and irregular larval output (Siddique *et al.*, 1997; Uparanukraw *et al.*, 1999). The sensitivity of parasitological methods with a single stool examination was only 30% on average (Dreyer *et al.*, 1996; Nonaka *et al.*, 1998; Blat and Cantos, 2003; Lim *et al.*, 2004). It could be more effective in confirming proven *S. stercoralis* infection when combinations of direct fecal smear, formalin-ether concentration, and filter paper culture methods were used. However, the sensitivity can be increased up to 50% with examination of three stool samples (Egido *et al.*, 2001) and may reach up to 90% when seven serial stool samples are examined (Lim *et al.*, 2004).

Although direct fecal smear and concentration methods have their own limitations, they are still the most commonly and widely used diagnostic methods in most service giving health institutes because, the examinations is quite easy and less costly (Nonaka *et al.*, 1998). The absence of larvae in direct fecal smear does not necessarily indicate the absence of infection (Koosha *et al.*, 2004) rather it needs further diagnosis especially when *S. stercoralis* infection is suspected in areas of endemicity (Roman-Sanchez *et al.*, 2003). The infection of *S. stercoralis* can be classified as light, moderate and severe

when 1, 1 to 3, and more than 3 larvae per field are found, respectively during direct fecal smear (Egido *et al.*, 2001).

Although direct identifications of larvae with direct and concentration methods provide the only definitive diagnosis, poor sensitivity and of repeated stool examination highlight the need for the development of other more sensitive methods. Based on these facts, new methods like agar plate culture and Bermanization were developed with higher sensitivity even with a single stool examination than the usual methods.

As stated by Moustafa (1997) agar plate method is a more sensitive parasitological methods for the detection of *S. stercoralis* infection in endemic areas but less efficient for other helminths. The agar plate culture gives consistently higher sensitivity (78% to 100%) than simple direct fecal smear (0% to 52%) and formalin-ether concentration method (13% to 55%) (Uparanukraw *et al.*, 1999). Based on these, agar plate culture is considered as the most important parasitological tool for the detection of chronic *S. stercoralis* infection (Dinleyici *et al.*, 2003). According to Blat and Cantos (2003), Baermann and agar plate detect up to 49% and 70% positive result respectively from a single stool specimens. The result of agar plate can be increased up to 96% when three serial stool samples are taken (Moustafa, 1997; Siddiqui and Berk, 2001). In some literatures, the efficacy of the agar plate culture are more or less similar to Baermann methods, but Baermann method has some advantages in terms of cost effectiveness and the time needed to obtain the result (Egido *et al.*, 2001). Both these methods are laborious, time consuming and difficult to use for routine laboratory work (Dryer *et al.*, 1996) but important for research and epidemiological work.

Several studies support the view that detection of parasite-specific antibodies becomes a useful complement to the traditional parasitological diagnosis of *S. stercoralis* infection (Dinleyici *et al.*, 2003). The most sensitive, specific and

commonly used immunodiagnistic method is an enzyme linked immunosorbent assay (ELISA). However, this method has also limitations, which includes, lack of discrimination between past and present infections (Ferriera, 2003), cross-reaction with other helminths infections (Siddique *et al.*, 1997; Johnston *et al.*, 2005), and availability only at specialized centers (*Siddique* and Berk, 2001).

In Ethiopia, direct fecal smear, formal ether concentration, charcoal culture and modified Baermann methods were used by several investigators but the sensitivity of these methods is not very high and may not be comparable to agar plate. According to Assefa *et al.* (1991) about 60% of the total positive results were obtained in Wonji-Shoa sugar estate by modified Baermann method.

The other problem in diagnosis of *S. stercoralis* is to discriminate it from hookworm larvae in most clinical works, although hookworm larvae not commonly found in fresh direct fecal smears. However, the rhabiditiform larvae of *S. stercoralis* easily differentiated from hookworm larvae with characteristics features. The rhabiditiform larvae measuring 300-350 micrometers, has short buccal cavity, hourglass-shaped esophagus, prominent genital primordium in the mid section of the larvae and notched tail structure (Dinleyici *et al.*, 2003). The filariform larvae can also be distinguished from hookworm larvae by their elongated esophagus.

The treatments of *Strongyloides stercoralis* infection is also difficult because of poor sensitivity of diagnostic methods as well as the chronic autoinfective pathway of the life cycle that lead to persistent and severe strongyloidiasis. As stated by Ferriera (2003) and Keiser and Nutman (2004), the most common and frequent chemotherapic agents used to treat strongyloidiasis are thiabendazole, albendazole, and ivermectin. The administration of these drugs may repeat every two weeks until complete eradication of the parasite is

confirmed (Keiser and Nutman, 2004). This is because the autoinfective larvae require at least two weeks for shedding larvae in stools.

Thiabendazole is effective for complete eradication of the parasite. However, its side effects like nausea, headache, hypotension and hypersensitivity reaction (Mahmoud, 1996), gastrointestinal problems and liver dysfunction (Zaha *et al.*, 2000) limiting the wide use of this drug. In the contrary, ivermectin appears to be equally effective as thiabendazole for treating strongyloidiasis and is associated with fewer side effects (Mahmoud, 1996; Zaha *et al.*, 2000). The World Health Organization is currently considered ivermectin as the drug of choice for the treatment of strongyloidiasis (Siddique and Berk, 2001; Ferriera, 2003). In the case of disseminated strongyloidiasis, the use of broad-spectrum anti-microbial drugs such as cephalosporine and quinolones in combination with anti-helminths therapy (Ferriera, 2003) is recommended in order to combat secondary bacterial and fungal infections caused by enteric microorganisms, which frequently accompanied to severe manifestations of the disease. Other measures such as prevent from acquiring the infection, reducing the dose of immunosuppressive drug therapy and early treatment of secondary bacterial invasions (Mahmoud, 1996) are also essential elements in the prevention of *S. stercoralis* infection. Effective screening and treatment of individuals with at high risk of acquiring this infection such as HIV/AIDS patients is also very important (Keiser and Nutman, 2004).

2. Objectives

General objective: To evaluate different parasitological methods for the detection of *Strongyloides stercoralis* infection in selected health institutions in Addis Ababa.

Specific objectives: **1.**To determine specific and sensitive diagnostic methods for detection of *S. stercoralis* infection.

2.To assess possible association of *S. stercoralis* infection with immunocompromised status of individuals, particularly in HIV/AIDS patients.

3.To assess possible association of *Strongyloides stercoralis* infection with diarrhea

3. Materials and methods

3.1.The Study Area: This study was carried out in selected hospitals and health centers, in Addis Ababa from October to May, 2005/2006. The health institutes included in this study were Yekatit-12, Minilik-II, Zewditu Memorial Hospitals, and Kirkos Health Center. The laboratory works were processed within the Ethiopian Health and Nutrition Research Institute (EHNRI), Parasitological Laboratory.

3.2.The Study population and sample collection: The study population size was determined by the statistical technique used for the determination of sample size, $N=Z^2x\ p\ (1-p)/d^2$ (Machado and Costa-Cruz, 1998).

Where, Z=1.96(at 95% confidence interval)

P=13%(average prevalence of *S. stercoralis* infection in Ethiopia)

d=5%(tolerance or worst acceptable result)

1-P=87%(non observed value)

Based on this, the study population defined was 173, but the number was increased to 351. Among these, 226 were HIV positive and the rest 125 were HIV negative individuals. Both diarrheal and non-diarrheal individuals were included in the study. The study subjects were randomly selected when they come to voluntarily counseling and testing (VCT) centers for HIV testing. In addition, HIV positive individuals who seek medical care in the selected hospitals were also included.

Single fresh stool samples were collected in a labeled cap from each consented subject, of both sexes and different age groups. The fresh stool samples were transported to the Parasitological Laboratory of the Ethiopian Health and Nutrition Research Institute (EHNRI) within 2 to 3 hours after collection.

3.3. Sample Preparation and Identification: After collecting fresh stool specimens, the samples were processed and examined for the presence of *S. stercoralis* larvae, and ova and cysts of other intestinal parasites by the following parasitological methods.

3.3.1. Direct Fecal Smear: One drop of normal saline was placed on the center of clean slide and 1-2 mg of fresh stool sample was mixed with an applicator stick. The suspension was covered with a cover slip and examined for the presence of *S. stercoralis* first stage (L1) larvae, and other parasites ova, cysts and trophozoites under the microscope using 10 x and 40 x magnifications.

3.3.2. Formal-Ether Concentration Method: Two gram of fecal samples from each specimen was mixed in a vial containing 8 ml formal-saline solution. The mixture was shaken well, and the fecal suspension was filtered through two layers of cotton gauze into a centrifuge tube. To the suspension, 3 ml of ether was added; the tube was closed with rubber stopper and shaken vigorously for one minute. After removing the stopper, the tube was centrifuged for 2 minutes at 2000 revolution per minute (rpm). The supernatant was discarded by inverting the tube. The remaining sediment was examined under a light microscope at 10 x and 40 x magnifications for the presence of *S. stercoralis* first stage (L1) larvae and the presence of ova and cysts of other intestinal parasites (Cheesbrough, 1990).

3.3.3. Charcoal Culture Method: In this technique, ten gram of feces was thoroughly mixed with distilled water until it forms a thick suspension. The suspension was mixed with equal quantity of granulated charcoal. After covering the bottom of the dish with filter paper and moisten them with distil water, the fecal-charcoal mixtures were placed at the center of

the petri dish. Water can also be added until the charcoal glistens and there should be a layer of water over the bottom of the petri dish. The petri dish was placed in dark room after being sealed with vinyl tape. The petri dish was checked every day and the evaporated water was replaced by spraying to the surface without further mixing upto 13 days. Within seven to ten days, the strongyloides larvae developed into the infective stage (L3) and the larvae were harvested and its morphological features were observed. The petri dish was examined using a dissecting microscope and larvae can be seen at the edge of the filter paper (htt://www.btinternet.com). Finally, on the 13th day, water is added to the mixture and exposed to light for approximately 2 hours, which allows the larvae migrate into the water. Then, the water is collected in the test tube and centrifuged for 5 minutes at 2000 rpm. The types of larvae as well as their stages were identified microscopically from the sediments.

3.3.4. Harada Mori filter paper method: In the Harada-Mori technique, filter paper containing fresh fecal material was placed in test tube containing distilled water. The water should not directly touch the fecal materials, rather through capillary action it moisten part of the filter paper with stool sample. The test tubes were incubated at 28°C to 30°C for 10 to 13 days that create conditions suitable for the development of larvae, which can migrate to either side of the filter paper (Siddiqui and Berk, 2001). The amount of water inside the test tube was checked every day and replaced before it becomes dry. Finally, the larval stages were identified from the water after centrifugation for 5 minutes at 2000 rpm.

3.3.5. Baermann Method: After filling the funnel with warm water (37°C), a tea sieve mesh was placed on the mouth of the funnel touching part of the water. Then, the sieve was covered with two layer of cotton gauze. Ten gram of fresh feces was placed at the center of sieve mesh, which is partly immersed in the water. The fecal specimens were left for one hour at room

temperature, which makes the larvae crawl out of the fecal suspension and migrate to warm water through geotropisms and hydrotropism. Finally, part of the water discarded from the top by retaining 5 to 10 ml at the bottom of the funnels. It is transferred to a 15 ml test tube and centrifuged for 5 minutes at 2000 rpm to harvest & identify the larvae from the sediments using light microscope at 10 x and 40 x magnifications (Siddiqui and Berk, 2001).

3.3.6. Agar Plate Culture Method: The nutrient agar plate was prepared based on the protocol of the manufacturer (Oxoid LTD). Briefly, 28gram of nutrient agar was dissolved in 1liter of distilled and autoclaved water. Nine milliliter of the mixture was placed in a sterile petri dish, allowed to air dried and kept at cold room temperature until use. Four grams of feces were placed at the center of a nutrient agar plate and sealed with adhesive tape to prevent the migration of larvae to out side surface. The plates were incubated for 2 to 5 days at room temperature and observed daily with inverted microscope for the presence of tracks, moving larvae or free-living adults. After 5th day, 5ml of 10% formalin solution was added to all plates and kept for five minutes. The excess formalin solution was placed in centrifuge tubes and centrifuged at 1500 rpm for two minutes. Finally, the sediments were analyzed for the presence of *S. stercoralis* larvae, adults and/or eggs. The larvae were distinguished from that of hookworm by staining with lugol iodine (Blatt and Cantos, 2003).

3.3.7. Modified Ziehl-Neelsen: In this technique, direct fecal smears were made on a clean slide and allowed to dry overnight under room temperature. The dried smears were fixed with methanol for 5 minutes and stained with carbol-fuchsine for 30 minutes. The carbol-fuchsine was rinsed with tap water and then decolorized in acid alcohol for 3 minutes. After rinsing in tape water, counterstained with methylene blue for 1

minute, then rinsed with water and allowed to air dry. The slides were examined with 100x magnification with oil immersion for the presence of oocysts of *I. Belli* and *C. parvum* (Henry *et al.*, 1986).

3.4. Ethical consideration: This project was ethically cleared by ethical committee of Biology Department, Addis Ababa University. After informing the study subjects about the benefit of the study, individuals volunteer to participate and gave their written consents were included in the study. Samples were taken by trained laboratory technicians. All positive cases for intestinal parasites were treated free of charge. All the results were kept strictly confidential.

3.5. Data analysis: Data were analyzed by using the statistical package for social science (SPSS) Version-11 X^2-test and interpreted for specificity and sensitivity of the techniques for the detection of *S. strecoralis* infection. The associations of *S. stercoralis* infection with HIV/AIDS, diarrhea and non diarrheal patients as well as to see the associations of *S. stercoralis* infection with other parasites with a defined value of $P<0.05$ was considered as significant.

Calculation of sensitivity and specificity: The sensitivity and specificity of different parasitological methods were calculated in relation to the Baremann method, which is the standard parasitological method used for the detection of *S. stercoralis* infection. By taking two by two distribution table in which both the standard and the test method were included. The sensitivity and specificity were calculated as: **Sensitivity** =a/ a+c x 100

Specificity =b/b+d x 100

Test method		Standard method	
		Positive	Negative
	Positive	a	b
	Negative	c	d

Equipments: Camera fitted microscope(LEICA DMLS2) and computer from the Ethiopian Health and Nutrition Research Institute were used for taking pictures from the prepared slides.

4. Results

Out of 351 study subjects, 226 were HIV positive and 125 were HIV negatives. The age range of the study participants was 10 to 65 years, of which 34.5% were male and 65.5% females (Table 1). Forty percent of the HIV positive subjects and twenty six percent of the HIV negative subjects have diarrhea of single or multiple episodes.

The majority of HIV positive subjects were in the age range of 25 to 34 years and most of the HIV negative subjects were in the age range of 15 to 24 years. Majority of the study populations were females both in the HIV positive (65.9%) subjects and HIV negative (64.8%) subjects (Table 1).

Table 1: Age and sex distribution of HIV positive and HIV negative study subjects in selected health institutions in Addis Ababa, 2005/2006

Age	**HIV positive (n=226)**		**HIV negative (n=125)**		**Total (n=351)**	
	Male	Female	Male	Female	Male	Female
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
15-24	3 (1.3)	25 (11.1)	19 (15.2)	43 (34.4)	22 (6.3)	68 (19.4)
25-34	26 (11.5)	67 (29.6)	17 (13.6)	24 (19.2)	43 (12.3)	91 (25.9)
35-44	32 (14.2)	45 (19.9)	2 (1.6)	11 (8.8)	34 (9.6)	56 (15.9)
>45	15 (6.6)	11 (4.9)	4 (3.2)	3 (2.4)	19 (5.4)	14 (3.9)
Total	77 (34)	149 (65.9)	44 (35.2)	81 (64.8)	121 (34.5)	230 (65.5)

Among the 351 stool specimens examined, 43(12.3%) subjects were positive for *S. stercoralis* infection detected by combination of agar plate and Baermann method. The detection rates of different techniques were: nutrient agar plate 42(97.7%), Baermann method 32(74.4%), formal-ether concentration technique 24(55.8%), direct-fecal smear 22(51.2%), Harada Mori filter paper method 19(44.2%) and charcoal culture method 17(39.3%) (Table 2). The sensitivity and specificity of different parasitological methods were evaluated as compared to the standard Baermann method. This result showed that nutrient agar plate was both more sensitive and specific method for the detection of *S. stercoralis* infection (Table 3).

Intestinal parasites were detected in 44.3% of HIV positive and 31.2% of HIV negative subjects (Table 4). The common intestinal protozoa detected were: *Entamoeba histolytica/dispar* (8.6%), *Blastocystis hominis* (6%), *Cryptosporidium parvum* (2.9%), *Isospora belli* (1.7%), *Giardia lamblia* (3.1%) *and Entamoeba coli* (2.3%). Among intestinal helminths *Strongyloides stercoralis* (12.3%), *Ascaris lumbricoides* (6%), hookworms (3.4%), and *Trichuris trichiura* (1.1%) were detected in the study population. More than 90% of the *S. stercoralis* and all *C. parvum and I. belli* positive subjects were detected from HIV positive patients (Table 4).

Among the 351 study subjects, 124 (35.3%) had diarrhea with a single or multiple frequency and the remaining 227(64.7%) were non-diarrheal subjects. Higher proportion of intestinal parasites such as *S. stercoralis* (24.2%), *E. histolytica/dispar* (10.5%), *G. lamblia* (8.1%), *C. parvum* (7.3%), *and B. hominis* (7.3%) were detected from diarrheal patients as compared to non-diarrheal subjects. On the contrary, higher proportions of *A. lumbricoides* (7.1%), *hookworms* (4.9%), and *E. coli* (3.5%) were detected among none diarrheal subjects.

Out of the 226 HIV positive subjects, 91 (40.3%) were diarrheal and 135(59.7%) non-diarrheal. The most common intestinal parasites detected from HIV positive diarrheal patients were *S. stercoralis* (32.9%), *C. parvum* (9.9%), *E. histolytica/dispar* (10.9%), *B. hominis* (7.7%), and *G. lamblia* (7.7%). On the other hand, common intestinal parasites such as *A. lumbricoides* (5.9%), hookworm infection (3.7%), and *E.coli* (2.2%) were detected in HIV positive non-diarrheal subjects (Table 5).

Among the 125 HIV negative individuals, 33(26.4%) were diarrheal and 92(73.6%) non-diarrheal. The most common intestinal parasites detected from HIV negative subjects were hookworm (5.6%), *A. lumbricoides* (8%), *B. hominis* (5.6%), *E.coli* (4%), and *S. stercoralis* (3.2%). Higher proportions of these infections were detected from HIV negative non-diarrheal as compared to HIV negative diarrheal subjects. *S. stercoralis* and *E. coli* were exclusively detected from HIV negative non-diarrheal subjects whereas high proportions of *E. histolytica/dispar* and all infections of *G. lamblia* were detected from HIV negative diarrheal subjects (Table 6).

Among the 43 individuals positive for *S. stercoralis* infection, 34(79.1%) infected with only *S. stercoralis*, 9(20.9%) were infected with *S. stercoralis* and one or more other intestinal parasites (Table 7). Moreover, more single *S. stercoralis* infections were detected among HIV/AIDS patients with diarrhea.

The most frequent intestinal parasite detected by nutrient agar plate in the present study was *S. stercoralis*, which had a higher prevalence than any of the parasites identified in HIV positive diarrheal patients and the second prevalent in HIV positive non-diarrheal patients (Table 5).

Among the 226 HIV positive subjects, 39(17.3%) were positive for *S. stercoralis* infections whereas only 4(3.2%) of the 125 HIV negative subjects were positive (Table 4). The risk of getting *S. stercoralis* among HIV positive subjects are

about 6 times higher than HIV negative subjects (OR=6.3). *S. stercoralis* is strongly associated with HIV positive diarrheal patients as compared to HIV positive non-diarrheal subjects (P<0.001) (Table 5). The risk of getting *S. stercoralis* appears to be higher among HIV positive with diarrhea than without diarrhea (OR=6.9). In contrast, no *S. stercoralis* infections were detected from HIV negative diarrheal patients (Table 6).

Table 2: Detection rate of *S. stercoralis* infection by different parasitological methods in selected health institutions in Addis Ababa, 2005/2006

Parasitological methods	***Strongyloides stercoralis* detected (n=43)**	
	No.	%
Agar plate culture	42	97.7
Baermann's method*	32	74.4
Concentration method	24	55.8
Direct fecal smear	22	51.2
Harada Mori Filter paper	19	44.2
Charcoal culture	17	39.3

* 29 watery diarrheal cases were not done by Baermann's methods

Table 3: Determinations of sensitivity and specificity of parasitological methods as compared to the Baermann method for the detection of *S. stercoralis* infection among 322 study subjects

A. Nutrient agar plate

		Baermann technique		
		Positive (n=32)	Negative	Total
Nutrient agar plate	Positive	31	11	42
	Negative	1	279	280
	Total	32	290	322

NB: Sensitivity of nutrient agar plate =31/32=96.9%

Specificity of nutrient agar plate =279/290=96.2%

B. Concentration method

		Baermann technique		
		Positive	Negative	Total
Concentration method	Positive	24	0	24
	Negative	8	290	298
	Total	32	290	322

NB: Sensitivity of concentration method =24/32=75%

Specificity of concentration method =290/290=100%

C. Direct fecal smear

		Baermann technique		
		Positive	Negative	Total
Direct fecal smear	Positive	22	0	22
	Negative	10	290	300
	Total	32	290	322

NB: Sensitivity of direct fecal smear=22 /32=68.75%

Specificity of direct fecal smear=290/290 =100%

D. Harada Mori filter paper

		Baermann technique		
		Positive	Negative	Total
Harada Mori filter paper	Positive	19	0	19
	Negative	13	290	303
	Total	32	290	322

NB: Sensitivity of Harada Mori filter paper=19/32=59.4%

Specificity of Harada Mori filter paper=290/290=100%

E. Charcoal culture

		Baermann technique		
		Positive	Negative	Total
Charcoal culture	Positive	17	0	17
	Negative	15	290	305
	Total	32	290	322

NB: Sensitivity of charcoal culture=17/32=53.1%

Specificity of charcoal culture=290/290=100%

Table 4: Intestinal parasites detected among HIV positive and HIV negative subjects in selected health institutions in Addis Ababa, 2005/2006

Parasite identified	HIV status		
	HIV positive (n=226)	**HIV negative (n=125)**	**Total (n=351)**
	No. (%)	No. (%)	No. %
Strongyloides stercoralis@	39 (17.3)**	4 (3.2)	43 (12.3)
Isospora belli	6 (2.7)	0	6 (1.7)
Cryptosporidium parvum	10 (4.4)*	0	10 (2.9)
Hookworm species	5 (2.2)	7 (5.6)	12 (3.4)
Entamoeba histolytica/dispar	20 (8.8)	10 (8)	30 (8.6)
Entamoeba coli	3 (1.3)	5 (4)	8 (2.3)
Endolimax nana	1 (0.4)	0	1 (0.3)
Ascaris lumbricoides	11 (4.9)	10 (8)	21 (6)
Trichuris trichiura	4 (1.8)	0	4 (1.1)
Giardia lamblia	8 (3.5)	3 (2.4)	11 (3.1)
Chilomastix mesnili	0	1 (0.8)	1 (0.3)
Blastocystis hominis	14 (6.2)	7 (6.4)	21 (6)
Hymenolepis dimunta	1 (0.4	0	1 (0.3)
Hymenolepis nana	0	1 (0.8)	1 (0.3)
Sarcocyst	0	1 (0.8)	1 (0.3)
Total infected	100 (44.3)	39 (31.2)	139 (39.6)

* P<0.05

** P<0.001

@ Nutrient agar plate culture

Table 5: Intestinal parasites detected among HIV positive diarrheal and non-diarrheal patients in selected health institutions in Addis Ababa, 2005/2006

Subjects	**Diarrheal (n=91)**	**Non-diarrheal (n=135)**	**Total (n=226)**
	No. (%)	No. (%)	No. (%)
Strongyloides stercoralis@	30 (32.9)**	9 (6.7)	39 (17.3)
Isospora belli	5 (5.5)*	1 (0.7)	6 (2.7)
Cryptosporidium parvum	9 (9.9)**	1 (0.7)	10 (4.4)
Hookworm species	0	5 (3.7)	5 (2.2)
Entamoeba histolytica/ dispar	10 (10.9)	10 (7.4)	20 (8.9)
Entamoeba coli	0	3 (2.2)	3 (1.3)
Endolimax nana	0	1 (0.7)	1 (0.4)
Ascaris lumbricoides	3 (3.3)	8 (5.9)	11 (4.9)
Trichuris trichiura	3 (3.3)	1 (0.7)	4 (1.8)
Giardia lamblia	7 (7.7)**	1 (0.7)	8 (3.5)
Blastocystis hominis	7 (7.7)	7 (5.2)	14 (6.2)
Hymenolepis diminuta	0	1 (0.7)	1 (0.4)
Total	59 (64.8)*	41 (30.4))	100 (44.3)

* P<0.05

** P<0.001

@ Nutrient agar plate culture

Table 6: Intestinal parasites detected among HIV negative diarrheal and non-diarrheal subjects in selected health institutions in Addis Ababa, 2005/2006

Subjects	**Diarrheal (n=33)**	**Non diarrheal (n=92)**	**Total (n=125)**
	No. (%)	No. (%)	No. (%)
Strongyloides stercoralis@	0	4 (4.3)	4 (3.2)
Isospora belli	0	0	0
Cryptosporidium parvum	0	0	0
Hookworm species	1 (3)	6 (6.5)	7 (5.6)
Entamoeba histolytica/ dispar	3 (9.1)	7 (7.6)	10 (8)
Entamoeba coli	0	5 (5.4)	5 (4)
Ascaris lumbricoides	2 (6.1)	8 (8.7)	10 (8)
Giardia lamblia	3 (9.1)**	0	3 (2.4)
Blastocystis hominis	2 (6)	5 (5.4)	7 (5.6)
Hymenolepis nana	0	1 (1.1)	1 (0.8)
Chilomastix mesnili	0	1 (1.1)	1 (0.8)
Sarcocyst	0	1 (1.1)	1 (0.8)
Total	10 (30.3)	29 (31.5)	39 (31.2)

** P<0.001

@ Nutrient agar plate culture

Table 7: Single *S. stercoralis* and multiple intestinal parasitic infections among HIV positive and HIV negative subjects in selected health institutions in Addis Ababa, 2005/2006

HIV Status	Total positive subjects		
	Only *S.stercorals* infection (n=34)	*S. stercoralis* + one or more other parasite (n=9)	Total (n=43)
	No. (%)	No. (%)	No. (%)
Positive	32 (94.1)	7 (77.8)	39 (90.7)
Negative	2 (5.9)	2 (22.2)	4 (9.3)

Of the total 43 patients infected with *S. stercoralis*, co-occurrence with other protozoa and helminths was detected only in 9 patients. Among these, the co-occurrence of *S. stercoralis* and *B. hominis* was slightly higher, 3 of the 9 subjects. These co-occurrences were more commonly observed in HIV positive subjects than in HIV negative subjects with no significant difference in diarrheal and non-diarrheal patients ($P>0.05$).

Out of the 43 patients positive for *S. stercoralis* infection, the majority of *S. stercoralis* infections 30(69.7%) were detected in the age range 25 to 44 years among HIV positive patients whereas there is no such difference among HIV negative study subjects. However, there was no statistical significant difference of *S. stercoralis* infection on the age of the study participants.

Baermann method had significantly higher detection rate than direct fecal smear, formal ether concentration, Harada Mori filter paper, and charcoal culture ($P<0.001$). However, in the present study, higher *S. stercoralis* infections were detected by nutrient agar plate even compared to the standard

Baermann method (Table 2). Moreover, agar plate has an advantage over Baermann method to detect *S. stercoralis* infection from diarrheal samples as well as very low infection rate. However, one case of *S. stercoralis* infection was missed by nutrient agar plate, but detected by Baermann method. Seventy five percent of *S. stercoralis* infections from HIV negative non-diarrheal groups were detected by nutrient agar plate and Baermann method, and none of them were identified by the direct and concentration methods.

The nutrient agar plate method support the switching of developmental phases, i.e. changes from parasitic life into free-living stage and may stay for long period. In the present study, the free-living stages were kept about one month. The free-living adults were observed after five days of culturing and most of the abdomen of the female worm was full of single or double rows of eggs (Figure 2) and sometimes these eggs were hatched inside the body of adult female worm (Figure 3).

Massive rhabditiform and filariform larvae were observed on the surface of nutrient agar plate within three days, which makes the detection rate with agar plate more sensitive than other parasitological methods (Figure 4). In the present study, all stages of the life cycles (eggs, rhabditiform larvae, filariform larvae and adult stage) were observed from a single nutrient agar plate after keeping the plate for seven days at room temperature (Figure 5). The adult and the larval stages were clearly distinguished based on their size, shape and motility with the help of dissecting microscope on the surface of nutrient agar plate or washing the plate with distilled water or formaline and centrifuged for further identification of the stage of the larval and to distinguish from hookworm larvae.

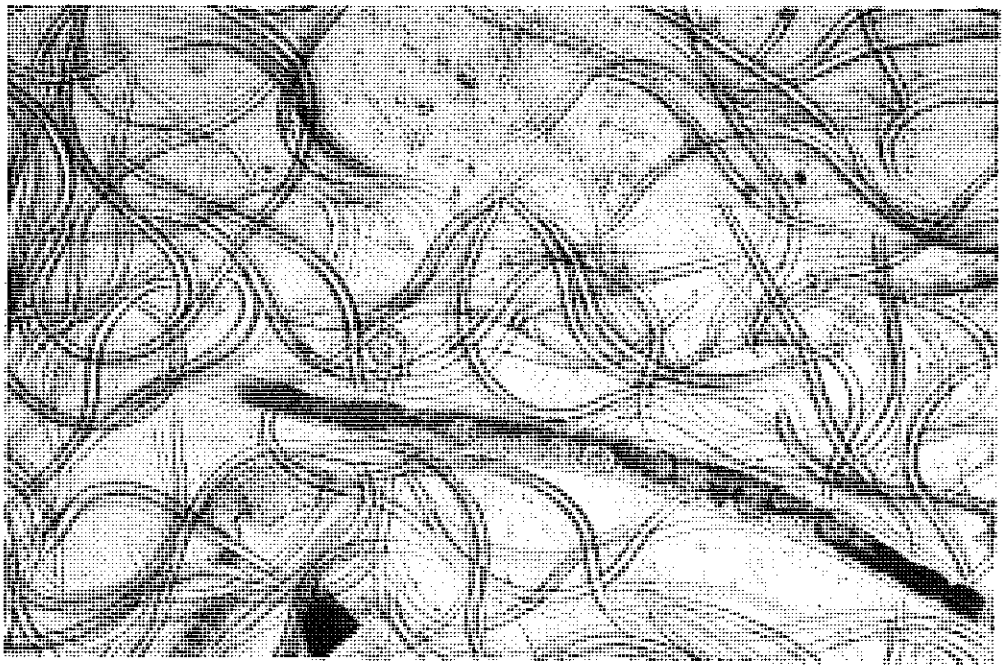


Figure 2: Massive rhabditiform larvae, filariform larvae and adult *S. stercoralis* in agar plate (Magnification 40X)

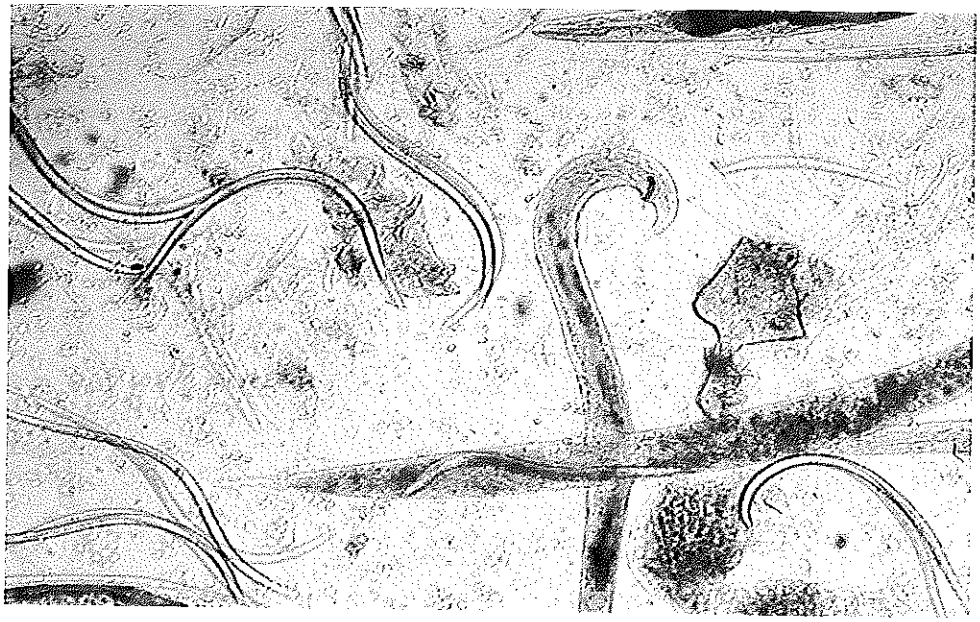


Figure 3: Rhabditiform larvae of *S. stercoralis* hatched within the body of adult worm taken from agar plate (Magnification 40X)

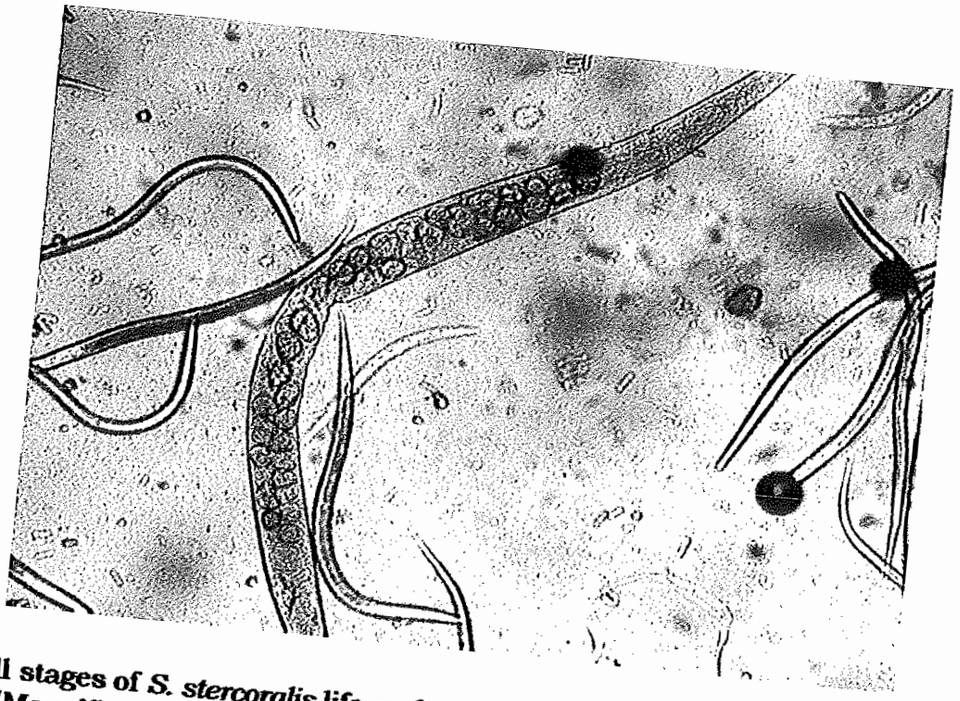


Figure 4: All stages of *S. stercoralis* life cycle in one agar plate (Magnification 40X)

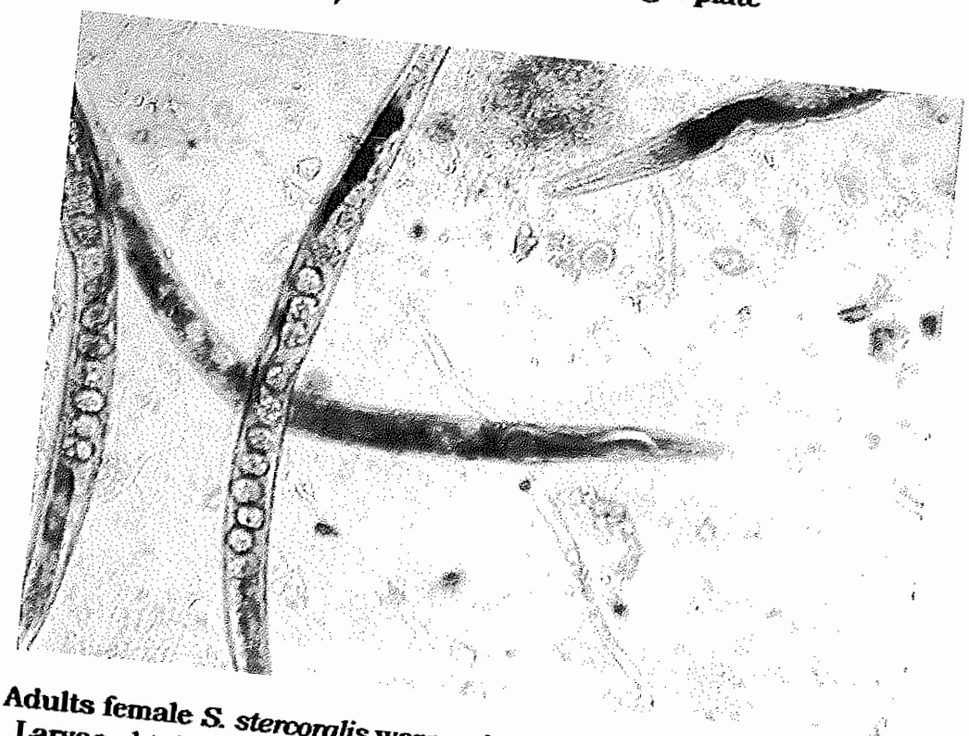


Figure 5: Adults female *S. stercoralis* worm with eggs and rhabiditiform Larvae obtained from agar plate (Magnification 40X)

5. Discussions

Chronic diarrhea is one the major problems and probably the major cause of morbidity and mortality among HIV/AIDS patients. Intestinal parasites, bacteria, fungi or viral infections are responsible for the majority of this chronic diarrheal illness (Awole *et al.*, 2003). Among the intestinal parasites *Cryptosporidium parvum, Isospora belli,* Microsporidial parasites, *Entamoaba histolytica/dispar, Giardia lamblia, Strongyloides stercoralis* and *Blastosystis hominis* are implicated with chronic diarrheal illness (Cimerman *et al.*, 1999). In this study, it is noted that most of these intestinal parasites were detected with diarrheal patients, especially with HIV/AIDS. Among the intestinal parasites *C. parvum, I. belli,* Microsporidium species and *Cyclospora cayetanensis* are considered to be opportunistic intestinal parasites (Awole *et al.*, 2003). According to Smith (1993), intestinal parasites like *C. parvum* and *I. belli* appear to be the most predominant opportunistic parasites, particularly among HIV/AIDS patients with chronic diarrhea in the developed world. This fact is more pronounced in Africa and other tropical countries (Cimerman *et al.*, 1999).

The common intestinal protozoan parasites detected in the present study were *E. histolytica/dispar, B. hominis, G. lamblia* and *E. coli. Cryptosporidium parvum* and *Isospora belli* were among the opportunistic intestinal parasites exclusively detected from HIV positive subjects, which is in agreement with other studies in Addis Ababa (Fisseha *et al.*, 1999). Intestinal helminths such as *S. stercoralis, A. lumbricoides,* hookworms and *T. trichiura* were also common in the study population, which is in agreement with other studies in Southwestern Ethiopia (Awole *et al.*, 1999). Higher proportions of intestinal parasites were detected from patients with diarrhea than those without diarrhea in the present study, which is similar with study from Iran that showed 53% in diarrheal patients and 12.6% in non-diarrheal patients (Zali *et al.*, 2004).

Diarrhea is the most frequent manifestations of diseases among AIDS patients. Such a high frequency of diarrhea is associated with the presence of intestinal parasites (Feitosa *et al.*, 2001). The intestinal parasites commonly detected with a higher proportion in HIV/AIDS diarrheal patients were *S. stercoralis, I. belli, C. parvum, E. histolytica/dispar, G. lamblia* and *B. hominis* compared to HIV positive non-diarrheal subjects. Almost similar observations were reported from Addis Ababa and Southwestern Ethiopia (Fisseha *et al.*, 1999; Awole *et al.*, 2003). However, high infection of *S. stercoralis* was observed in this study, which might be due to the difference in sensitivity of diagnostic methods used. Moreover, very low *C. parvum* infection was detected as compared to previous work that might be attributed to the immune status of the study subjects. Most of the HIV positive individuals enrolled in this study might have better immune status and may not develop into AIDS stage, that might be the probable reason for low detection rate of opportunistic parasites such as *C. parvum* and *I. belli.*

There was high proportion of HIV/AIDS patients who had diarrhea in the absence of identifiable parasitic infections, indicating the existence of other diarrhoeagenic agents or mechanisms. The intestinal parasites detected in the present study are not the only cause of diarrhea. Other infections such as Microsporodial parasites, bacterial infections, fungal infections, viral infections might be responsible for this diarrhea, which are the limitations in this study.

All cases of *I. belli* and *C. parvum,* and the majority of *S. stercoralis* infections were detected among HIV patients. Moreover, significantly higher infections of these parasites were detected from HIV positive diarrheal patients as compared to HIV positive non-diarrheal cases ($P<0.05$). This implies that opportunistic intestinal parasites are assumed the major causes of diarrhea

among HIV patients and usually associated with severe immunosuppression (Brink *et al.*, 2002).

In this study, *Isospora belli* (2.7%) were detected from HIV patients with lower prevalence as compared to previous studies done elsewhere such as, 7% in Southwestern Ethiopia (Awole *et al.*, 2003), 7% in Zaire, 13% in Uganda and 12% in Haiti (Wiest *et al*, 1991). However, it is higher than a study conducted in Addis Ababa (0.7%) (Fisseha *et al.*, 1999), but similar results were reported in Cuba 1.5% and in Jordan 3% (Escobedo and Nunez, 1999). This might be due to the difference in immune status of the study population, sensitivity of the methods used or difference in prevalence of the infection in the area studied.

Cryptosporidium parvum (4.4%) were detected from HIV patients, which is significantly lower than other studies carried out in Ethiopia and other developing countries such as 25.9% among AIDS patients in Addis Ababa Fisseha *et al.* (1999), 11% in Southwestern Ethiopia Awole *et al.* (2003), 10% in Jordan and 11% in India (Escobedo and Nunez, 1999). The differences observed might be from the conditions of the study participants. In this study, most of the study populations were only HIV positive subjects who may have not yet developed into full-blown AIDS stage with relatively better level of immune status. Intestinal parasitic infections especially the opportunistic ones are strongly associated with depleted immune status (Brink *et al.*, 2002).

Other protozoa's like *Entamoaba histolytica/dispar* and *Giardia lamblia* were associated with chronic diarrhea and significant differences were not observed be it HIV positive or HIV negative ($P>0.05$). However, slightly higher prevalence of *E. histolytica/dispar* and *G. lamblia* were detected from HIV positive patients compared to HIV negative subjects. This observation is in agreement with other studies that high prevalence of Entamoeba parasites were detected in HIV/AIDS patients than in HIV negative patients in Ethiopia (Fontanet *et*

al., 2000). However, as indicated by Kebede *et al.* (2003) there was misdiagnosis and over diagnosis of the true *E. histolytica* from the same locality. *Giardia lamblia* showed a significant association with diarrhea in the present study ($P<0.001$).

Blasitosystis hominis were detected from both HIV positive and HIV negative subjects and there is no significant difference observed ($P>0.05$). However, there is a slightly higher infection of *B. hominis* among HIV positive diarrheal patients as compared to HIV positive non-diarrheal subjects, and no difference was observed in HIV negative subjects whether they are diarrhea or non-diarrhea. *B. hominis* infection leads to chronic diarrheal manifestations in AIDS patients as indicated by Cimerman *et al.* (1999), which is in agreement with the present study.

The prevalence of *Strongyloides stercoralis* infection varies from locality to locality and based on the immune status of the populations. In general, the infection of *S. stercoralis* is with in the range 3% to 44% in most of the central plateau of Ethiopia (McConnell and Armstrong, 1976). The detections of this helminth in this study was within similar range showed that 12.3% in the study group as a whole, 17.3% among HIV/AIDS patients and 32.9% in HIV positive diarrheal patients. On the other hand, earlier studies showed that the prevalence of *S. stercoralis* in Addis Ababa was very low(Fisseha *et al.*, 1999). This could be due to poor sensitivity and specificity of diagnostic methods which leads to under estimate the prevalence of *S. stercoralis* in Ethiopia.

Most confirmed cases of *S. stercoralis* infections reported so far in association with AIDS occurred in the United States, where prevalence of helminthic parasites is comparatively very low (Gompels *et al.*, 1991). However, concurrent cases of *S. stercoralis* infection and AIDS in developing countries are believed to be happening in remote areas with poor diagnostic and reporting facilities. As a result, frequent misdiagnosis and underreporting

cases may occur and that results in underestimation of the actual prevalence of the infection in most developing countries. The same situation is observed in Ethiopia, where lower prevalence was reported (Fisseha *et al.*, 1999; Hailemariam *et al.*, 2004; Legesse and Erico, 2004).

In the present study, *Strongyloides stercoralis* was detected in 12.3% of the study subjects. This is in agreement to previous report from Ethiopia and other countries: 13% in Wonji Shoa Sugar State (Fontanet *et al.*, 2000) and 11.1% in Southwestern Ethiopia (Awole *et al.*, 2003). However, this result is lower than a study carried out in Wonji Shoa Sugar state 17.3% (Assefa *et al.*, 1991). Most of the previous studies in Ethiopia were lower compared to the present study, such as, 9% in Jimma Town (Indris, 2001), 8.6% in Addis Ababa (Endeshaw *et al.*, 2004), and 3.4% in Addis Ababa (Fisseha *et al.*, 1999). This could be attributed to the difference in diagnostic methods used by the investigators.

The detection of *S. stercoralis* infection is significantly higher among HIV/AIDS patients compared to HIV negative cases ($P<0.001$). This result is in agreement with other study from Brazil, where 15% of the HIV patients were infected with this helminth as compared to 1.4% in the general population (Cimerman *et al.*, 1999). Moreover, the larval output was significantly higher in HIV patients as compared to HIV negative individuals. Similar observations were reported from Southwestern Ethiopia (Awole *et al.*, 2003). This showed that, HIV patients appeared to be more susceptible to this helminth infection as compared to the HIV negative subjects. In general, strong association was observed between S. *stercoralis* infection and HIV/AIDS by different investigators (Robinson *et al.*, 1990; Guerin *et al.*, 1997; Lindo *et al.*, 1998). From this and other studies, it can be concluded that, HIV patients have a higher prevalence of *S. stercoralis* infection than the HIV negative population.

In this study, the detection of *S. stercoralis* infection was higher among HIV positive diarrheal subjects as compared to HIV positive non-diarrheal subjects, which is higher than previous studies conducted among HIV positive subjects in Ethiopia (Fisseha *et al.*, 1999; Hailemariam *et al.*, 2004). This could be due to the difference in sensitivity of the diagnostic methods used. In the present study, nutrient agar plate was used for the detection of this infection, which has a higher sensitivity and specificity than any other parasitological methods used. This indicates the importance of this infection particularly among HIV positive diarrheal patients. Since the onset of AIDS pandemic, *S. stercoralis* has been considered to be an agent of opportunistic infection in AIDS patients (Cirioni *et al.*, 1996). Moreover, according to Ferreira (2003), opportunistic nature of *S. stercoralis* in patients with AIDS is doubtless. This study also supports that *S. stercoralis* is an important intestinal parasite in HIV/AIDS diarrheal patients.

Strongyloides stercoralis infection was the top most frequent parasites in this study particularly among HIV/AIDS patients. Other reports such as the annual report of the Parasitological Division of National Research Institute of Health for 1987-1988(Unpublished) indicated that *S. stercoralis* was the third commonest intestinal helminths encountered in Ethiopia. Moreover, Taticheff *et al.* (1981) reported that *S. stercoralis* is the third common intestinal helminths among the study group following to *A. lumbricoides* and *T. trichiura.* All these facts showed that *S. stercoralis* infection is of public health importance in Ethiopia. Moreover, the problem of HIV/AIDS pandemic in Ethiopia leads to a countless immunosuppression, which directly increases the multiplication and dissemination of silent and chronic infections of *S. stercoralis* throughout the body of the infected individuals.

S. stercoralis infections are assumed to be age dependent, the most vulnerable age groups are children due to higher exposure to larvae contaminated soil and older ages due to decline of immune status and nature of occupations

(Hammad and Lenox, 1999). However, in the present study, higher infection was detected within the age range of 25 to 44 years. Similar studies conducted in Addis Ababa showed that 59% of the *S. stercoralis* infection was concentrated within the age of 21 to 40 years (Endeshaw *et al.,* 2004). However, in this study, almost 89.5% of the study populations were within the age range of 15 to 44 years, which may increase the possibility of detecting more *S. stercoralis* infection and it is difficult to see the age dependency of *S. stercoralis* infection among the study subjects.

Although males are more vulnerable to high infections of *S. stercoralis* than females (Keiser and Nutman, 2004), there was no significant difference in this study. However, there was a slightly higher detection rate from male compared to females.

In the present study, *Strongyloides stercoralis* infection was more frequent in the single parasitic infection as compared to multiple infections. There was no strong co-occurrence of *S. stercoralis* infection with other intestinal parasites observed in this study ($P>0.05$). Of the total *S. stercoralis* infections, co-occurrences with other intestinal parasites were detected only from 9 patients. Moreover, almost all co-infections were observed from HIV positive cases with no significant difference between diarrhea and non-diarrheal subjects ($P>0.05$). Co-occurrence of *S. stercoralis* with *B. hominis* was the predominant as compared with other intestinal parasites. In one of the HIV positive diarrheal subjects co-infection of *S. stercoralis* with *B. hominis*, *E. histolytica/dispar*, and *G. lamblia* was detected. In general, this finding showed that it could be a cause of abdominal complaints and diarrhea especially in HIV/AIDS patients.

Strongyloides stercoralis is one of the difficult parasitic infections in its diagnosis. Most of the parasitological methods have a problem of poor sensitivity probably due to intermittent and very low excretions of larvae with

feces and it may be impossible to detect light infections during routine laboratory work. Direct fecal smear and formal ether concentrations are the common and usual parasitological method in most service giving health institutions in Ethiopia. However, these methods have a problem of poor sensitivity, which detects 0% to 66% or an average of 30% of the proven *S. stercoralis* infection (Dryer *et al.*, 1996; Uparanukraw *et al.*, 1999), and most of the time leads to false negative results. In the present study, 51.2% and 55.8% of the proven *S. stercoralis* infections were detected by direct fecal smear and formal ether concentration methods, respectively which is in agreement with the above-mentioned study. The detection of *S. stercoralis* larvae with direct fecal smear from a single stool specimen may be an indication of heavy infection with this parasite.

Other parasitological methods such as Harada Mori filter paper and charcoal culture have also problem of poor sensitivity and difficult to implement for routine purpose, because it needs prolonged culturing time. In this study, 44.2% and 39.3% of the proven *S. stercoralis* infections were detected by Harada Mori filter paper and charcoal culture methods, respectively.

Baermann method was the standard patasitological method commonly used for research and epidemiological work for the detection of *S. stercoralis* infection (Drier *et al.*, 1996). In the present study, it detected 32(74.7%) of *S. stercoralis* infection in the study subjects. The sensitivity and specificity of other parasitological methods were evaluated as compared to the standard Baermann method. The result showed that nutrient agar plate had higher sensitivity and specificity as compared to other parasitlogical methods for the detections of *S. stercoralis*, which is in agreement with other study (Moustafa, 1997). Although Baermann method is considered to be the standard parasitological method for the detection of *S. stercoralis* infection, it has lower detection rate and not applicable for watery diarrheal samples compared to

nutrient agar plate. These limitations justifying the evaluation of other specific methods like nutrient agar plate.

The nutrient agar plate was the most sensitive method for the detection of *S. stercoralis* infection in this study, which detects 42 (97.7%) out of 43 positive cases ($P<0.001$), which is similar to other works reported (78% to 100%) elsewhere (Moustafa, 1997; Uparanukraw *et al.*, 1999). Even though, agar plate need 3 to 5 days for culturing, it is more important for any types of stool samples whether watery diarrhea or loose and not as such cumbersome. According to Egido *et al.* (2001) Baermann's and nutrient agar plate methods have more or less similar positivity, which is similar to our findings in non-watery diarrheal samples. In the present study, massive rhabditiform and filariform larvae were found when this method was used. The increase in the detection rate of *S. stercoralis* larvae with the agar plate method could be due to the possibility of detecting of free-living species of the larvae, which can be found in the indirect life cycle. The nutrient agar plate support the switching of developmental stages from parasitic stage to free-living stage (Blatt and Cantos, 2003). The free-living adult may continue laying of an embryonated eggs and rhabditiform larvae. Moreover, both sexes of the adult free-living worm clearly observed in the agar plate and can be distinguished by the presence or absence of eggs in their abdomen (Figure 2-5).

The agar plate has also additional advantages. All stages of the life cycle (eggs, rhabditiform larvae, filariform larvae and free-living adult stages of both sexes) from a single nutrient agar plate was observed which is important for further detail studies of the worm and for harvesting the filariform larvae for antigen preparation. In some cases, the rhabditiform larvae were clearly seen inside the body of adult female worms after keeping the plate for seven days which shows *S. stercoralis* is not only oviparous but also it is larviparous (Figure 3).

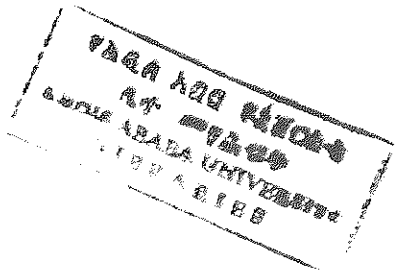
The major difficulty of nutrient agar plate technique is the required time for culturing (3 to 5 days) and needs great care for the technicians to avoid contaminations. Once the method is established for routine purpose, agar plates is not costly, it needs 0.28 gram of nutrient agar for the detection of one sample, which costs not more than 40 cents excluding the cost of the petri dish and other accessories.

6. Conclusions and Recommendations

The prevalence of intestinal parasites is usually high among HIV/AIDS patients as compared to the general population. The most common intestinal parasites detected in this study were *Strongyloides stercoralis, Entamoeba histolytica/dispar, Blastocystis hominis, Giardia lamblia, Cryptosporidium parvum,* and *Isospora belli.* Among these *S. stercoralis* infection is the most common and the most prevalent infection particularly among HIV patients both in the diarrheal and non-diarrheal subjects detected by nutrient agar plate. It was found in about 32.9% of the HIV positive diarrheal subjects whereas in only 6.7% of the HIV positive non-diarrheal subjects. This study showed that *S. stercoralis* infection has significantly higher association with HIV/AIDS and diarrhea. This leads to the needs of special attention for *S. stercoralis* infection when considering opportunistic parasites such as *C. parvum, I. belli* and Microsporidial parasites among HIV/AIDS patients.

S. stercoralis infection usually causes asymptomatic chronic infection of the gastrointestinal tract, may not be detected by using common parasitological methods. Undetected and silent infections may lead to serious and fatal forms such as disseminated disease and hyperinfections when diseases such as HIV/AIDS impair the immune status of the host. Therefore, appropriate and sensitive diagnostic method such as nutrient agar plate is very essential for an early detection of chronic and silent infection. A nutrient agar plate is the most sensitive parasitological method for the detection of *S. stercoralis* infection and commonly used in most endemic countries. In the present study that 97.7% (42 of the 43) of the *S. stercoralis* infections were detected by nutrient agar plate. Only one case was missed by agar plate, which was detected by Baermann method. The higher detection rate of nutrient agar plate may be due to the detection of free-living adults as well as second

generation of larvae from the free-living adults. Moreover, agar plate is applied to any type of samples whether watery diarrhea or soft. The cost is not such costly compared to Baermann and other parasitological methods, but the major problem is the time (3 to 5 days) needed for culturing. Thus, the nutrient agar plate method can be recommended for the detections of *S. stercoralis* infection among HIV/AIDS patients, particularly for epidemiological and research study.



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APPENDIXES

Appendix A

Data collection form for parasitological analysis

No.	S. code	Age	Sex	HIV status	Stool appearance	Intestinal parasites detected				Remark
						Dir.	Conc.	M. Ziehl-Neelsen	Fecal culture	

NB: S. code =Sample code (patient identification number)

Dir. = Direct fecal smear

Con.= Concentration method

M. Ziehl-Neelsen =Modified Ziehl- Neelsen

Appendix B

Data collection form for *S. stercoralis* and other helminths larvae

No.	S. code	Age	Sex	HIV status	Stool appearance	Parasitological methods used for the detection of *S. stercoralis* larvae						Remark
						Dir.	Con.	HMFP	Baer.	Char.	N. agar	

NB: Dir.= Direct fecal smear Con.= Concentration method

HMFP=Harada Mori filter paper culture

Baer. = Baermann's method

Char. = Charcoal culture method

N.agar = Nutrient agar plate culture

Appendix C

Result Report for Parasitological Examinations

Code No._____ Sex_____ Age_____

Collection center (Hospital/Health Center)_____Sub city_____

Type of specimens Stool sample Appearances _____

Date of collection_____

Ova/parasite Positive_____Negative_____

Parasites identified	Direct smear	Concentration	Culture	Modified Ziehl-Neelsen
1.				
2.				
3.				
4.				
5.				

Others

_____

_____

_____

Remark

_____

_____

_____

Date Reported _____ Signature_____

የጥናት ተሳታፊ የስምምነት ቅጽ

መለያ ቁጥር_____

የተሳታፊው ስም _____ እድሜ _____ ጾታ_____

የህክምና ባለሙያው ስም _____ቦታ_____

እኔ _____ የተባለኩት የአዲስ አበባ ነዋሪ፤ በከተማችን ውስጥ ከሚከሰቱት የአንጀት ጥገኛ ህዋሳት አንድ በሆነው "ስትሮንግሎየድስ ስተርኮራልስ" ጥናት ውስጥ አንድሳተፍ ፈቃደኛ መሆኔን እና አለመሆኔን ተጠይቂአለሁ። ይህ ጥገኛ ህዋስ ኤች አይ ቪ ባለባቸውና በሌለባቸው ሰዎች ላይ ያለውን ልዩነትን እና እሱ በመኖሩ ሊከሰቱ የሚችሉ ምልክቶችን ለማወቅና አስፈላጊውን ጥንቃቄ ለመውሰድ አንደሚያግዝ በቅድሚያ ተነግሮኛል።

ለዚህ ጥናት ያገለግል ዘንድ በፈቃደኝነት ላይ የተመሠረተ የኤች አይ ቪ መርመራ በሚሰጡ ማዕከላት (VCTC) በፊላገቴ ለመመርመር በመጣሁበት ወቅት ከ20-30 ግራም የሚሆን ሰገራ ለመስጠት ተስማምቻለሁ። ከፍለግሁ ውጤቱን መስማትና ከዚያ በሃላ የሚደረገውን የህክምና ክትትል በአቅራቢያየ ባለ የጤና ተቋም አገኛለሁ። ከልፈለግሁ ደግሞ በሁለቱም ለመሳተፍ አልገደድም። በተጨማሪም ማንኛውም አይነት ውጤት በሚሰጠር አንደሚያገ ተነግሮኛል።

በዚህ ጥናት ምንም አይነት የገንዘብ ጥቅም የማላገኝ መሆኔንና ከመፈረሜ በፊት አንዳስብበት በቂ ጊዜ ተሰጥቶኝ አስቤበት የተስማማሁ መሆኔን በፊርማየ ለማረጋገጥ አወዳለሁ።

የተሳታፊው ስም _____ ፊርማ _____ ቀን _____

የአጥኝው ስም _____ ፊርማ _____ ቀን _____

የምስክር ስም _____ ፊርማ _____ ቀን _____