DIARRHOEA ASSOCIATED PARASITIC INFECTIOUS AGENTS IN PATIENTS WITH AIDS IN SELECTED ADDIS ABABA HOSPITALS

A THESIS SUBMITTED

TO

THE SCHOOL OF GRADUATE STUDIES

ADDIS ABABA UNIVERSITY

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE DEGREE OF

MASTERS OF SCIENCE IN BIOLOGY

BY

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MAY, 1996

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ACKNOWLEDGMENT

I am extremely grateful to the National AIDS control program (NACP) for providing me all the reagents and equipment required in the study and the Ethiopian Health and Nutrition Research Institute (EHNRI) for allowing me to work in the laboratories which has an attractive academic and social atmosphere.

I am also very grateful to my research advisor, Dr. Beyene Petros for his excellent guidance, encouragement, reading the manuscripts and giving valuable comments avoiding countless errors and confusing statements. I would like to extend my thanks to my co-advisor, Ato Tilahun W/michael for providing early stimulus and advice, encouragement and valuable comments in the course of the study.

The cooperation of Police, Armed Force General Hospital, Zauditu and Tikur Anbessa Hospitals in the collection of samples is greatly acknowledged. I gratefully acknowledge Ato Hussien Mohammed for his excellent technical assistance in the Parasitology laboratory. My thanks also goes to Ato Adane Bitew and Belete Tegbaru for their cooperation and discussion on the course of the study.

Thanks are due to the staff members of National Referral Laboratory for AIDS (NRLA), Ethio-Netherlands AIDS Research Project (ENARP) and Parasitology laboratory at the EHNRI.

I am also grateful to the school of graduate studies for financial support for this research.

ABSTRACT

The study was aimed at determining the prevalence of gastro-intestinal parasites in AIDS patients with chronic diarrhoea. This prevalence was compared with that in two control groups: HIV seronegative diarrhoeal patients and AIDS patients (HIV seropositive individuals) without diarrhoea. Stool specimens were screened for parasite infection from clinically diagnosed hospitalized AIDS patients in seven hospitals in Addis Ababa. Of 147 AIDS patients with chronic diarrhoea, 74 (50.3%) were infected with one or more kind of parasites. Out of the 56 non-AIDS (seronegative) diarrhoeal patients 23 (41.1%) and out of the 43 non-diarrhoeal (seropositive) patients 18 (41.9%) were infected by a variety of intestinal protozoa and helminths. The parasites detected in AIDS patients only were *Crptosporidium* spp, *Isospora* spp, and *Blastocystis* spp, *Ascaris lumbricoides*, *Giardia lamblia*, *Strongyloides stercoralis*, *Taenia saginata*, *Trichuris trichura*, *Entamoeba histolytica*, and Hook worm spp. Among the intestinal parasites, *Cryptosporidium* spp was exclusively associated with diarrhoeal AIDS patients ($P < 0.001$). None of the other parasites were significantly associated with AIDS patients. The high proportion of the study subjects who had diarrhoea in the absence of identifiable parasitic infections suggests that other infectious agents (eg. Bacteria and Virus) or mechanisms other than infectious agents are responsible for the diarrhoea.

INTRODUCTION

Acquired immunodeficiency syndrome(AIDS) was first reported in the last decade (Green, 1993) and since that time has been spreading in a pandemic manner throughout the world. Usually it clinically manifests itself in the form of opportunistic infections and/or neoplasia. After a course of some months to ten years, approximately 100% of the cases have lethal outcome (Curran, *et al.*, 1985; Piot, et al., 1988).

AIDS has now been responsible for the loss of many lives and remains a formidable threat for many more. Its social, economic and political significance is unprecedented. It is now more than a decade since AIDS sprang into medical and public awareness as a fatal disease of the immune system (Green, 1993; Negele and Kaschawa, 1987). Thus AIDS pandemic has reached catastrophic proportions. Globally, World Health Organization (WHO) had estimated the actual number of infected individuals by the human immunodeficiency virus (HIV) may have been 17 million by mid 1992 (WHO, 1995).

Although antiviral and other supportive medications may modestly prolong the lives of those infected, the great majority of them will eventually have developed full-blown AIDS within six months to 7 years (Gallo, 1987). Reasonable estimates of the number of cases of HIV infection likely to

occur by the year 2000 ranges from 40 million to more than 110 million, which is about 2% of the world's present population (Green, 1993 ; WHO, 1995). In Ethiopia, a total of 19,433 AIDS cases have been reported to the Ministry Of Health (MOH) since the first AIDS reported case, i.e. on January, 1986 (MOH, 1996).

The human immunodeficiency virus (HIV), the etiological agent of AIDS, is a member of the family Retrovirridae and subfamily lentivirus. Retroviruses are RNA viruses and information flow is from RNA to DNA and then back to RNA and finally to proteins (Gallo, 1986; Levy, *et. al*.1984). These viruses have a unique enzyme called reverse transcriptase (RT) that can transcribe DNA copies of the RNA viral genome. This is the reversal of the normal information flow and hence, the name retrovirus.

Most clinical manifestation of HIV infection result either from the reactivation of preexisting latent pathogens as the individual becomes immunosuppressed or is exposed to locally predominant pathogens. Consequently, clinical presentations of AIDS and the pathogens responsible for it in different geographical areas, are the reflection of the differing prevalence of endogenous infections (Hunter, *et al*., 1992).

HIV infection is world wide with the patterns of the infection varying depending on the region involved. In developing countries, the prevalence of HIV infection is low in rural areas except where civil war, break up of families, and movements of the population have facilitated the spread of the virus into rural areas (Cheesbrough, 1990).

HIV has been isolated from blood, semen, saliva, tear, breast milk, vaginal/cervical secretions, urine, cerebrospinal fluid (CSF) and brain tissues (Groopman, *et al.*,1985). Among these, blood and semen are to be the most important sources of transmission.

The modes of transmission include: sexual contact, direct contact with contaminated blood and blood products, and transfer of the virus via breast milk or across the placenta from infected mothers to their offspring (Ho, *et al.*,1984; levy, *et al.*, 1985). On the other hand there is no evidence of transmission of HIV through kissing, touch, breathing, sharing of eating and drinking utensils, sharing of shawers and towels and the like. There also is no evidence for arthropod transmission.

The genome of HIV contains two types of genes: the structural genes and the regulatory genes. The structural genes are responsible for the direction and synthesis of proteins (P)

and glycoproteins (gp) that will give the virus its physical characteristics, that is, shape, size, structural integrity, compartmentalization, etc. The regulatory genes are responsible for the subsequent production of proteins that can affect the activities of viral components, or can specifically turn other genes on and off. Among other activities, the regulatory genes are responsible for the profound pathogenicity of HIV (Constantine, *et al.*, 1992; Oroszlan and Luftig, 1993).

Structurally, HIV, is composed of two major parts, an outer envelope and an inner core (Fig 1). The external envelope components are embedded in the lipid matrix of the membrane and are involved in the binding of the virus to the host cell during infection. The outmost viral components are arranged as spikes or knobs and are extended from the lipid membrane. These external spikes are proteins with sugar molecules and are therefore glycoproteins (gp).The core components of the virus are located internal to the outer membrane and are bound by a protein coat encompassing two identical copies of the nucleic RNA genome. Also within the core are three viral enzymes: RT, integrase, and protease. These enzymes are responsible for transcription, integration of the virus into the host cells, and cleavage of other proteins, respectively (Earl, *et. al.*, 1990).

Only after months to years does the virus so significantly damage the immune system that opportunistic malignancies and infections appear(Mills and Masur,1990). The damage takes place when the number of T4 lymphocytes in the blood is reduced to very low levels during the long subclinical phase of chronic infection, from about 1,000 per cubic millimeter to less than 100. Thus,it has been established that the decrease in T4 cells is responsible for the decline in immune function that happens over the same period.

Symptoms associated with AIDS are diverse and vary because of the involvement of many organ systems. The most frequently reported causes of death in AIDS patients are infectious diseases caused by the ultimate destruction of the immune cells leading to the manifestation of immune incompetence. The clinical symptoms of AIDS are usually caused by opportunistic pathogens, organisms that rarely cause disease in an immunocompetent individual. At the time the virus is activated and symptoms appear, a precipitous decline usually occurs in the number of T cells. A direct correlation between viral burden, decreasing T cells, and disease progression has been demonstrated (Cooper, *et al.*, 1985). AIDS is considered the diagnosis for patients who are HIV infected and have evidence of severe immunosuppression, or a variety of opportunistic infections.

According to CDC (1987), some diseases are so unlikely in the absence of AIDS. They also have been adopted as circumstantial evidence of the syndrome. Such indicator diseases include candidiasis of the oesophagus, trachea, bronchi, or lungs; extrapulmonary cryptococosis; protracted cryptosporidiosis with diarrhoea; Kaposi's sarcoma in patients less than 60 years of age; lymphoid interstitial pneumonia or pulmonary lymphoid hyperplasia *Pneumocystis carinii* pneumonia; and disseminated ***Mycobacterium avium*** complex. Several factors specifically affecting the CNS are cytomegalovirus infection, primary brain lymphoma, cerebral toxoplasmosis, and progressive multifocal leukoencephalopathy. Additional typical problems include intractable emaciation, recurrent non-typhoidal *Salmonella* septicemia, extrapulmonary tuberculosis, non-tuberculous mycobacterial diseases, several types of non-Hodgkin's lymphoma, protracted isosporiasis with diarrhoea, disseminated histoplasmosis, disseminated coccidiomycosis, and a variety of recurrent bacterial infections in children.

If patients exhibit any of these problems, they probably have AIDS unless they had a long-term immunosuppressant therapy of some sort within 3 months of developing the indicator diseases. They may have also another condition that will necessarily interfere with immune function. The diagnosis of AIDS is favored if an indictor disease develops along with

laboratory evidence of HIV infection (CDC, 1987).

Many of the infections and tumors that have to be diagnosed in order to diagnose AIDS are difficult to prove in places where facilities for investigation are limited. In order to improve diagnosis and AIDS surveillance in Africa, a WHO workshop in 1985 conceived a clinical case definition for AIDS in Africa. Based on the decision of this workshop chronic diarrhoea is one of the major clinical case definitions of AIDS.

Opportunistic infections and reactivated latent infections seen in HIV infected persons vary from region to region according to the different types of endemic pathogens found in theses regions (cheesbrough, 1990).

BY using assays for measuring anti- HIV antibodies and HIV antigens, individuals who have been infected by the virus can be identified. Widely used techniques include the enzyme linked immunosorbent assay (ELISA) (Constantine *et al.*, 1992) and the Western blot method (Barin *et al.*, 1985). Radioimmuno- precipitation techniques of varying complexity have also been useful in identifying individuals who have been infected by HIV.

The antibodies measured are primarily those formed against the viral envelope glycoproteins (Weiss, *et al.*, 1987). The presence of antibodies to envelope glycoproteins simply indicates an immune reaction to the virus and, therefore, infection by the virus.

However, that the patient has antibodies to HIV or HIV antigen retrievable from body fluids or tissues does not mean that the patient has AIDS. Individuals can carry the virus for months or years without developing AIDS (Polk, *et al.*, 1987). The earliest signs of immunodeficiency in the patient with HIV infection appear to be the declining number of CD4 T lymphocytes in the peripheral blood or an increase in the level of HIV antigens in the serum.

The fall in CD4 T lymphocytes to less than 250 cells/μl is often, but not always, reflected in a reduction in the total number of lymphocytes. According to Polk, *et al.* (1987) several techniques are available for measuring the ratio of CD4 (helper-inducer) T lymphocytes to CD8 (Suppressor cytotoxic) T lymphocytes in peripheral blood which may be as useful as detecting a fall in the absolute number of CD4 T lymphocytes. Normally the ratio of these lymphocytes population is 2.0 (CD4/CD8). A ratio of less than 0.9 to 1.0 is generally considered consistent with AIDS.

The patient's inability to resist opportunistic infections in AIDS is reflected in its inability to respond to standard tests of immune function, such as the anergy panels (Lechtenberg and Sher, 1988).

The gastrointestinal tract plays a critical role in the pathogenesis of the AIDS. After infection with HIV a complex sequence of immunologic events are initiated within the gastrointestinal mucosa, leading to suppression of mucosal immune function and impairment of nonspecific enteric defence mechanisms (Bartlett, *et al.*, 1988). The progressive decline in mucosal immune and defence mechanisms predisposes the host to gastrointestinal manifestations of HIV infection. The most common manifestation is diarrhoeal illness, which is experienced by approximately 50% of HIV infected patients in developed countries and nearly 90% of the infected patients in developing countries (Smith,1993).

The chronic malabsorption, malnutrition, and diarrhoea that occur several years later have generally been attributed to the opportunistic infections in the bowel as a result of immune deficiency. In Africa, for instance, this gastrointestinal problem has been termed "Slim disease" because of the substantial loss of weight resulting from infection persistent diarrhoea (Serwada, *et al.*, 1985).

Diarrhoea is the common clinical manifestation of HIV infection both in developing and developed countries. In tropical countries chronic diarrhoea is associated with significant weight loss and is often the presenting illness of HIV infected individuals (Dallabeta and Miotti, 1992). This diarrhoea-wasting in association with a positive HIV serological test is one of the AIDS criteria in the World Health Organization's classification (WHO,1986).

The etiologies of diarrhoeal diseases and the classification of diarrhoeal types are many. In acute Watery diarrhoea, most episodes last less than seven days. It involves the small intestine and is characterized by the frequent passage of loose watery stools without visible blood. Acute watery diarrhoea causes dehydration, base-deficit acidosis potassium depletion, and when food intake is reduced, contributes to malnutrition. Among these, dehydration is the most dangerous because it can cause decreased blood volume (hypovolemia), cardiovascular collapse,and death if not treated promptly. In developing countries, the main causes are rotaviruses, enterotoxigenic *E. coli*, *Shigella* spp., *Campylobacter jejuni*, and *Cryptosporidium* spp. In some countries *Vibro cholerae*, *Salmonella* spp. and *E. coli* are important (WHO, 1990; Geyid, 1995).

Acute dysenteric diarrhoea is a form of diarrhoea with visible blood and mucus in the faeces. The infection is mainly localized to the large intestine. Anorexia, rapid weight loss, and direct damage to the intestinal mucosa by the invasive bacteria are typical features of the disease. The most important causes of acute dysentery are *Shigella* spp., *Campylobacter jejuni* and, *Entamoeba histolytica*. Acute dysentery has a more harmful effect on the nutritional status than acute watery diarrhoea. Diarrhoeal episodes that begin with dysentery are more likely to become persistent than those with watery forms (Guerrant and Bobak,1991).

In persistent diarrhoea the episode may begin either as watery diarrhoea or as dysentery. Marked weight loss is frequent. There is no single microbial cause for persistent diarrhoea. All known enteric pathogens like *E. coli*, *Shigella* and *Cryptosporidium* spp. have been associated with persistent diarrhoea (WHO, 1990).

Irrespective of its cause, persistent diarrhoea is associated with extensive changes in the bowel mucosa, especially flattening of the villi and reduced production of disaccharidases. This causes reduced absorption of nutrients and may perpetuate the illness after the original infectious cause has been eliminated. Immunological impairment due to AIDS is one of the risk factors for persistent diarrhoea.

For parasitic diarrhoeal infections,there is no convincing evidence that their prevalence or incidence is increasing due to the HIV epidemic (Zula and Croft, 1992) . Available data on parasitic infections in patients with AIDS suggests a predominance of *Pneumocystis carinii, Toxoplasma gondii* and *Cryptosporidium* spp. However, for reasons which are unclear, parasitic infections such as *Plasmodium falciparum, Strongyloides stercoralis* and *Entamoeba histolytica*, where cell-mediated immune responses are also thought to be significant, do not appear to be opportunists of importance (Leaner and Tapper, 1984).

As many as 20% of AIDS patients in the United States and as many as 55% of patients in developing countries are infected by the protozoan parasite *Cryptosporidium* spp (smith, 1993). Although a relatively common cause of self-limiting diarrhoea in immunocomptente persons, *Cryptosporidium* spp causes debilitating diarrhoea in patients with AIDS. The diarrhoea is usually chronic, voluminous, non-bloody and watery.As a result, dehydration, electrolyte imbalance and wasting are common symptoms (Dallabetta and Miotti, 1992). Asymptomatic carriage has also been reported for *Cryptosporidium* spp.

Intestinal microsporidiosis, which occurs almost exclusively in HIV infected persons, is characterized by a chronic, watery, non-bloody diarrhoea that may be accompanied by

substantial fluid and weight loss (Orenstien, *et al.*, 1992). Although the symptoms are sporadic, they are generally indistinguishable from those of cryptosporidiosis. Microsporidians may account for as much as 50% of unexplained diarrhoea in patients with HIV infection (Orenstien, *et al.*, 1992; smith, 1993).

Enteric infection with *Isospora belli* occurs in fewer than 3% of AIDS patients in the United States but in as many as 15% of patients in developing countries such as Haiti and Zambia (DeHovitz, *et al.*, 1986; Conlon, *et al.*, 1989). Similar to Cryptosporidiosis, isosporiasis is a chronic illness characterized by profuse, non-bloody and watery diarrhoea. Other common symptoms include weight loss, nausea, and cramping abdominal pain. Steatorrhoea and occasionally eosinophilia may also be present (Smith, 1993).

Multiple parasitic opportunistic infections in patients with AIDS cause considerable problems. Firstly, there is the problem of the correct diagnosis of which organism is producing symptoms and there is the possibility of more than one organism being responsible (Gazzard, 1990).

Infections with *Mycobacteriam avium* complex are also associated with diarrhoea (Gray and Rabeneck, 1989; Horsburg, 1991). Although localized gastrointestinal involvement

occurs, enteric disease is usually associated with disseminated infection.

Salmonella spp, *Shigella flexneri* and *Campylobacter jejuni* infections occur in AIDS patients substantially and more frequently; cause a more prolonged or recurrent illness; and are more frequently associated with bacteremia and antibiotic resistance (Dworkin, *et al.*,1986; Celum, *et al.*,1987 and Blaser *et al.*,1989).

Of viral infections, Cytomegalovirus (CMV) is one of the most common and potentially serious pathogens that cause diarrhoea in HIV infected people. Among AIDS patients with colitis or enteritis, CMV has been identified in biopsies in as many as 45% of cases (Smith, *et al.*, 1988).

Adenoviruses have been identified in inflamed colon tissues of AIDS patients and in patients with chronic unexplained diarrhoea. The diarrhoea in these patients was watery, non-bloody, non-mucoid, and associated with weight loss (Janoff, *et al.*, 1991). Adenoviruses have also been detected in stools from AIDS patients without diarrhoea. However, a causal relation between adenovirus-induced histopathology and diarrhoeal illness in HIV infected patients has not been established.

In general, the incidence of diarrhoeal diseases in the World has increased greatly with AIDS and it has been a diverse etiology (Smith, 1993). In many AIDS patients, chronic diarrhoea is associated with marked weight loss that can be severely disabling In particular very low longevity rate in sub-sahara African countries has been reported for AIDS patients with diarrhoea (Atzori, *et al.*, 1993). For example, as many as 40% of AIDS patients have been reported to develop a syndrome of chronic diarrhoea and wasting which,in most cases, rapidly leads to death (Henry, *et al.*, 1986).

The cause of prolonged diarrhoea in Ethiopian AIDS patients is largely unknown since the infectious etiologies have never been systematically studied. This information is important because, an early identification of the infectious agents known to be associated with diarrhoea will allow an early medical intervention, increasing the survival time of AIDS patients. Furthermore, despite the importance of chronic diarrhoea in the longevity of AIDS patients, how often opportunistic parasites and other pathogens that cause diarrhoea and associated with AIDS, is not known in Ethiopia. In light of this, this study aims at determining the prevalence of pathogenic gastrointestinal parasitic agents in AIDS patients with chronic diarrhoea.

MATERIALS AND METHODS

1. STUDY SUBJECTS:

The study subjects were given pre-test counselling and only those who signed the informed consent were included in the study. They were first informed about the aims, methods and the anticipated benefits about the study. The subjects were also informed that they are free to withdraw consent at any time and their records and specimens will be examined by authorized persons, and the personal information on them will be treated strictly confidentially and will not be made public in any form.

Of the 246 stool samples examined in this study, 147 were from AIDS patients with chronic diarrhoea, 56 were from HIV sero- negative diarrhoeal patients (control group I) and 43 were from HIV seropositive individuals without diarrhoea (control group II).

A questionnaire relating to the history and clinical status was completed for each subject included in the study. Laboratory results and all clinical and laboratory data were identified only by a code assigned to each subject. The sample collection assistants and individuals in parasitology laboratory were blind to HIV status of the participants of

the study in the course of collecting and examining samples respectively.

The study included 147 hospitalized AIDS patients (37 females and 110 males; mean age 31 years, range 18-63 years) out of the approximately 283 AIDS hospitalized patients identified based on WHO (1986) case definition for AIDS. The patients were from 7 selected hospitals (St.Paul, Ras Desta, Zauditu Memorial, Minilik II, Tikur Anbessa,Police, and Armed Forces General Hospital) in Addis Ababa. Inclusion criteria to the study was chronic diarrhoea with the presence of 3 or more unformed stools a day for at least 30 days (WHO, 1986).

Fifty six patients with diarrhoea, but negative for HIV serology, selected from the Out Patient Department (OPD) of the hospitals were included in the study to serve as controls. Likewise, 43 non-diarrhoeal but HIV seropositive individuals, some of whom were hospitalized (n=19) and others attending the counseling program (n=24) were also included as a second control group.

2. STOOL EXAMINATION FOR PARASITES:

Stools were collected fresh and processed in the clinical parasitology laboratory in the Ethiopian Health and Nutrition Research Institute (EHNRI) within 4 hours of collection.

Specimens were examined as saline wet mounts by using normal saline and by formol-ether concentration method. In formol-ether concentration method (Cheesbrough, 1990), where approximately 2 grams of fecal specimen in 7 ml of formol saline solution in a screw cap tube was used. The suspension was passed through a sterile gauze, collecting the strained fluid in a beaker which later was transferred into a centrifuge tube. The tube was stoppered and shaken back and forth 5 times after adding about 3 ml of ether. Holding the tube over a sink the stopper was carefully removed and centrifuged immediately at medium speed (2000 rpm) for 5 minutes. Following centrifugation, the debris layer was carefully loosened by using an applicator stick and poured away. Then the entire sediment was transferred onto a slide using a sterile pasteur pipette; covered with a cover glass, and examined microscopically.

Air dried thin smears were stained by modified Ziehl-Neelsen technique (Henry, *et al.*,1986) for *Cryptosporidium* and *Isospora* oocysts. That is, the smears were fixed in methanol for 3 minutes, stained with carbol-fuchsin for 60 minutes, decolorised in 2% H_2SO_4 for one minute, washed in running tap water, counter- stained with 5% malachite green for 5 minutes, washed in running tap water, air dried and observed with immersion objectives. The sizes of the oocysts were measured (4-6 um for *Cryptosporidium* and 20 - 30 um by 10 -

19 um for *Isospora*). Microscopic examinations were done independently by two individuals and the findings reconciled. (Mersha and Tiruneh, 1992).

3. HIV SEROLOGY:

The HIV serology was performed using the enzyme linked immunosorbent assay (ELISA) (Constantine, *et al.*, 1992) and the dot (HIV SPOT) assay (Nkengasog, *et al.*, 1992) at the National Referral Laboratory for AIDS (NRLA) at EHNRI. For this a 10 ml blood sample was taken using vacutainers from each study subject and transported to NRLA to be centrifuged at 1500 rpm for 10 minutes. The serum was removed and stored at -20°C. These sera were tested for the presence of anti-HIV antibodies in two stages.

First, all sera were screened for the qualitative levels of antibodies to HIV antigen by the ELISA (Vironostika® HIV Uni-Form II) test which is a commercially available kit from Organon Tekinika (Boxtel, Holland). These kits were donated by the National AIDS Control Program (NACP), Ministry of Health. All the ELISA (Vironostika® HIV Uni-Form II) positive samples and some number of negative sera were retested by the same kind of test to rule out technical errors.

Ninety six well microtiter plates of Vironostika^{R} HIV Uni-Form II (Organon Teknika, Boxtel, Holland) with the required reagents (conjugate, substrate and washing buffer) were obtained for the qualitative determination of antibodies to HIV type 1 and/or 2 in human serum. Each microelisa well contains horseradish peroxidase (HRP)-labeled conjugate sphere of the same HIV-antigen mixture. To each well 100 µl of specimen diluent was dispensed using multichannel pipette, which dissolved the conjugate sphere. Then, 50 µl test samples and appropriate controls containing anti-HIV-1 and/or anti-HIV-2 were added, the plates covered with plate sealer and incubated in a water bath at 37°C for an hour. After incubation the wells were washed 6 times with wash phosphate buffer. Following the wash procedure 100 µl Tetramethyl-benzine (TMB) substrate was pipetted into each well and incubated at room temperature (18-25°C). Color develops which turns yellow when the reaction is stopped by adding 100 µl of 1 mol/l sulfuric acid to each well.The plates were read within 2 hours. Spectrophotometer (Titertek Multiskan MCC/340, Helsinki, Finland) was used for reading the absorbance of the solution in each well at 450 nm after blanking the reader on air.

The cutoff value is calculated according to the recommendation of the manufacturer of the test kit. A test sample was registered reactive if sample absorbance is

greater or equal to the cutoff value and a test sample was registered as non-reactive if sample absorbance is less than the cutoff value. A non-reactive result indicates that the sample tested contains neither anti-HIV-1 nor anti-HIV-2 or contains anti-HIV-I and/or anti-HIV-2 below the detectable limits of Vironostika HIV Uni-Form II. A reactive result means that the samples tested either contains anti-HIV-1 and/or anti-HIV-2 or contains a nonspecifically reacting factor. Secondly, all positive sera from the first ELISA were assayed by the HIV-SPOT test which is more rapid and specific than the Vironostika ELISA (Nkengasong et al., 1992). The test involves trapping of antibodies to HIV-1 and/or HIV-2 by the capture reagents which are adsorbed to the porous membrane. This assay is supplied with essential materials for testing and capture reagents that are recombinant protein of HIV-1, corresponding to a region overlapping the junction between the gp120 and gp41 fragment of the envelope protein, plus a highly purified peptide which corresponds to a region of the envelope transmembrane protein of HIV-2. One device, a solid support where the antigen is coated, was used for each test sample and control code number written on them for identification. According to the procedure 3 drops of reconstituted buffer (Phosphate buffer saline) was added and allowed to soak. This was followed by the addition of 1 drop of undiluted sample and the control. Then 2 drops of reconstituted liquid buffer and 2 drops of

wash solution were added. Finally, addition of 2 drops of reconstituted gold conjugate the devices were washed by using 3 drops of wash solution . Results were read within 10 minutes visually.

A distinct red spot appears on the membrane indicating positive or a clear membrane after the procedure or overall pink color indicating negative for antibodies to HIV-1 and/or HIV-2. Positive samples showed typical spots within the range of the weak positive and strong positive compared with the controls provided with the test kit.

An ELISA positive or doubtful serum was repeated by the same test before it is analyzed by the second test (HIV-SPOT). The sera which were positive twice were considered as ELISA positive and tested by HIV-SPOT. The manufacturer of the kit recommends a cut off value of an absorbance value reading of greater or equal to 0.100 plus the mean absorbance of the negative controls.

Statistical analysis was performed by using EPI-INFO to test differences between the cases and the two control groups.

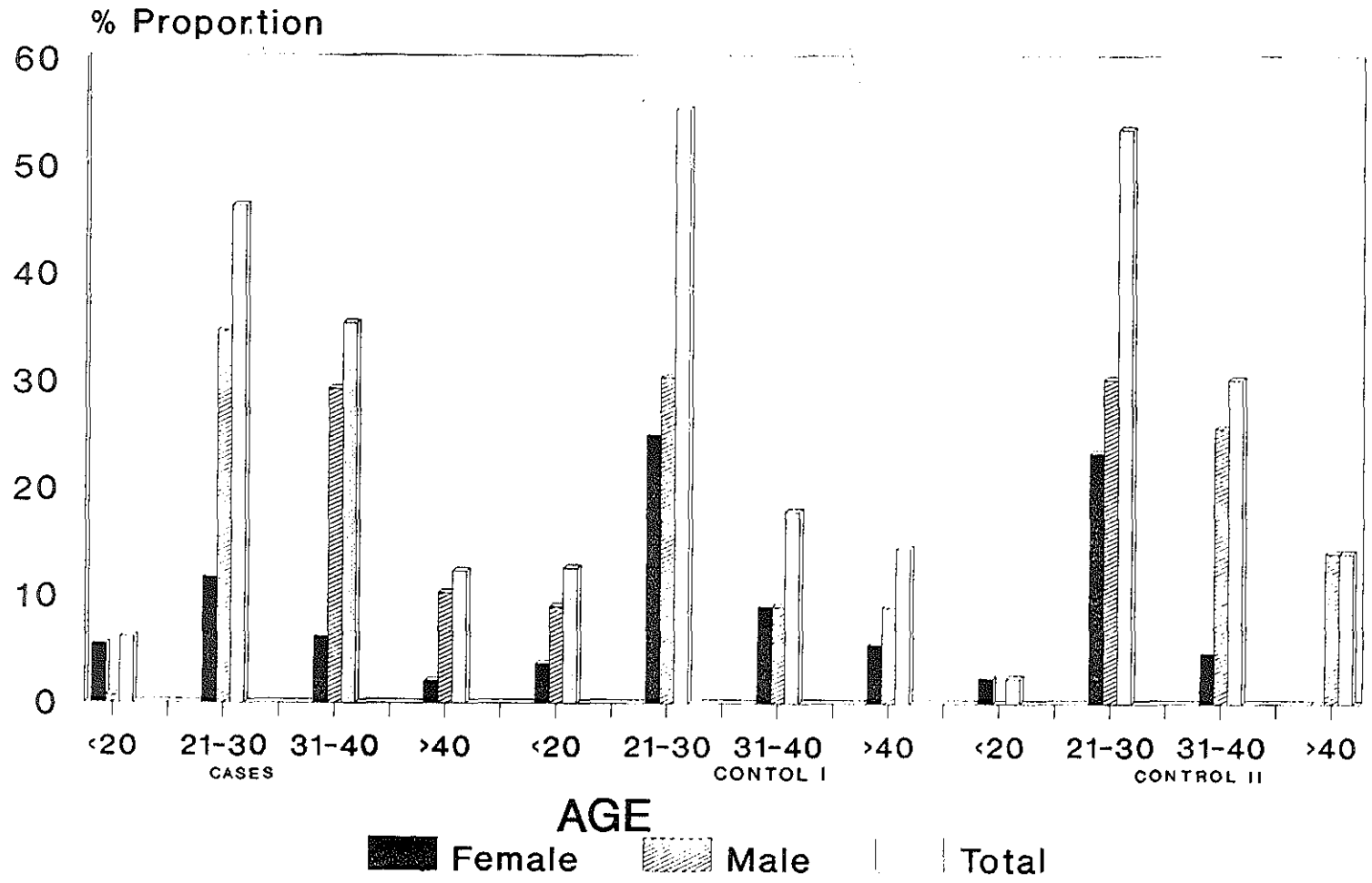
RESULTS

The age profile of the study and control subjects is similar to HIV/AIDS profile in the general population reported by the Ministry of Health (MOH, 1995) and the normal pattern observed for a typical Ethiopian populations as reported by the Central Statistical Authority (CSA) (CSA, 1988) respectively, where both young and older individuals are represented in the sample (Fig 2).

Seventy four (50.3%) of the AIDS patients with diarrhoea were parasite-positive (Table 1). The detected parasites in stools of all diarrhoeal AIDS patients in the 7 hospitals consisted of the common parasites *A. lumbricoides* (11.8%), *E.histolytica* (8.2%), *G.lamblia* (4.1%), *S.stercoralis* (3.4%), *Taenia saginata*, (3.4%) and *T.trichura* (7.5%). In addition, opportunistic parasites frequently associated with AIDS were also detected. These include, *Cryptosporidium* spp (25.9%), *Isospora* spp (1.4%) and *Blastocystis* spp (0.7%) (Table 2).

Out of 203 diarrhoeal stools collected approximately 35% of the specimen were watery diarrhoea, 25% bloody diarrhoea, and about 16% were mucoid. Associations of these types of diarrheas was observed with some kinds of parasitic infection like *Cryptosporidium* with watery, *E. histoltica* with bloody and *G.lamblia* with mucoid diarrhoea. In parasite-negative

FIG 2. Age and Sex distribution among
cases and the control groups



stools 13(12.5%) samples of AIDS patients and also 4 (16.6%) samples of the seronegative diarrhoeal patients had many cells and red blood cells during the microscopic observation.

TABLE 1: Parasites detected in AIDS patients with diarrhoea from 7 hospitals in Addis Ababa (n= 147).

Parasite species	**Number infected**	**Per cent infected**
S.stercoralis	5	3.4%
G.lamblia	6	4.1%
A.lumbricoides	17	11.8%
T.trichura	11	7.5%
Taenia saginata	5	3.4%
E.histolytica	12	8.2%
Cryptosporidium spp	38	25.9%
Isospora spp	2	1.4%
Blastocystis spp	1	0.7%
Total infected patients	74	50.3%

The intestinal parasites detected from the seronegative diarrhoeal patients are depicted on Table 2. *A.lumbricoides* (21.4%), *T.trichura* (12.5%), Hook worm spp (3.6%), *Taenia saginata* (3.4%), *S.mansoni* (1.8%), *E.histolytica* (5.4%), *S.stercoralis* (1.8%) and *G.lamblia* (3.6%) were the common parasites detected. Among all the stool specimen examined from diarrhoeal patients that were negative for HIV serology, a total of 23 (41.1%) were positive for one or more parasites. There were two diarrhoeal non-AIDS patients who tested positive for HIV antibodies during the initial screening to select the proper control group.

TABLE 2: Parasites detected in HIV seronegative diarrhoeal OPD patients from 7 Hospitals in Addis Ababa (n = 5 6) .

Parasite species	Number infected	per cent infected
S.stercoralis	1	1.8%
G.lamblia	2	3.6%
A.lumbricoides	12	21.4%
T.trichura	7	12.5%
Taenia saginata	2	3.6%
E.histolytica (trophozoite)	3	5.4%
S.mansoni	1	1.8%
Hook worm spp	2	3.6%
Total infected patients	23	41.1%

TABLE 3: Parasites detected in HIV seropositive individuals without diarrhoea from 7 Hospitals Addis Ababa (n= 43).

Parasite species	HIV-positve/ AIDS(n=19) No.(%)	HIV positive (n=24) No.(%)
G. lamblia	1(5.3)	1(4.2)
A. lumbricoides	3(15.9)	4(16.7)
T. trichura	2(10.6)	4(16.7)
Taenia saginata	2(10.6)	1(4.2)
E. histolytica	1(5.3)	2(8.4)
Isospora spp	1(5.3)	0
Blastocystis spp	0	1(4.2)
S. mansoni	0	1(4.2)
Hook worm spp	0	2(8.4)
Total infected patients	8(42.1)	10(41.7)

The common intestinal parasites *A.lumbricoides* (32.6%), *T. trichura* (27.3%), *Taenia saginata* (14.8%), *G.lamblia* (9.5%), *E.histolytica* (13.7%), Hook worm spp (8.2%), and *S.mansoni* (4.2%) were detected among the HIV seropositive non-diarrhoeal subjects. In addition, the opportunistic parasites, *Isospora* spp (5.3%) and *Blastocystis* spp (4.2%) were also recorded. Out of the 19 samples collected from hospitalized non-diarrhoeal AIDS patients, 11 were negative for parasites, while in 8 of them one or more parasitic infections were detected. One infection with *Isospora* spp was also observed (Table 2).

The chi-square test performed to assess differences between the prevalence of each parasite in the cases and the controls showed *Cryptosporidium* spp to be highly prevalent in AIDS patients with diarrhoea, and the difference was highly significant ($P < 0.001$) (Table 4).

The only parasite significantly prevalent in the cases was *Cryptosporidium* spp ($P < 0.001$) and no significant difference was observed between the cases and controls with regards to other common parasites. Although significance cannot be established, *Isospora* spp and *Blastocystis* spp are only associated with HIV/AIDS positivity (Table 4).

TABLE 4: Comparison of parasite detection in AIDS patients with diarrhoea (Cases), HIV seronegative diarrhoeal patients (Control I) and HIV seropositive individuals without diarrhoea (Control II).

Parasites Species	**Cases (n=147)**	**Control I (n=56)**	**Control II (n=43)**
S.stercorlais	5(3.4%)	1(1.8%)	0
G.lamblia	6(4.1%)	2(3.6%)	2(4.6%)
A.lumbricoides	17(11.8%)	12(21.4%)	7(16.3%)
T.trichura	11(7.5%)	7(12.5%)	6(14.0%)
Taenia saginata	4(3.4%)	2(3.6%)	3(7.0%)
E.histolytica	12(8.2%)	3(5.4%)	3(7.0%)
Cryptosporidium spp	38(25.9%)**	0	0
Isospora spp	2(1.4%)	0	1(2.4%)
Blastocystis spp	1(0.7%)	0	1(2.4%)
S.mansoni	0	1(1.8%)	1(2.4%)
Hook worm spp	0	2(3.6%)	2(4.6%)
Total infected	74(50.3%)	23(41.1%)	18(41.9%)

**: $P < 0.001$

TABLE 5: Single parasite infection in cases and the controls by species, number and per cent.

Parasites	Cases NO.(%)	Control I No.(%)	Control II NO.(%)
S.stronyloides	1(1.8%)	1(5.9%)	0
G.lamblia	3(5.6%)	0	2(20%)
A.lumbricoides	6(11.1%)	10(58.8%)*	2(20%)
T.trichura	5(9.1%)	2(11.8%)	2(20%)
Taenia saginata	2(3.7%)	0	0
E.histolytica	10(18.5%)	3(17.6%)	2(20%)
Cryptosporidium spp	25(46.3%)**	0	0
Isospora spp	1(1.9%)	0	1(10%)
Blastocystis spp	1(1.9%)	0	1(10%)
Hookworm spp	0	1(5.9%)	0
Total infection	54(73.0%)	16(69.6%)	10(55.6%)

*: $P < 0.05$

**: $P < 0.001$

TABLE 6: Double parasite infections of the cases and controls by species, number and per cent.

Parasites	Cases No.(%)	Control I No.(%)	Control II No.(%)
S.stercoralis & Cryptosporidium spp	2(13.3)	0	0
G.lamblia & A.lumbricoides	1(6.7)	0	0
G.lamblia & T.trichura	0	2(28.6)	0
G.lamblia & E.histolytica	1(6.7)	0	0
A.lumbricoides & T.trichura	2(13.3)	1(14.4)	1(12.5)
A.lumbricoides & Taenia saginata	1(6.7)	1(14.3)	2(25)
A.lumbricoides & E.hisyolytica	0	0	1(12.5)
A.lumbricoides & Cyptosporidium spp	4(26.7)	0	0
A.lumbricoides & hookworm spp	0	1(14.3)	1(12.5)
T.trichura & Taenia saginata	0	1(14.3)	1(12.5)
T.trichura & Cyptosporidium spp	1(6.7)	0	0
T.trichura & Isospora spp	1(6.7)	0	0
T.trichura & S.mansoni	0	1(14.3)	1(12.5)
T.trichura & Hookworm spp	0	0	1(12.5)
T.saginata & Cryptosporidium spp	1(6.7)	0	0
E.histolytica & Crytosporidium spp	1(6.7)	0	0
Total infection	15(18.9)	7(30.4)	8(44.4)

TABLE 7: Distribution of single and multiple parasitic infections in patients with parasitic infections diagnosed.

Parasites detected in the study subjects.

Subjects	One No.(%)	Two No.(%)	Three No.(%)	Four No.(%)
AIDS patients (n =74)	54(73.0)	15(20.3)	4(5.4)	1(1.4)
Control group I (n = 23)	16(69.6)	7(30.4)	0	0
Control group II (n = 18)	10(55.6)	8(44.4)	0	0

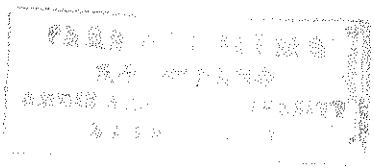


Table 7 shows the prevalence of single and multiple parasitic infections in the case and control groups. The control groups had single and double infections only. Whereas, in diarrhoeal AIDS patients the multiple infection goes up to four species of parasites where patients with 1 species make up 73%, with 2 species 20.3%, with 3 species 5.4%, and with 4 species 1.4%.

In a single parasite infection *Cryptosporidium* spp was highly associated with diarrhoeal AIDS patients. Whereas, *Ascaris* was associated with non-AIDS diarrhoea and no difference was observed between the cases and the controls with regards to other parasites (Table 5). More number of double parasite infections was detected in cases than the controls and also more number of *Cryptosporidium* spp double infection associated with other parsites in the cases (Table 6).

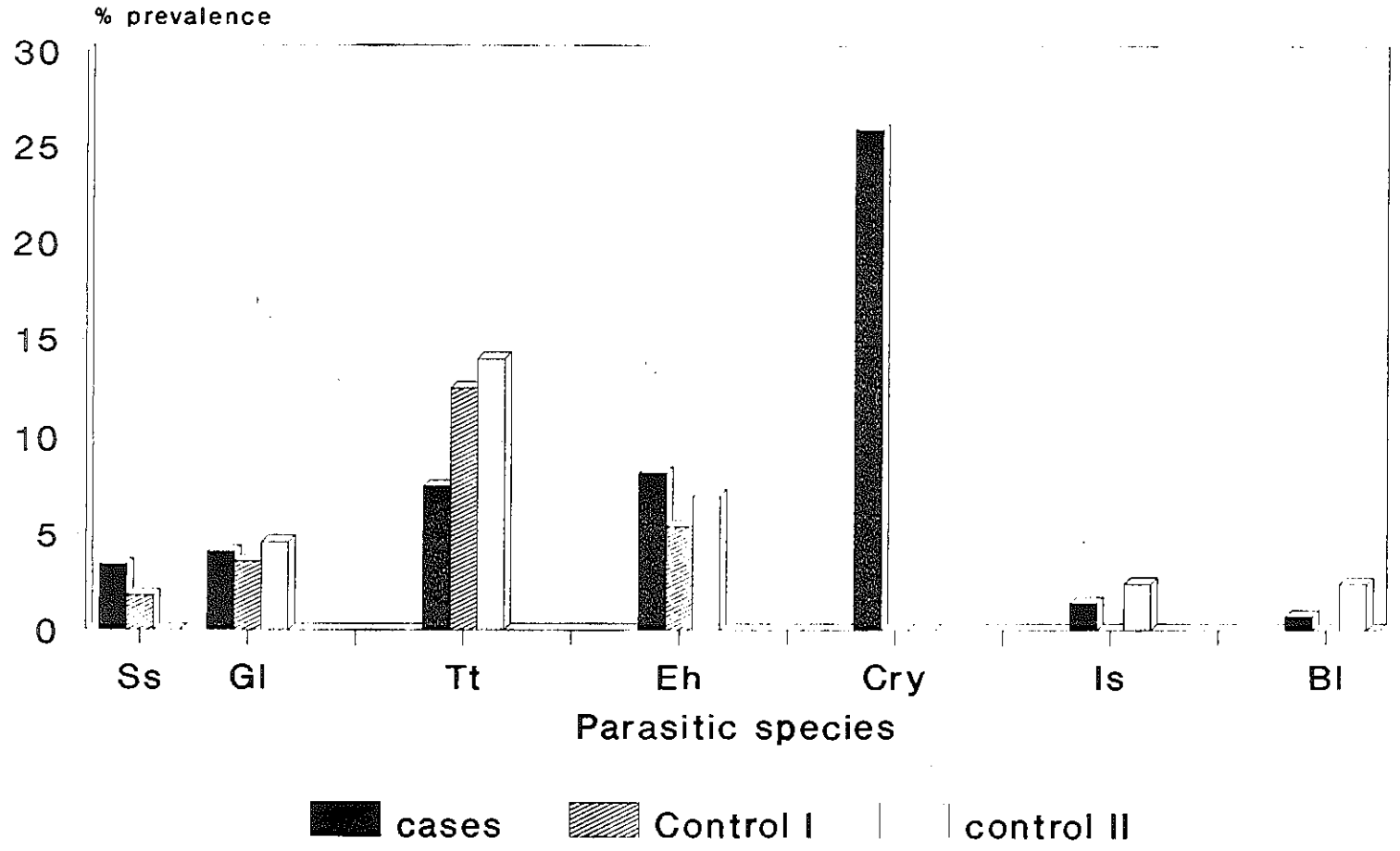
In the four species parasite infection (1.4%) *S.stercoralis*, *Ascaris* and *Trichuris* were found together with *Cryptosporidium*. *Giardia* and *Trichuris* were observed with *Cryptosporidium* in three species parasite infections. In the two control groups there were only single and double parasitic infections. In the controls groups,infections with two species of parasites were mainly observed between the two more prevalent parasites, *Ascaris* and *Trichuris*, and the remaining common intestinal parasites.

High AIDS prevalence ($P < 0.001$) associated with *Cryptosporidium* spp prevalence was encountered in the young age group (Fig 2; Fig 4). *Isospora* spp and *Blastocystis* spp infections were both equally prevalent ($P < 0.001$) in males and females while *Cryptosporidium* spp infection was more prevalent in males than in females (Fig 5).

S.stercoralis and *G.lamblia* were more frequent in the young age group (21-30) while *E.histolytica* infection was observed with similar prevalence among all age groups. But *T.trichura* was highly prevalent in the younger age group, 21-30 years (Fig 4).

All 147 serum samples from clinically proven AIDS patients with diarrhoea and the non-diarrhoeal patients that constituted group II controls tested positive for anti-HIV antibodies. Of the 58 serum samples collected from diarrhoeal patients (group I controls), only two tested positive for anti- HIV antibodies. The optical density reading of almost all the positive samples showed a value ranging from 0.457 to 2.673. One of the two HIV positive diarrhoeal patients was negative for parasites while the second patient was infected with *E.histolytica* and *A.lumbricoides*). These 2 subjects were excluded from the study and their cases reported to the managing hospitals.

FIG 3: Percentage prevalence versus parasite spp that are usually associated with diarrhoea



Ss= S. stercoralis, Gl= G.lamblia, Tt= T.trichura Eh= E.histolytica

Cry=Cryptosporidium sp., Is=Isospora sp., Bl=Blastocystis sp

FIG 4. Percentage parasite occurrence
with age groups in the cases & controls

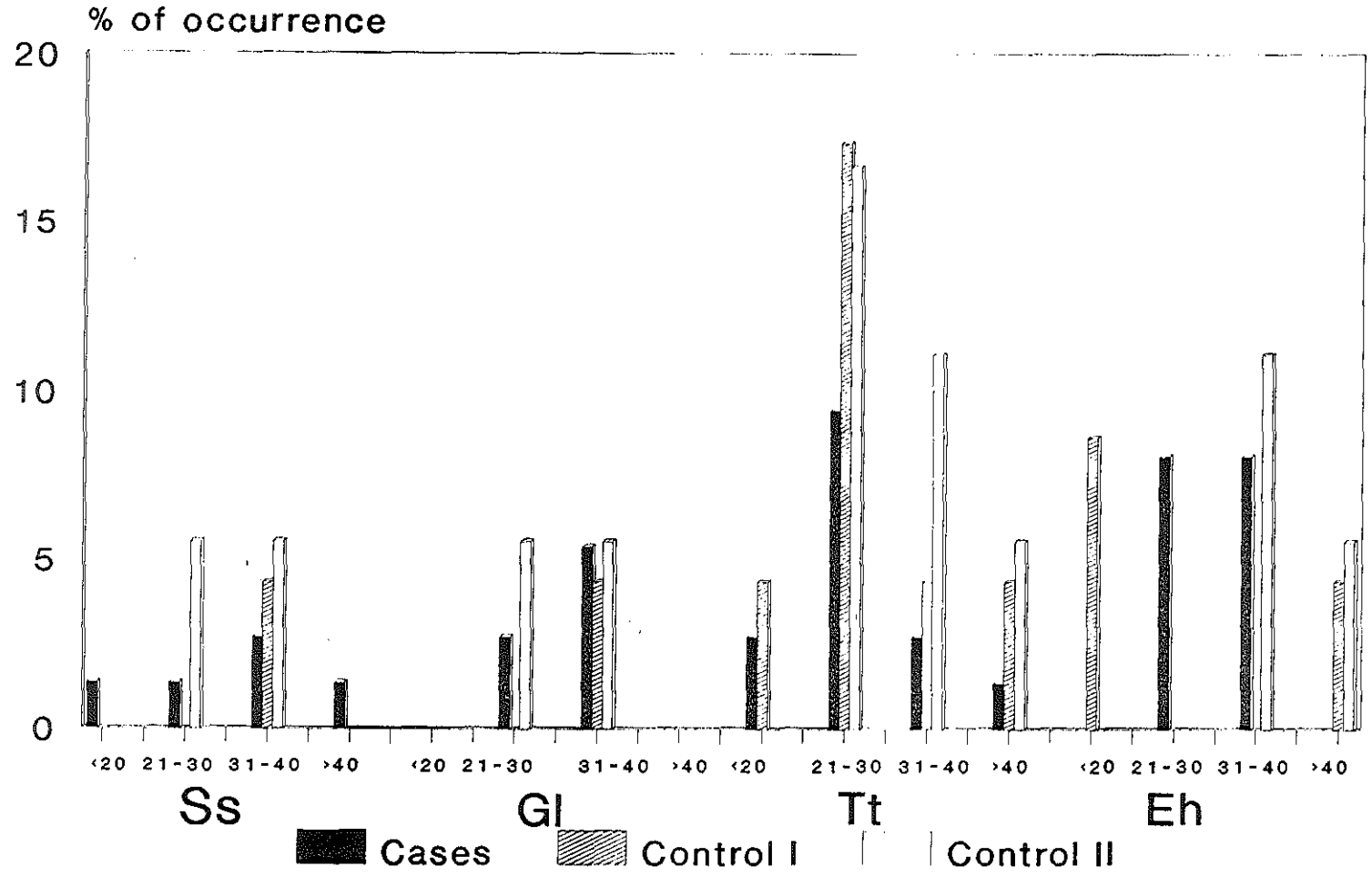


Fig 4. Continued

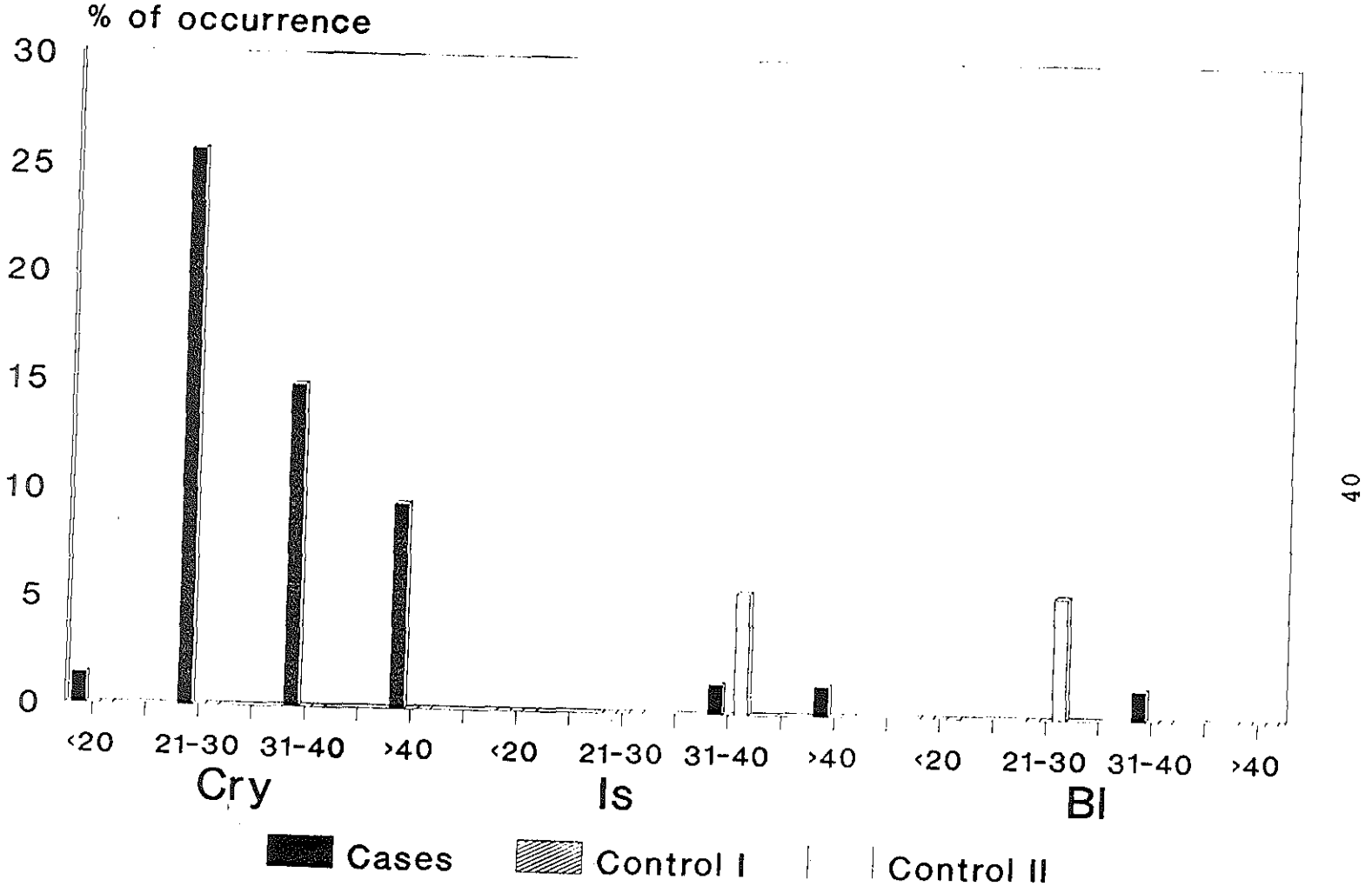
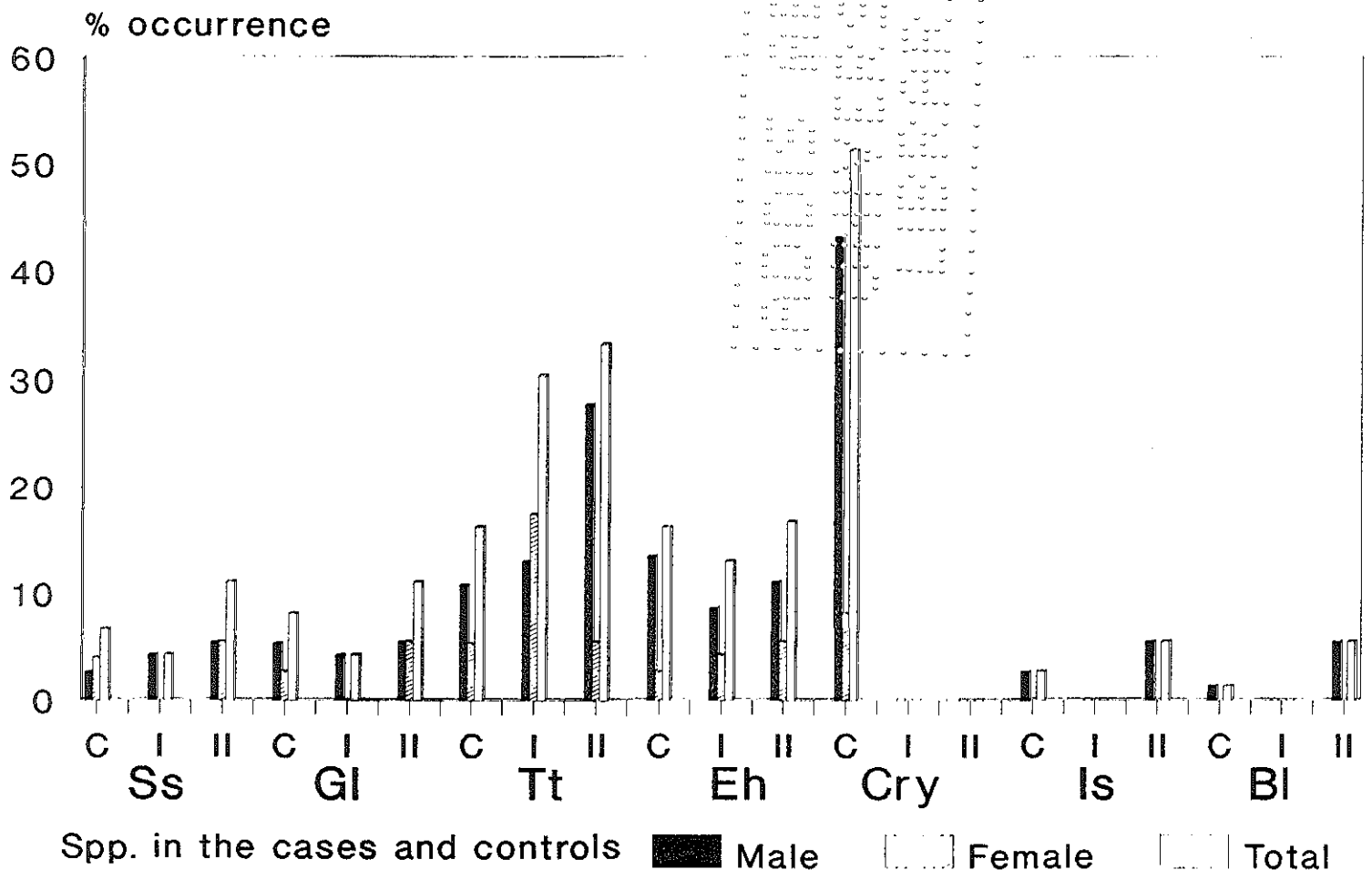


FIG 5. Percentage parasite occurrence
by sex in the cases and controls



DISCUSSION

Diarrhoea remains a major clinical problem for AIDS patients. The emergence of new opportunistic agents for which there is no effective treatment, and the enlarging pattern of drug resistance of many enteric pathogens continue to be the challenges in managing AIDS patients. In light of this, better understanding of HIV-induced mucosal immuno-suppression, sound clinical judgement, careful diagnostic evaluation, development of newer antimicrobial agents, and judicious patient management are the accepted approaches to face the challenge (Perre, 1995).

Among intestinal parasites, the coccidian parasites *Cryptosporidium* spp and *Isospora belli* appear to be the opportunistic parasites frequently encountered among adult HIV positive (AIDS) patients with chronic diarrhoea in the USA and Europe (Smith, 1993).

Previous studies from Africa on intestinal parasitic infections in patients with AIDS and chronic diarrhoea (Hunter, *et al.*, 1992; Dallabetta and Miotti, 1992; Khunmalo-Ngwenya, *et al.*, 1994) had shown varying detection rates of *I.belli* and *Cryptosporidium* spp. As in the USA and Europe, these parasites have been more significantly associated with AIDS in Africa than the more common intestinal parasites.

The results of the present study has demonstrated a similar situation. For example, the studies conducted on cryptosporidiosis in AIDS patients with chronic diarrhoea in Zaire, Uganda, Haiti, Zambia (Dallabetta and Miotti, 1992), and Tanzania (Atzori, *et al.*, 1993) had revealed prevalence rates of 22%, 48%, 42%, 32%, and 42%, respectively. The 26% prevalence determined in our study for the same parasite, is comparable with what was reported from Zaire and Zambia.

On the whole, the findings of our study clearly indicates that AIDS patients and HIV positive individuals, diarrhoeal or non-diarrhoeal, in Addis Ababa hospitals, do not have a greater overall intestinal parasitic load than diarrhoeal patients without AIDS. As would be expected, associations between AIDS, the common parasitic infections and chronic diarrhoea were not evident. But *Cryptosporidium,* a well established opportunistic parasite was found to be highly prevalent in AIDS patients with diarrhoea (Fig 3).

Although there are other important causes of diarrhoea, such as viral and bacterial infections, this study considered only the parasitic infections for which the diagnostic techniques are readily available. Due to the problems associated with supportive antibiotic treatment the AIDS patients have been receiving in the hospitals, determination of bacterial diarrhoeal agents that was initially designed in the study

was interrupted after trying to analyze more than 30 samples.

Diagnosed cases of isosporiasis (1.4%) were considerably fewer (Fig 3) in our study than what was reported for other African countries like Zaire (7%), Uganda (13%) and Zambia (16%) and for Haiti (12%) (Dallabetta and Miotti, 1992). This could possibly be either due to their more sensitive detection method (i.e.taking rectal swab and stool sample at the same time) or is a reflection of low prevalence in our study population. This needs to be established with further studies.

Blastocystosis, is ordinarily known to be endemic to tropical and subtropical countries. However, it has also been reported from immunocompetent individuals from the United States and Europe. In recent years, however, *Blstocystis spp* has been reported as the causative agent of diarrhoea in a number of cases in patients with AIDS (Garavelli, *et al.*, 1990). In this study, one case each was detected in an AIDS patient with diarrhoea and in an HIV seropositive individual, but none in HIV seronegative diarrhoeal patients (Fig 3). The finding suggests that *Blastocystis* spp may be associated with HIV infection and AIDS. It will be necessary to conduct further study to determine the importance of this parasite in relation to HIV/AIDS in Ethiopia.

The parasites *E.histolytica*, *G.lamblia*, and *S.stercoralis* were not more associated with HIV infected patients than with the seronegative population. Consequently, their presence in people at risk for AIDS may not be predictive of HIV infection. For giardiasis, it has been shown that acute antibody response to it is depressed in AIDS patients (Janoff, *et al.*, 1988 and Quinn, 1992) implying that the role of anti-*Giardia* antibodies is possibly immunopathological (Sullivan *et al.*,1987) and hence the immunosuppression of it would not exacerbate giardiasis. On the other hand, virtual restriction of infections with *Cryptosporidium* spp, *Isospora* spp and *Blastocystis* spp to AIDS/HIV seropositive individuals suggests the opportunistic relationship of these pathogens to the immunodeppressed status of the study subjects.

When comparing the parasitological findings of AIDS patients with HIV seronegative diarrhoeal patients and HIV seropositive patients without diarrhoea, *Cryptosporidium* spp was observed only in AIDS patients with chronic diarrhoea. However,the situation with *I. belli* and *Blastocystis* spp was different in that they were detected both in some AIDS patients with diarrhoea and some HIV seropositive individuals without diarrhoea. Therefore,since the present information is limited, it is an aspect that needs further investigation to see if theses parasites could serve as indicators of HIV/AIDS. *S. stercoralis* was found in similar percentage in

both groups. Similarly *G.lamblia*, *E.histolytica*, *Taenia saginata*, *A lumbricoides*, and *T.trichura* were detected in both groups. Likewise, Hookworm spp and *Schistosoma mansoni* were only detected in the stool of seronegative diarrhoeal patients and HIV seropositive individuals without diarrhoea.

This study showed *Ascaris* to be a more common infection both in diarrhoeal AIDS patients and the control groups. In particular, it was more prevalent in HIV seronegative diarrhoeal patients, and the difference from the other control group was statistically significant ($P < 0.05$). This probably shows the association of *Ascaris* with diarrhoea more than any other factor; an observation atypical of ascariasis. It also was noticed that seventy five per cent of those infected by *Ascaris* were females suggesting the probability of mothers being easily contaminated while handling their infected children. In the current changing trend of hitherto known host-parasite relationship with the advent of immunosuppressive phenomena, it will be interesting to closely look into the atypical observation encountered in ascariasis and diarrhoea.

Lucas (1990) had reported that for reasons which are unclear, parasites such as *S.stercoralis* and *E.histolytica* where cell-mediated immune responses are thought to be significant, do not appear to be opportunists of importance. Although

strongyloidiasis and AIDS often coexist in regions of high endemicity such as central Africa, only few cases of disseminated disease in people infected with the HIV have been reported (Torres, *et al.*,1993). On the other hand, most confirmed cases of *Strongyloides* infection reported so far in association with AIDS occurred in the United States, where prevalence of helminth parasites is comparatively low. Since concurrent cases of *S.stercoralis* infection and AIDS in developing countries are presumed to happen in remote or isolated areas with poor diagnostic and reporting facilities, gross undereporting and/or frequent misdiagnosis may indeed occur (Gompels et al., 1991). In the present study *S.stercoralis* infections were not associated with HIV infection. However, since the diagnostic method applied was not specific for the detection of *S. stercoralis,* a conclusive statement cannot be made.

Ascaris, *E.histolytica*, *G. lamblia*, *Trichuris*, *S.stercoralis* and other helminths were identified in up to 12-21 % in AIDS patients and the controls. Infection with these common parasites is a reflection of poor environmental hygiene which is common to both AIDS and non-AIDS patients in the endemic tropical and subtropical countries.

Studies conducted in Addis Ababa by Lemma, *et al.*, (1968) and Taticheff, *et al.*, (1981) on parasitological survey of Addis

Ababa school children and pre-school children, respectively showed 73% of the children to harbor one or more parasites. Fifty-five per cent of the examined children showed *Ascaris*, 15% *Trichuris* and about 9% *Giardia*. The other parasites found were in much smaller percentages. Although our findings in the adult population studied, showed lower prevalence rates particularly for *Ascaris* and *Trichuris*, the rates for other parasites in Addis Ababa and out of Addis Ababa are comparable to that reported by Kloos and Tesfa-Yohannes (1988).

The high proportion of AIDS patients who had diarrhoea in the absence of identifiable parasite infections strongly indicates the existence of other diarrhoeagenic agents or mechanisms. The detection of these will require a more comprehensive and better controlled studies. The newly recognized organisms, such as *Enterocytozoon bieneusi* (Microsporidian) which have been reported from American AIDS patients (Orenstein,*et al.*, 1990), may also have an important role in Ethiopian AIDS patients.

Recent studies have now demonstrated the presence of HIV itself in the bowel mucosa of patients with gastrointestinal disorders as well as in asymptomatic individuals (Heise, *et al.*, 1991). Thus, the virus can "home out" in this organ as well as in the brain. Its presence in entero-

chromaffin cells in bowel mucosa is well established. These hormone-producing cells are distributed throughout the intestinal tract and are responsible for normal motility and bowel function. The lamina propria has also shown evidence of HIV infection, most probably in infected macrophages (Fox, *et al.*, 1989), since, at the time of bowel symptom, very few CD4 lymphocytes are present in the infected gastrointestinal tract.

As with CNS diseases, however, some investigators argue that infections of macrophages and T cells in the intestine of these patients induces the production of cytokines that have destructive toxic effects on the bowel mucosa. Macrophage-tropic HIV strains are readily isolated from bowel tissue. Also, histochemical studies of the gastrointestinal tract show chronic inflammation (Kotler, *et al.*,1984) that is not unlike that of bowel disorders caused by a variety of infections and toxic agents. Nevertheless, the extensive diarrhoea and malabsorption observed in AIDS does suggest a direct effect of the virus on intestinal cell membrane integrity, perhaps in the handling of sodium ions and water. The watery diarrhoea often observed could be the effect of a toxin, perhaps a product of infected cells or a viral protein.

is recommended to: (3.1) determine the true prevalence of cryptosporidial infection in AIDS patients in Ethiopia; (3.2) describe the clinical profile associated with it and, (3.3) elucidate the association with other enteropathogens causing diarrhoeal disease in AIDS patients.

4. Furthermore, the role of bacteria, viruses and immunological aberrations as mechanisms for diarrhoea in HIV-seropositive individuals must also be investigated.

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APPENDIX 2

REQUEST FOR HIV TESTING OF AIDS PATIENTS

(STRICTLY CONFIDENTIAL)

Lab ser. No.————————————————

Date:————————————————

A. PATIENT'S DETAILS

Code No.———————— Age:———————— Sex:————————

Kef.———— Keb.———— House No.———— Tel. No.————

Occupation:————————————————————————————

Marital status:———————————— No. of children:————

Previous B/D transfusion: Yes/Not/Not known

Where———————————— When:————————————

Symptoms present	Signs
Weight loss	Weight loss————
Fever	Temp.(max.)————
Night sweats	Pneumonia————
Diarrhoea	Diarrhoea————
Lymph node engagement	Lymph nodes————
Cough	Skin rash————
Others	Hepatomegaly————
	Splenomegaly————
	Candidiasis————
	Failure to thrive————

B. DETAILS OF MEDICAL PRACTITIONER'S NAME AND ADDRESS

Name:_____

Address:_____ Tel._____

Signature_____Date:_____

****

C. TEST RESULT

Screening test_____ Date_____Pos / Neg

Screening test_____ Date_____Pos / Neg

Confirmatory test_____ Date_____ Pos / Neg

Reported by:_____

Signature:_____

Date:_____

To:_____

Address:_____

Comments:

_____

_____

_____

APPENDIX 3

RESULT REPORT FOR HIV TESTING OF AIDS PATIENTS

(STRICTLY CONFIDENTIAL)

Patient's code No.———————— Sex———— Age———

Hospital———————————— Physician————————

RESULT

☐ Negative Reaction On ELISA

☐ Positive Reaction On ELISA

☐ Negative reaction on confirmatory Assay

☐ Positive reaction on confirmatory Assay

COMMENT

_____

_____

_____

Result reported by:————————

Signature: ————————————

Date:————————————

APPENDIX 4

RESULT REPORT FOR PARASITOLOGICAL EXAMINATION

Code No.———— Sex———— Age————

Address Kef.———— Kebele———— Tel.————

Type of specimen———— Appearance ————

Date of collection ————

****

Ova/parasite Positive———— Negative————

Parasite identified	Direct count	Concentratio count
1.		
2.		
3.		
4.		
5.		

Others

———————————————————————————————

———————————————————————————————

———————————————————

Remark

———————————————————————————————

———————————————————————

Date Reported———— Signature————

APPENDIX 5

DATA COLLECTION FORMAT FOR PARASITOLOGICAL ANALYSIS

Ser No.	Hosp. code No.	Lab. code No.	sex	Age	Direct microscopy	Concentration method	Modified Ziehl-Neelsen stain	Remark

APPENDIX 6

DATA COLLECTION FORMAT FOR SEROLOGICAL ANALYSIS

Hospital Code Number	Laboratory Code Number	Sex	Age	SEROLOGICAL OBSERVATION			
				ELISA O.D	HIV-spot	Final result	Remark