CHAPTER-I
INTRODUCTION

1.1 Introduction

Human Immunodeficiency Virus (HIV) is a retrovirus, a member of the lentivirinae subfamily, which has spherical virion measuring about 80-90 nm in diameter. It contains linear, positive sense, 9-10 kb, diploid, single stranded RNA as a genome.

Acquired Immunodeficiency Syndrome (AIDS) is a constellation of diseases which reflect the severe late manifestations of HIV. Patients with AIDS are considered to have CDC group IV and its diagnosis remains, in essence, a clinical definition. AIDS is one of the most important public health problems worldwide at the start of 21st century. The national HIV/AIDS strategy (2002-2006) in Nepal has identified migrant populations, especially labour migrants to India, as one of the vulnerable groups for HIV infection (NCASC, 2003). As of June 30, 2006, 6990 HIV seropositive cases have been detected, with 1085 cases of AIDS; the estimated number is much higher than 60,000 (NCASC, 2006).

HIV infection leads to the depletion of CD_4+ T helper cells as it is the target cell for HIV infection. The depletion of T- helper lymphocytes results in the causation of opportunistic infections (OIs). OIs are those infections which are caused only when there is immunological and anatomical defects. When the immunological and anatomical defences are impaired or compromised as a result of congenital or acquired diseases or by use of immunosuppressive therapy or surgical techniques there is the manifestation of OIs. The OIs among HIV seropositive subjects and AIDS are caused by bacteria, fungi, parasites and other viruses as well.

However, the frequency of OIS varies with the geographic area, the prevalence of microorganisms in an environment and the stage of HIV infection (Fleming, 1990).

Gastrointestinal involvement in HIV/AIDS is almost universal and significant disease occurs in 50-96% of patients. Diarrhoea can be a manifestation or a life threating complication of infection with HIV sometimes during the course of the disease. Infectious causes of diarrhoea have been found in 30-80% of patients depending on the extent of the study and patient characteristics. Such pathogens include opportunistic agents that consistently cause severe, chronic or frequent gastrointestinal disease and non opportunistic agents that usually cause acute, treatable diarrhoea illness (Fleming, 1990; Gilks and Ojoo 1991; Gurerrant and Boback 1990; Harries and Beeching 1991; Smith *et al*, 1988). The etiology for such diarrhoea could be either parasitic, bacterial, fungal, enteric virus or HIV itself may contribute to the diarrhoea. In addition to microbes, other factors such as medication, immune dysregulation, autonomic disorder and nutritional supplementation play substantial role in diarrhoea of HIV/AIDS patients (Essex *et al*, 1994; Harries 1991; Soave and Framm 1997).

Several species of protozoa have been associated with acute and chronic diarrhoea in HIV diseases. These include: *Cryptosporidium parvum, Isospora belli, Microsporidium* species, *Giardia lamblia, Entamoeba histolytica, Cyclospora* species, *Blastocystis hominis* and *Dientamoeba fragilis* (Awole *et al*, 2003). Nematode like S*trongyloides stercoralis* can also cause diarrhoea and overwhelming infestation in patients with variety of immunosuppressive disorders including HIV/AIDS (Ambrioise, 2001; Pollock and Farthing 1997). Other nematodes such as hookworms, O*pisthorchis veverrini* and *Ascaris lumbricoides* can also be seen in stool of HIV patients (Wiwanitkit, 2001). Severe helminthic infection, expressing either as more egg/s in faeces or infestation simultaneously by several helminthes, correlated positively with the load of HIV particles in plasma (Fincham, 2003). This is because both progression of HIV infection to AIDS and helminth infections are associated with increased T helper cell 2 (Th_2) cytokine productions (Conlon *et al*, 1990). Helminths infection like ascariasis has also been shown to polarise the immune response in young adults to Th_2, which should increase the risk of sexual transmission of HIV. Ascariasis also suppresses interleukin-2, a Th_1 cytokine that can be used as a treatment for HIV/AIDS because it improves count of CD_4 T cells and restores immune function substantially (Fincham, 2003).

Several bacterial enteric infections occur with increased frequency in persons infected with HIV and some of them are more likely to be severe, recurrent, persistent and associated with extra-intestinal disease. Salmonellosis is likely to cause severe invasive disease in HIV infected persons. CDC surveillance definition has recently included recurrent non-typhoidal *Salmonella* septicaemia as an AIDS defining illness. *Shigella* bacteraemia, a rare complication of shigellosis in adults, may be more common in HIV infected patients. *Campylobacter* spp. are known to be one of the more prevalent organisms in HIV diarrhoea. *Campylobacter* enteritis and *Campylobacter* bacteraemia are more common in patients with AIDS than in the general public (Smith *et al*, 2002).

In Nepal many groups are considered as high risk population to HIV infection. These include commercial sex workers, intravenous drug users, immigrants, highway truckers, blood and organ recipients, housewives, newborn and the clients of sex workers (NCASC, 2006). This study tried to reveal the prevalence of enteric parasitic infections among the high risk group population. Due to time constraint and unavailability of the samples only the female sex workers and highway truckers were included in this study.

CHAPTER-II
OBJECTIVES

2.1 General objective

The general objective of this study was to establish the prevalence of intestinal parasitic infections among HIV positive and high risk groups for HIV infection in Nepal.

2.2 Specific objectives

1. To reveal the macroscopic character of stool specimen.
2. To perform stool examination for trophozoites, cysts and oocysts of protozoa and ova and larvae of helminths.
3. To find out the correlation of gastrointestinal tract symptoms with parasitic infection.
4. To establish the prevalence of parasitic infection among rehabilitated and non-rehabilitated study population.
5. To correlate the findings with ethnic groups.
6. To look for statistical significance if parasite infections are associated with gastrointestinal symptom, gender and age.

CHAPTER-III
LITERATURE REVIEW

3.1 HIV and AIDS

AIDS was recognized in the United States in June 1981 with a sudden outbreak of opportunistic infections, *Pneumocystic carinii* pneumonia and Kaposi's sarcoma. The first indication that AIDS could be caused by a retrovirus came in 1983, when Barresinoussi *et al* (1983), at the Pasteur Institute recovered a reverse transcriptase containing virus when a lymph node of a man with persistent lymphadenopathy syndrome. Gallo *et al* (1984), reported the isolation of HIV from individuals with AIDS.

3.1.1 Life cycle

The life cycle of HIV-1 can be considered in two distinct phases. The initial early events occur within a short time and include viral attachment, entry, reverse transcription, entry into the nucleus and integration of the double stranded DNA (the provirus). The second phase occurs over the lifetime of the infected cell as viral and cellular proteins regulate the production of viral proteins and new infectious virions.

3.1.2 Modes of transmission

The transmission of virus can be greatly influenced by the amount of infectious virus in a body fluid and the extent of contact with that body fluid. HIV is present in semen, vaginal/cervical secretions and blood, and these are the main vehicles of transmission. Virus may be present in CSF, saliva, tears and urine, but usually to a lower titre than in blood and there is epidemiological evidence of infection from these sources. Major routes of transmission are as follows:

3.1.2.1 By sexual contact

WHO (2003), has reported that majority of HIV infection (70%) worldwide occur through heterosexual contact and the groups most affected are sexually active men and women in their 20s to 40s. HIV has been isolated from semen (both fluid and cellular components) and vaginal and endocervical secretions. There is sexual polarity to transmission, females acquiring the infection much more readily from males than vice-versa. Risk of infection increases if either partner has another STD that causes genital ulceration. Transmission rates are higher when the infected has advanced HIV disease.

Anal intercourse between homosexual men is a common mode of transmission especially in western countries. This mode carries a high risk because semen from one partner can enter the blood through a damaged blood vessel in the wall of the rectum of the uninfected partner.

In various studies, transmission rates from this mode range from 10% to around 50%. The baby is more at risk if the mother has been recently infected or is in advanced stage of AIDS, as the virus is in higher concentration (WHO, 1996). Perinatal transmission may occur in utero (transplacental), during parturition or via breast milk (postnatal).

3.1.2.2 Transmission through blood

The virus is present both in white cells and in the plasma. If the inoculum is large, as in blood transfusion, the recipient of infected blood almost inevitably becomes infected. The minute volumes of blood transferred by accidental needle stick injuries only very rarely lead to infection. Accidental micro-inoculations with needles and attached syringes containing blood do occasionally lead to infection in health care workers.

3.1.3 Prevention of HIV infection

There is no cure for HIV infection. The only way to prevent HIV infection is through total avoidance of sources of transmission. AIDS prevention and control broadly includes:

- informing the general public about HIV transmission and risky behaviours.
- ensuring the safety of blood and blood products.
- counseling HIV infected persons, and
- taking action to reduce HIV transmission among injecting drug users.

3.1.4 Laboratory diagnosis

HIV infection can be detected in the laboratory either by detection of antibodies to HIV or by detection of the virus, its antigen and its DNA. Detection of specific antigens, viral nucleic acid, isolation/culture of virus are all confirmatory tests in that the presence of the virus is detected. But they are risky because of the danger of infection to laboratory workers, are very laborious and difficult to perform, require skilled expertise and hence are to be done only in laboratories specified research purposes.

The indirect predictors of HIV infection (CD_4 cell count, β_2 microglobulin etc.) are monitors of immunity status of patients and are to be done at routine intervals to monitor the progression of disease.

3.1.4.1 Detection of HIV-specific antibodies

In HIV infection, there is an initial period of viral replication during which antibody is undetectable. This period of seronegativity, called window period, may last for 1-3 months, but in a small minority of patients for up to a year, and rarely for years.

This is done by performing initial screening tests, which if positive, are followed up by supplemental tests to confirm the diagnosis.

Screening tests: Enzyme linked immunosorbent assay (ELISA) is the most commonly performed test to detect HIV antibodies. There are various kind of ELISA based on the principle of test:
Indirect ELISA
Competitive ELISA
Antigen sandwich ELISA

Antigen and antibody capture ELISA

ELISA takes up to three hours to yield results. It has a major advantage of being economical although rapid tests give result within minutes. Commercial kits are available for ELISA and rapid tests. Rapid tests include:

Dot blot assays
Particle agglutination
HIV spot and comb tests
Fluorometric microparticle technologies

In addition supplemental tests are also done for the confirmation.

Second and third ELISA/Rapid
Western blot
Indirect Immunofluorercence
Radio Immuno Precipitation Assay

3.1.4.2 Virus culture

Virus culture is another method for identifying HIV infection. The culture method is , however, expensive, labour intensive, can take weeks for complete results and potentially exposes laboratory workers to high concentrations of HIV. Virus culture is used for research (drug sensitivity, vaccine studies etc).

3.1.4.3 Polymerase chain reaction (PCR)

PCR can detect proviral DNA during window period, can differentiate latent HIV infection from active viral transcription and can quantitate the copy number of HIV DNA when used with external standards. PCR can successfully differentiate between HIV-1 and HIV-2 infections. Proviral DNA can be detected in peripheral blood mononuclear cells before seroconversion. Limitation to the diagnostic use of PCR are rare false negatives, some of which can be avoided by the use of multiple primer pairs from conserved regions of the genome and false positive due to cross contamination of the PCR reaction mixture.

HIV-1 can be detected by PCR in the CSF of HIV infected patients independently of disease stage; spread of HIV-1 to the brain represents an early event during infection which occurs in most asymptomatic individuals. PCR can also be used to detect HIV infection in neonates borne to HIV infected mothers.

3.1.4.4 Viral load assay

Quantitation of HIV RNA in plasma is useful for determining free viral load, assessing the efficacy of antiviral therapy and predicting progression and clinical outcome. Because baseline HIV viral load is predictive of survival at 10 years in patients with nearly identical CD_4 counts, assessment of baseline viraemia prior to initiation of therapy is useful in patient management.

3.1.4.5 Indirect predictors of HIV infection

Decreased CD_4 cells
Increased β_2 microglobulin
Increased serum neopterin
Increased IL_2 receptors
Indicator diseases for AIDS

3.1.5 Prophylaxis and therapy

3.1.5.1 Vaccines

No vaccines for human use is available. Vaccines containing gp160, gp120 or gp 41 are prepared by recombinant DNA technology or as synthetic peptides. Mutation in HIV has been a great obstacle in the areas of vaccine production. Despite the difficulties, several vaccines are under trail.

3.1.5.2 Therapy

Drugs are available that prolong survival and reduce the number of opportunistic infections. The most extensively used drug is azidothymidine, though it is toxic to bone marrow. Viral protease inhibitors are also promising. Drugs are expensive. Toxicity of the drugs to host and emergence of resistant strains of virus have been the major problem in chemotherapy.

3.1.6 Epidemiology

Globally, only a small number of HIV infections are estimated to have occurred during the late 1970s and early 1980s. During the late 1990s, HIV prevalence increased remarkedly in Subsahran Africa. HIV has been well established in Asia for many years. The HIV/AIDS epidemic is spreading rapidly in all South Asian countries. India has the single largest proportion of HIV positive cases within its boarder, second globally to South Africa. Over 4 million estimated HIV infections are existing within the region and about 13,000 AIDS cases have been reported by the year 2000 (STC, 2003). The first case of AIDS in Nepal was reported in 1988. By mid 1990s, Nepal has entered the "Concentrated epidemic" stage with consistent HIV prevalence in female sex workers, injecting drug users and migrants. The HIV prevalence is estimated around 0.5% in the general adult population (NCASC, 2003 and WHO/UNAIDS, 2003). There were around 60,018 people living with HIV/AIDS and 2,598 AIDS related deaths by 2002. Yearwise data indicates that the cases of HIV/AIDS have increased sharply since mid 1990s. In 1992, more than double members of new cases were reported than the formerly reported cumulative cases. Similarly, higher numbers of cases were reported in 2004 as compared to other years.

3.1.7 Parasitic infections of the gastrointestinal tract associated with HIV/AIDS

Parasitic diarrhoeas are all important cause of morbidity in developing countries. Some of the parasites are well-established enteric pathogens eg. *E. histolytica*, *G.lamblia* and

Balantidium coli and others are opportunistic pathogens i.e. *Cryptosporidium*, *Isospora*, *Microsporidium* and *Cyclospora* etc. Only a small percent of individuals harbouring the established enteric pathogenic parasites suffer from symptomatic disease in all immunocompetent hosts. However, with the advent of HIV/AIDS, the scenario has changed. Chronic, recurrent infections with all the enteric parasites have been reported from all over the world with varying frequencies. The rate of infection with a particular enteric parasite in HIV/AIDS patient will depend upon the endemicity of that particular parasite into community.

It has been estimated that the probability of developing a serious infection is 33% at 1 year and 58% at 2 years, if CD_4 counts are below 200.

Chronic parasitic gastro-intestinal infections mainly diarrhoea have been reported in 26% to 66% of patients with AIDS in North America and Europe and in 63% to 93% of patients in Haiti and Africa (developing countries). Protozoa are the most common cause particularly in developing countries. Most of the opportunistic enteric protozoa have gained importance during recent times on account of their association with HIV/AIDS.

Most common enteric opportunistic parasites which have been associated with HIV/AIDS include:

- *Cryptosporidium* spp.
- *Isospora belli*
- *Microsporidium* spp.
- *Cyclospora* spp.
- *Strongyloides stercoralis*
- *G. lamblia*
- *E. histolytica*

3.1.8 Bacterial enteric infections in HIV infected persons

Several bacterial enteric infections occur with increased frequency in persons infected with HIV and some of these are more likely to be severe, recurrent, persistent and associated with extra intestinal disease.

Enteric pathogens recovered from HIV infected persons include:

- *Shigella flexneri*
- *Salmonella* spp.
- *Campylobacter* spp.
- *Enterohaemoragic Escherichia coli*
- *Enteroinvasive Escherichia coli*
- *Clostridium difficile*
- *Vibrio chderae*
- *Staphylococcus aureus*
- *Plesiomonas shigelloides*
- *Aeromonas hydrphilla*
- *Yersinia enterocolitica*

(Source: Manual on laboratory diagnosis of common opportunistic infections associated with HIV/AIDS).

3.2 Gastrointestinal tract infection among HIV/AIDS patients

One of the major health problems among HIV infected patients is the intestinal parasite infestations. It can be seen that intestinal helminth infestation in HIV infected patients is common. However, the reported prevalence is usually similar to those of non HIV-infected patients in the same setting. The infestations are ordinary not opportunistic, hence, thus usually show no correlation to immune status of the patients. The suppression of immunity due to HIV infection shows no significant role in increasing the intestinal helminth infestations. On the other hand, having occult intestinal helminth infestations does also not worsen the outcome of HIV infection. Concerning the clinical manifestation, most of the helminth infestations are asymptomatic and the diagnosis is usually based on the stool examination. Treatments of the infestations as well as the outcomes are usually similar to immunocompetent host. Intestinal protozoal infections are also important problems for HIV-infected patients. Some infections are ordinary, while the others are opportunistic infections. The important opportunistic intestinal parasites including *C. parvum*, *I. belli*, *Cyclospora* and *Microsporidium* are found at high prevalence among the HIV-infected patients, especially in low immune cases with persistent diarrhoea. Concerning the clinical manifestation, most of the infections bring diarrhoea and the diagnosis is usually based on the stool examination with special stains. The treatment of the opportunistic infection can usually get control of the present illness but not prevent the re-infection. Luckily, with the present wide distribution of HAART, the prevalence of the opportunistic intestinal protozoa infections is significantly decreased (Wiwanitkit, 2006).

Infections of the gastrointestinal tract are common in patients with AIDS. It has been estimated that 30-50% of patients with AIDS in the USA and about 90% in Africa and Haiti suffer from chronic diarrhoea (Janoff and Smith 1988; Smith *et al*, 1992). The isolation rate of intestinal parasites in patients with AIDS and chronic diarrhoea vary from 40-83% (Kazanjian, 1993; Kotler and Orenstein, 1994) and the parasitic agents also differ markedly from region to region (Sorvillo *et al*, 1995).

In times when HIV has caused the largest and most devastating pandemic in the history of humankind, parasitic infection still remains one of the most prevalent types of infection in the world.

In a recent study of 22 HIV infected patients with diarrhoea in Thailand, *Microsporidium* was the most common pathogen (27%), followed by *Cryptosporidium* (9%) and *Isospora* (4.5%) (Punpoowong *et al*, 1998).

Kaminsky *et al* (2004) from Hondurus reported the overall prevalence of 44.3% for *T. trichiura*, 24% for *A. lumbricoides*, 12% for hookworms and 7.5% for *S. stercoralis* among HIV positive population. There was a strong correlation between eosinophilia and helminthiasis. Both helminthic and protozoan infection are highly prevalent in Hondurus and parasites such as *S. stercoralis*, *A. lumbricoides*, *T. trichiura* and hookworms known to be strongly associated with Th_2 cytokine shift accompanied by eosinophillia could represent a condition associated with rapid immunocompetence deterioration as observed empirically in Hondurus in those HIV infected individuals.

Many of the population at high risk for HIV also live in highly endemic areas of intestinal parasitic infections into adulthood (Bundy *et al*, 2000).

There is mounting evidence that these helminthic infections influence the HIV disease progression by regulating the host immune response both at the cellular level and humoral level as a result of a strong T helper 2 type cytokine profiles (Kalinkowich *et al*, 1998).

In developing countries, it is impossible to ignore the burden of parasitic infections usually categorized as the disease of poverty (Evans and Jamison, 1994).

The presence of individuals infected with intestinal parasites in situation of poverty, with deficient sanitary infrastructure, lack of latrines or poor use of them, overcrowding and soil characteristics favourable for the proliferation of parasites provide condition for the maintenance and transmission of such infections (Beaver *et al*, 1985).

In situation of chronic helminthic infections the Th_2 subset dominates and thought to develop Th1 function facilitating HIV disease progression (Actor *et al*, 1993; Baum *et al*, 2003; Bentwich *et al*, 1996; Borkow *et al*, 2001). Th_2 response has been associated with wasting and more rapid HIV disease progression, confirming previous research that eosinophil percentages were directly and significantly correlated with viral load and inversely correlated with CD_4 cell count (Actor *et al*, 1993; Bentwich *et al*, 1996; Borkow *et al*, 2001; Kalinkowich *et al*, 1998). *C. parvum* was found infecting 12.5% of 80 AIDS patients in Tegucigalpa (Kaminsky, 1999) and 25.3% of 79 AIDS patients in

San Pedro Sula but not 21 HIV positive nor 100 healthy commercial sex workers (Kaminsky 1999, 2000).

The newly emerging relationship of helminthic infections to HIV, however and its potential impact on HIV progression especially in countries where coinfection with HIV is growing reality justify efforts for strengthening such programmes and increasing measures to prevent and remedy conditions that foster transmission of parasitic infections (Kaminsky *et al*, 2004).

Intestinal parasitic infections could play an important role in the progression of infection with HIV by further disturbing the immune system whilst it is already engaged in the fight against HIV. The intestinal parasites identified in the study population were amoebic parasites (24.6%), hookworms (23.8%), *A. lumbricoides* (22.2%), *T. trichiura* (19.5), *S. stercoralis* (13.0%), *T. saginata* (4.5%), *G. lamblia* (3.0%) and *E. vermicularis* (1.3%). Overall, the HIV positives were no more or less likely to carry intestinal parasites than the HIV negative (76.2%vs 69.9%, P >0.05). However, when each parasite was considered separately, amoebic parasites were found to be more common in the HIV seropositive than seronegative (43.7%vs 24.0%).There was a moderate interaction between intestinal parasites and HIV at the asymptomatic stage of HIV infection (Fontanet *et al*, 2000).

Few studies, however, have assessed the prevalence and predictors for intestinal helminthic infections among HIV infected adults in urban African settings where HIV infection rates are highest. The investigators found at least one type of intestinal helminth in 24.9% of HIV-infected adults. Thirty-nine (52.7%) were infected with *A. lumbricoides* and 29 (39.2%) were infected with hookworm. More than 80% were light-intensity infections. A recent visit to a rural area, food shortage, and prior history of helminth infection were significant predictors of current helminth status. The high helminth prevalence and potential for adverse interactions between helminths and HIV suggests that helminth diagnosis and treatment should be part of routine HIV care (Modjarrad *et al*, 2005).

No statistically significant difference in prevalence of individual parasite species was detected between cases and control excepting that of *Blastocystis* spp. which were significantly higher in HIV/AIDS patients. Helminthic intestinal parasites were more common than protozoans both in HIV/AIDS patients (48.7%vs 28.2%) and in controls (42.3%vs7.8%). Multiple parasitic infections were observed in a total of 15 HIV/AIDS (15/78) and 2 HIV negative controls (2/26).The species of parasites most frequently seen in multiple infection in HIV/AIDS patients were *A. lumbricoides* and *Blastocystis* spp. (Gatechew *et al*, 2004).

Chronic infection with helminthes illustrates best some of the elements of chronic immune activation that may also be found in HIV infection (Betwich *et al*, 1998). This type of immune activation has been suggested as a major factor for the increased susceptibility and progression of HIV infection in Africa and other developing countries (Betwich *et al*, 1995).

Intestinal parasites were significantly more common among patients with diarrhoea than those without diarrhoea (53.6 vs.12.9%) in Iran. The overall prevalence of the parasitic infections was 18.4% in this population group (Zali *et al*, 2004). Intestinal parasitic infections didn't appear to be highly prevalent: an intermediate to low level of prevalence, in comparison with data obtained from prevalence studies carried out in other regions was found (Brink *et al*, 2002; Brandonosio *et al*, 1999; Mohandas *et al*, 2002; Punpoowong *et al,* 1998). This could be correlated to the relatively high level of immune status among the study population and the fact that diarrhoea and intestinal parasitic infections are strongly associated with lower CD_4 counts (Brink *et al*, 2002).

The study carried out in Southwestern Ethiopia revealed that HIV infected and HIV non infected in the age ranges of 25 to 34 years of both sexes were the predominant age group (37.6% and 39.9%) respectively. Diarrhoea is more prevalent in HIV infected 99 (51.1%) than HIV non infected patients 53 (29.5%). Regardless of their diarrhoea status, the general prevalence of intestinal parasites in HIV infected and HIV non infected were 44.8% and 34.4% respectively. Helminthic infections were more common than protozoan infection in both HIV infected and HIV non infected (65.1% and 62%) respectively (Awole *et al*, 2003).

The HIV infected patients were found to have virtually all parasites such as *G. lamblia, C. parvum, Chilomastix mesnili, Entamoeba coli, Iodamoeba butshlii, E. histolytica/dispar, Endolimax nana, B. hominis, E. vermicularis, A. lumbricoides* and *H. nana.* In HIV infected patients, the content of CD_8 lymphocytes was increased, but that of CD_{20} lymphocytes was normal. Parasites were detectable with different levels of CD_4 lymphocytes, but *C. parvum* was found only if its count was <200/ml. In the HIV infected patients, the hyperproduction of IgE was caused mainly by helminthes rather than protozoa. In these patients, the increased level of IgE was also noted in the absence of parasites (Nurtaev *et al*, 2005).

The prevalence of parasitic infections in HIV infected patients carried out in South Korea revealed the prevalence of 31.4% and *C. parvum* being the most commonest (Guk *et al*, 2005).

In order to verify the occurrence of intestinal parasitic infections in HIV/AIDS patients, 100 HIV/AIDS patients (Group 1) and 85 clinically healthy individuals (Group 2) were submitted to coproparasitological examination. Intestinal parasites were detected in 27% of patients from Group 1 and in 17.6% from Group 2. In Group 1 the most frequent parasites were *S. stercoralis* 12%, with 2 cases of hyperinfection, *Isospora belli* 7%, *Cryptosporidium* spp. 4% with 1 asymptomatic case and hookworm 4%. Of the infected patients from Group 1 who reported to be chronic alcoholics, 64.3% had strongyloidiasis. Only 6 of the 27 infected patients from Group 1 were on HAART. In Group 2 the most frequent parasites were *S. stercoralis* 7.1%, hookworm 7.1% and *G. lamblia* 3.5% (Silva *et al*, 2005).

Ascariasis and HIV/AIDS are often co-endemic under conditions of poverty in South Africa; and discordant immune responses to the respective infections could theoretically be affecting the epidemic of HIV/AIDS in various ways. It is well-known that sensitisation to helminthic antigens can aggravate or ameliorate several non-helminthic diseases and impair immunization against cholera, tetanus and tuberculosis (Adams *et al*, 2006).

Since the first AIDS cases were described, a high prevalence of gastrointestinal alterations has been reported, especially diarrhoea associated with parasitosis.

This became more evident when the appearance of a syndrome named "Slim Disease", characterized by an intense weight loss accompanied by chronic diarrhoea, prolonged fever and diffuse muscle weakness, was observed in Africa, especially in Uganda (Grunfeld and Schanbelan, 1994; Kanradt *et al*, 1985; Mhiri *et al*, 1992). Studies conducted in Zaire and Uganda have shown the presence of some pathogenic agents responsible for the "Slim Disease", such as *Isospora, Cryptosporidium, Salmonella, Shigella* and *Campylobacter* species, amounting to a prevalence of 60 to 80% (Bolinger and Quinn,1994). "Slim Disease" has been observed in advanced stages of HIV infection. The expression "Wasting Syndrome" was adopted in substitution by WHO in 1988 on the basis of criteria laid down by the CDC (Mhiri *et al*, 1992).

In a study conducted in USA among one hundred individuals confirmed to be infected with HIV, the prevalence of enteric parasites was 55 (55%) for *G. lamblia*, 6 (6%) for *Cryptosporidium*, 10 (10%) for *I. belli* and 3 (3%) for *E. histolytica* (Esfandiari *et al*, 1995).

According to the study conducted in Africa, invasive, non-typhoidal *Salmonella* (NTS) infections are a common but life-threatening complication in adults who are seropositive for HIV. The high prevalence of human infection with intestinal helminths which penetrate the gut could explain the greater importance of NTS bacteraemia in Africa compared with that in industrialized countries. If helminth infection is a major risk factor for NTS it would provide a locally relevant, public-health target. Intestinal helminth carriage in 57 HIV-positive patients with NTS bacteraemia (the cases) was compared with that in 162 HIV-positive controls who were similar to the cases in terms of age, sex, urban dwelling and socio-economic factors. The prevalence of helminth infection, 29% overall, was lower among the cases (18%) than among the controls

(33%). Five (9%) of the cases and 12 (7%) of the controls were infected with nematodes which penetrate the gut (*A. lumbricoides* and *S. stercoralis*). The study do not exclude the possibility that helminths play a role in invasive NTS infections, but are not consistent with helminths being a sufficient risk factor in this population to be a public-health target (Dowling *et al*, 2002).

In France, 81 HIV/AIDS patients were studied for parasites, viruses, and bacteria. Pathogens were found in 70.6% of AIDS patients with diarrhoea or malabsorption. The respective prevalence of protozoa in AIDS patients with diarrhoea was *Cryptosporidium* spp. 37.3%, *B. hominis* 13.7%, *G. lamblia* 5.8%, *I. belli* 2% and *Enterocytozoon bieneusi* 2%. Microsporidia were noted in one patient with severe malabsorption but no diarrhoea. Other pathogens included cytomegalovirus in 27.4% and *Mycobacterium avium* in 5.8%. Patients with identified pathogens were more immunosuppressed and more severely malnourished than those with unexplained diarrhoea. Multiple pathogens were found in 13 of 81 patients (16%). Twenty-six of 66 identified pathogens (40%) were diagnosed only on biopsy specimens. Chronic diarrhoea in HIV patients could be explained in the vast majority by appropriate gastrointestinal investigations. Cryptosporidia played a major role, while microsporidia appeared to be less common (Cotte *et al*, 1993).

According to the study conducted in Brazil among AIDS patients, 40% were infected with at least one pathogenic species. The total prevalence of parasites was 16% for *G. lamblia*, 13% for *E. coli*, 7% for *C. parvum*, 3.5% for *H. nana*, 2.5% for *A. lumbricoides*, 2.5% for *S. stercoralis*, 2% for *I. belli*, and 0.5% for *B. hominis* (Cimerman *et al*, 1999).

According to the study done in Zambia total of 178 children with diarrhoea were enrolled. Of these 44 (25%) were HIV seropositive and 134 (75%) were seronegative for HIV. Out of 44 HIV-seropositive patients, 20 (45%) had acute diarrhoea and 24 (55 %) had chronic diarrhoea. Of the 134 HIV-seronegative patients, 68 had acute diarrhoea (51 %) and 66 (49 %) had chronic diarrhoea. At least one intestinal parasite was found in 34 out of the 178 children enrolled. The commonest parasites identified were *Ascaris* and *Cryptosporidium* (Chintu *et al,* 1995).

A prospective observational study was conducted to determine the prevalence and the clinical impact of intestinal parasitic infections in diarrhoeal illness among HIV-infected and HIV-uninfected children hospitalized with diarrhoea in Bangkok, Thailand, intestinal parasites were identified in the stool specimens of 27 of 82 (33%) HIV-infected and 12 of 80 (15%) HIV uninfected children. *Microsporidium* and *Cryptosporidium* were the most commonly found parasites. Eighty-two percent of HIV infected and 97% of HIV uninfected groups presented with acute diarrhoea and 76% of each group had watery diarrhoea (Chokephaibulkit *et al*, 2001).

According to the study conducted among 105 workers of 29 roadside restaurants along a highway in Assam were interviewed for their habits, educational status and were examined for their hygienic status and presence of communicable diseases (STDs, TB etc). Most of them were young, males with 40% literacy; more than half were unmarried. About 30% of them were alcoholic and smokers and 2.9% were addicted to cannabis. The hygienic conditions of the workers were poor. More than one third had sexual contact with multiple sex partners or commercial sex workers and 2% were engaged in homosexual activity. Most of them did not use condom. A 25.7% of them had genital lesions suggestive of sexually transmitted disease, 11.8% showed gram negative diplococci in urethral smears and 5.1% were VDRL reactive. Skin infections followed by gastrointestinal disorders and respiratory tract infections were other prevalent problems. A total 70.6% were positive for intestinal parasites and 22.2% were sputum positive for acid fast bacilli (Biswas *et al*, 1999).

In the course of a long-term study of parasitic infections among HIV-infected persons in Austria, 618 persons infected with HIV were examined. In 9% of 219 patients, *Cryptosporidium* spp. was found. In two persons, an infection with *S. stercoralis* was diagnosed. Except these AIDS-associated opportunistic infections, the incidence of parasitic infections in the Austrian HIV infected population was found to be low, and, except for *E. histolytica*, not significantly exceeding the frequency of parasitic infections in HIV non infected Austrians (Aspock and Hassl, 1990).

According to the study conducted in North-Eastern Tanzania to determine the prevalence of pathogenic intestinal parasites among adult patients with enteropathic AIDS, a total of 352 patients were recruited of whom 158 (45%) had chronic diarrhoea. Of the 352 patients, 123 (35%) had intestinal parasites. Of the 123, 77 (62.6%) patients had chronic diarrhoea. The types of parasites detected were *Cryptosporidium*, *I. belli*, *S.stercoralis*, *Schistosoma mansoni*, *T. trichiura*, *A. lumbricoides*, hookworms and *E. histolytica*. The prevalence of intestinal parasites was significantly higher in patients with chronic diarrhoea than in those without diarrhoea (Tarimo *et al*, 1996).

According to the study conducted in Africa among seventy AIDS patients, wasting syndrome was the clinical presentation in (97%) almost all AIDS patients who had chronic diarrhoea. Intestinal parasites were detected in 41 out of 70 diarrhoeal specimens in AIDS patients. Multiple parasitic infections were detected in three diarrhoeal specimens. Intracellular parasite, (29%) *C. parvum*, and mucosal parasite, (17%) *S. stercoralis* were the frequently isolated parasites in diarrhoeal specimens of AIDS patients, accounting for 80% diarrhaoegen pathogens among positive specimens. *C. parvum*, under-estimated cause of chronic diarrhoea in immunocompetent adults, was found to be the prominent diarrhoeagen in AIDS patients in this study, similar with other studies in different African countries (Tadesse and Kassu, 2005).

A broad spectrum of gastrointestinal pathogens can cause diarrhoea in patients with AIDS. A systematic approach utilizing symptomatology and the appropriate diagnostic tests will maximize the clinician's chance of identifying the specific pathogens. Enteric infections in AIDS patients are often incurable and require prolonged therapy and chronic suppression. Experimental agents show promise of decreasing the morbidity and mortality attendant on diarrhoea in AIDS patients (Sachs and Dickinson, 1989).

Intestinal parasite infections are very frequent in HIV patients with severe immunodeficiency (CD_4 < $100/mm^3$) causing chronic diarrhoea and malabsorption in the majority of cases. The most frequent microorganisms are *Microsporidium* and *C. parvum* while *C. cayetanensis* and *I. belli* are more prevalent in subtropical and tropical areas and rare in industrialized areas. The diagnosis can be obtained by stool examination (differences in size and form of cysts), although microsporidia is frequently demonstrated by intestinal biopsy and/or duodenal aspirate (Moreno-Camacho *et al*, 1998).

In Mexico, the study done was to evaluate the frequency of *E. histolytica/dispar* intestinal infection in HIV/AIDS subjects and their HIV negative close relatives or sexual partners. Enteric parasites were investigated in stool samples by microscopic examination and *E. histolytica* and *E. dispar* were identified by PCR. The investigators found by microscopic analysis in HIV/AIDS group that the *E. histolytica/dispar* complex was present in 5.9% of the members, while in the HIV- group was 2.9%. With PCR they found that the *E. histolytica* prevalence was 25.3% in the HIV/AIDS group and 18.5% in the HIV- group. The difference in the results obtained with the microscopic and PCR was due to the different sensibility of the procedures. Besides, they found patients who were infected with *E. histolytica* in both groups were asymptomatic cyst passers. Their results suggest that *E. histolytica* strains prevalent in the studied community appear to be of low pathogenic potential (Moran *et al*, 2005).

Previous studies from African countries where HIV-1 infection is prevalent have shown that infections with *C. parvum*, *I. belli* and microsporidia are frequently associated with chronic diarrhoea in AIDS patients. The information about the occurrence of these parasites in HIV-2 associated AIDS cases with chronic diarrhoea is limited. They have performed a study of stool parasites in patients from Guinea-Bissau, the country with the highest prevalence of HIV-2 in the world. Stool specimens from 52 adult patients with chronic diarrhoea of which 37 were HIV positive and fulfilling the clinical criteria of AIDS (5 HIV-1, 28 HIV-2 and four dually infected with HIV-1 and HIV-2) were screened for parasitic infections. Twenty five percent of the HIV-2 positive patients were infected with *C. parvum*, 11% with *I. belli* and 11% with *Microsporidium*, all three parasites were seen only in HIV positive patients. Other stool parasites such as *B.*

hominis, hookworm and *S. stercoralis* were observed both among HIV positive and HIV negative patients (Lebbad *et al*, 2001).

Kotler *et al* (1993), compared retrospectively, the effects of infection in jejunal mucosa with the protozoa cryptosporidia or microsporidia and with HIV upon mucosal structure and absorptive function in 29 AIDS patients. The presence or absence of protozoal infection was confirmed by transmission electron microscopy. Villus blunting and crypt hyperplasia were seen mainly in the parasite-infected groups, although two patients without parasites also had shortened villi. Absorptive functions, including disaccharidase-specific activities and D-xylose absorption, closely paralleled the degree of small intestinal alteration. Evidence of HIV-infected cells in jejunal mucosa was examined by RNA in situ hybridization and by antigen-capture ELISA of mucosal homogenates. They found evidence of HIV in almost half the patients, which did not correlate with intestinal injury or diminished absorption.

A longitudinal study was carried in Bamako (Mali), concerning HIV positive patients suffering from diarrhoea. Opportunistic infections have been relatively frequent with *C. parvum* with 20%, *I. belli* with 8.5% and *Microsporidium* with 11.5% of cases. Other non-opportunistic microbes were found. The frequency and the danger of those opportunistic infections require their efficient diagnosis and care management (Konate *et al*, 2005).

Previous studies have found a high prevalence of *I. belli* and *C. parvum* infections in African AIDS patients with chronic diarrhoea. So the investigators aimed to determine the prevalence of gastrointestinal parasite in AIDS patients in hospital, not only those with diarrhoea, and to compare them with the general community. Clinically diagnosed AIDS patients in a Zambian teaching hospital were interviewed and examined, and stool specimens were studied for parasite infection. A control group was recruited from adults in a township near Lusaka. Of 90 AIDS patients (58% male), 50 (56%) had chronic diarrhoea and 9 (10%) had diarrhoea of shorter duration. In the control group (105 adults; 85% female), only one complained of diarrhoea. A variety of intestinal protozoa and helminths was found in 57% of AIDS patients and 88% of the community members. Isosporiasis was detected in 7 and cryptosporidiosis in 2, AIDS patients, all with diarrhoea, but not in any control. *Strongyloides* was found in 2 AIDS patients with diarrhoea and one community resident. Hospital patients with AIDS had fewer *E. coli* and *E. histolytica* infections, probably because of previous chemotherapy (Hunter *et al*, 1992).

According to the study in Harare, Zimbabwe among 88 HIV-infected individuals presenting with diarrhoea of greater than 1 week duration, *C. parvum* was detected in 9% (7 out of 82) of samples evaluated, but no *Cyclospora* was detected. *E. bieneusi* was detected in 18% (10 out of 55) of stool by trichrome staining and in 51% (28 out of 55) of stool examined by PCR. *E. bieneusi* infection was common in HIV infected patients with diarrhoea in Zimbabwe and may be acquired through person-to-person and fecal-oral transmission (Gumbo *et al*, 1999).

A survey on intestinal parasites in a rural area of Tanzania revealed the presence of 8 protozoa and seven helminths in 287 subjects (81.8%). The prevalence of *E. histolytica* and *A. lumbricoides* was higher in HIV-negative than in HIV-positive patients. On the other hand, *C. parvum*, *I. belli* and *S. stercoralis* prevalence was higher in HIV-positive than in HIV-negative patients. The prevalence of these two opportunistic protozoa was also higher in AIDS patients than in HIV-positive patients without AIDS. Specific anti-*C. parvum* IgG were detected by ELISA in 18% and 56% of HIV-negative and positive patients, respectively, confirming the high number of contacts between this parasite and humans (Gomez Morales *et al*, 1995).

Diarrhoea is a common complication and one of the most important causes of malabsorption and malnutrition in AIDS patients. The investigators included 217 HIV/AIDS patients who reported diarrhoea between May 2002 and September 2005. They analyzed 1-3 stool samples per patient using six methods to detect oocysts, cysts, eggs and larvae of parasites. The average patients' age was 34.5 years. A total of 123 parasites were detected in 103/217 patients (47.5%) and 18/217 (8.3%) had mixed parasitic infections. *Cryptosporidium* spp. was the most frequent parasite detected. It was more prevalent in the group of patients who had diarrhoea for two or more weeks than those who had diarrhoea for less than two weeks. Other frequent parasitosis cases were isosporidiasis (10.6%), giardiasis (8.3%) and strongyloidiasis (6.9%) (Garcia *et al*, 2006).

Opportunistic intestinal parasites are a common cause of diarrhoea in HIV infected patients. To determine the prevalence of microsporidia and other opportunistic parasites infecting HIV patients in Bogota, Colombia, 115 patients were examined for these infections during the year 2001. Patients with complaint of gastrointestinal symptoms were asked to provide two consecutive stool samples. The prevalence of intestinal opportunistic parasites was 10.4% for *Cryptosporidium* sp. Initially, 29% of the samples were found to be positive for microsporidian spores using a modified Ziehl Neelsen chromotrope stain, but only 3.5% of them were confirmed as positive when a calcofluor/gram chromotrope stain was used. The general prevalence of intestinal parasites was 59.1%. The most frequently found pathogens were *B. hominis*, 25.2%, and *E. histolytica*, 13%. In other studies with HIV patients in Colombia, lower prevalences of *Cryptosporidium* spp. infection were observed (Florez *et al*, 2003).

A coproparasitological study was carried out on 67 AIDS patients admitted at the Institute of Tropical Medicine 'Pedro Kouri'. The results were compared with 136 HIV-seronegative patients (control group) who were also hospitalised in the same period. In both groups monoparasitism was more prevalent than polyparasitism and intestinal protozoa were more prevalent than helminths. At least one intestinal parasite was found in 34 (51%) of the 67 AIDS patients and in 65 (48%) of the control group patients. Intestinal coccidia were only detected in AIDS patients; *Cryptosporidium* spp. was the most prevalent, with 8 cases (11.9%), followed by *C. cayetanensis* with two cases (3.0%) and *I. belli* in one case i.e.1.5% (Escobedo and Nunez, 1999).

According to the study conducted by Buyukbaba *et al* (2004), enteric parasites were detected in 18 (47%) of the 38 patients, 16 patients harbored a single parasite, and 2 patients were found to be infected with more than one parasite. Only 1 (7%) of 15 AIDS patients without diarrhoea, were found to be infected with *G. lamblia*. On the other hand, 17 (74%) of 23 AIDS patients with chronic diarrhoea were found to be infected with various enteric parasites. *Cryptosporidium* spp. was detected in 9 (39%) of these 23 patients, and in 2 of them *Microsporidium* spp. accompanied *Cryptosporidium*. In 2 (9%) of these 23 patients *G. lamblia* were detected, while *I. belli*, *B. hominis*, *E. histolytica*, *S. stercoralis* and *T. trichiura* were detected in one patient each. As a result, the detection rate of emerging parasites, including *Cryptosporidium* spp, *Microsporidium* spp., *I. belli*, *B. hominis*, and *S. stercoralis* was significantly higher than conventional parasites and CD_4 T cell counts were found to be significantly lower among AIDS patients with chronic diarrhoea than those without diarrhoea.

A cross sectional survey was conducted to determine the association between enteric parasites and diarrhoea in HIV-infected adults in Caracas. Three hundred and four patients were evaluated: 104 had acute diarrhoea, 113 chronic diarrhoea and 87 were controls. *I. belli* infection was associated with acute and chronic diarrhoea, *E. histolytica/dispar* infection was also associated with both acute and chronic diarrhoea. *S. stercoralis* and *C. parvum* infections were associated mainly with chronic episodes. Weight loss, a non-infectious factor investigated, was significantly associated with diarrhoea. Eosinophilia, a laboratory parameter studied, was found to be associated with strongyloidiasis, giardiasis and isoporiasis (Arenas-Pinto *et al*, 2003).

In Brazil, the occurence of intestinal parasites in HIV patients was reported particullarly in the states of Rio de Janeiro and Sao Paulo. The study was undertaken to contribute to the knowledge of the occurrence of intestinal parasites in AIDS and HIV positive patients in Uberlandia, in the State of Minas Gerais, Southeast region of Brazil. A retrospective analysis of medical files was made of 291 patients who were attended in the Infirmary of Infectious and Parasitic Diseases of the Clinical Hospital at Federal University of Uberlandia, during the period of January 1990 to December 1994. Of these patients 238 were AIDS patients and 53 were HIV positive. As result of this research 39 (16.4%) patients were diagnosed with parasites in the AIDS group; of these, 78.9% were male and 21.1% were female. Their age varied between 4 and 60 years; 94.7% were from the State of Minas Gerais and 5.3% from the State of Sao Paulo.

In the group of HIV positive patients there were 5 (9.4%) patients with parasites; 80% were male and 20% female. Their age varied between 22 and 33 years; 80% were from the State of Minas Gerais and 20% from the State of Sao Paulo.

In the control group there were 69 (7.7%) patients with parasites; 58.7% were male and 41.3% were female. Their age varied between 1 and 76 years; 90.6% were from the State of Minas Gerais, 6.7% from the State of Sao Paulo and 2.7% from the State of Goias.

In the AIDS patients group there were 32 (82.1%) cases of monoparasitism, 5 (12.8%) cases of biparasitism and 2 (5.1%) cases of poliparasitism. The associations found are the following *S. stercoralis* and *H. nana* (1 case), *S. stercoralis* and *G. lamblia* (1 case), *S. stercoralis* and *Cryptosporidium* spp. (1 case), *A. lumbricoides* and *Cryptosporidium* spp. (1 case), *A. lumbricoides* and hookworm (1 case), *Cryptosporidium* spp., hookworm and *E. histolytica* (1case) and *S. stercoralis*, hookworm, *E. histolytica* and *T. trichiura* (1 case).

In the HIV positive patients group there were 5 cases of monoparasitism. In the control group there were 65 (94.2%) cases of monoparasitism, 3 (4.3%) cases of biparasitism and 1 case (1.5%) of polyparasitism. The associations found were: *G. lamblia* and *H. nana* (2 cases), hookworm and *S. stercoralis* (1 case) and *T. trichiura*, *A. lumbricoides* and *S. stercoralis* (1 case).

It was demonstrated that the occurrence of intestinal parasitic diseases in AIDS patients was higher than the observed in HIV positive patients and the control group. This difference was significant when compared to the occurrence of *S. stercoralis* in AIDS group and control group. It was also observed that patients with more than one parasite are more frequently encountered among AIDS individuals. The difference is significant in relation to the control group. The importance of research in *Cryptosporidium* sp.

should be emphasized as well as the routine diagnosis in patients that reveal diarrhoea. The frequency of *S. stercoralis* is also important, even though the specific parasitologic method for research in helminths larvae in feces were not used in the routine of the laboratory (Cruz *et al*, 1996).

Intestinal parasitosis is one of the major public health and socio-economic problems in Nepal though a hospital based study have shown a declining trend during a period of ten years. Intestinal parasites even in low or moderate number affect on both nutritional and thereby on immune status of individuals leading to various morbidity and mortality and, therefore, considered to be "unremittingly corrosive". This is attributed to the reduction in appetite, digestion, absorption, acute phase status and increasing intestinal nutrient loses. The reported prevalence of intestinal parasitosis in Nepal varies considerably with over 90.0% in some areas. Overall, intestinal helminth infections alone rank fourth in "top ten" diseases in Nepal (Rai, 2005).

Sherchand *et al* (1997), reported the prevalence of intestinal parasites among children in southern Nepal as 60.1%. Hookworm infestation superseded all parasites by showing a positivity of 11.6% followed by *A. lumbricoides*. Among protozoan infestations, *G. lamblia* was at the top 9.9% followed by *E. histolytica*, 7.2%. The infestation of *Cryptosporidium*, *Cyclospora* and *Isospora* were 4.4%, 3.8% and 1.9% respectively. *H. nana* showed 3.3%, tapeworm 2.2%, *Opisthorchis* spp. 1.6% and *Schistosoma* 0.5% whereas 28.9% of infestation included two or more intestinal parasites.

Sherchand *et al* (1996), reported the prevalence of *Cryptosporidium* as 6.8% from the analysis of stool samples of the patients with diarrhoea.

Sapkota *et al* (2004), reported the prevalence of enteric parasites as 32.0% among HIV/AIDS patients in Nepal. Among the parasite positive cases, 83.3% were diarrhoegenic and rest remained asymptomatic. The protozoan parasites detected were *C. parvum* (10.6%), *G. lamblia* (6.7%), *E. histolytica* (5.3%) and *C. cayetanensis* (2.6%). The helminthes detected were *S. stercoralis* and *T. trichiura* each with 2.6% prevalence, and a single case (1.3%) of hookworm.

Dhakal *et al* (2004), found that the prevalence rate for *C. parvum* infection was 10.4%; among which 56.3% specimens were found co-infected with other intestinal parasites such as *G. lamblia*, *E. histolytica*, *Cyclospora*, *E. coli*, *Ascaris*, hookworms, *T. trichiura* and *Trichomonas hominis*.

Opportunistic infections are the leading cause of mortality and morbidity among HIV/AIDS patients. The spectrum of opportunistic pathogens involved in such infections in Nepal is not well documented. A cross-sectional study was carried out at the AIDS clinic of Manipal Teaching Hospital, Pokhara, Nepal. A total of 45 opportunistic pathogenic isolates were recovered from the 74 patients. *Mycobacterium tuberculosis* was the commonest pathogen 60%, followed by *Cryptosporidium* spp. 13.3% and *Candida* spp. 11.1% (Das *et al*, 2005).

CHAPTER-IV
MATERIALS AND METHODS

The laboratory investigation part of this dissertation was carried out at the research laboratory of the Department of Microbiology, Nepal Medical College, Jorpati, Kathmandu. The study period was from June to November 2005.

4.1 Materials

The materials, equipments, chemicals and reagents used in this study are listed below:

4.1.1 Materials

1. Plastic containers with spatula
2. Wooden applicator sticks
3. Glass slides with cover slips
4. Beakers
5. Test tubes
6. Centrifuge tubes
7. Pipettes
8. Cylinders
9. Forceps
10. Droppers
11. Glass rods
12. Labelling sticker
13. Filter paper
14. Sieve and funnel

The glasswares purchased from local suppliers were of Borosil Company.

4.1.2 Equipments

1. Microscope : Olympus, Japan
2. Photomicrograph : Olympus, Japan
3. Centrifuge : REMI, Research Centrifuge, India
4. Refrigerator : Toshiba, Japan
5. Vortex shaker : Vortex, India
6. Electronic balance : Scout, USA
7. Water bath : Grant, OLS 200, England

4.1.3 Chemicals

1. Basic fuchin : Qualigens, India
2. Ethanol : Bengal, India
3. Ethyl acetate : Qualigens, India
4. Formaldehyde : Qualigens, India
5. Iodine crystals : Loba chenie,India
6. H_2SO_4 : Qualigens, India
7. HCl : Qualigens, India
8. KI Crystals : Qualigens, India
9. Methanol : Qualigens, India
10. Malachite Green : Loba chenie,India
11. NaCl powder : Qualigens, India
12. Phenol : Qualigens, India
13. Sucrose Crystals : Qualigens, India

The chemicals were of analytical grade and purchased from local suppliers.

4.1.4 Reagents (Composition in Appendix-I)

1. Carbol fuchsin
2. Ethanol (95%, 100%)

3. Formalin (10%)
4. 1% HCl in methanol
5. 5% H_2SO_4
6. Lugol's Iodine (5 times)
7. 0.5% Malachite Green
8. Physiological saline (0.85%)
9. Parrafin oil
10. Sucrose solution

4.2 Patient selection

This study has included HIV-seropositive individuals, AIDS patients and high risk group population to HIV infection visiting different rehabilitation centre of Kathmandu Valley and its outskirts. The samples of the high risk group population to HIV infection were taken from outside the Kathmandu Valley.

4.3 Categorization of patients

The questionnaire method was used to note the name, demographic data, age, sex height, body weight, gastrointestinal tract associated symptoms, secondary infections and the use of any medicine (ARV or others) by interviewing them or their care takers. Patients were then classified based on age, sex, ethnicity, HIV serostatus and AIDS.

4.4 Specimen collection and preservation

Patients were interviewed whether they had taken antiprotozoal, antihelminthic agents and antibiotics or not. If they had taken, it was noted on the questionnaire. Patients or their care takers were advised to collect about 30gm or nearly 30ml of the stool sample avoiding contamination with urine, water and other substances. They were advised to pass the stool on a sterile paper (to avoid contamination from toilet pan) and collect in the container with the spatula both provided to them. Single specimen was collected from each individual.

4.5 Macroscopic examination of stool sample

This include the visual examination of stool specimen based on the color, consistency, presence of blood and mucus, presence of adult worms and segments.

4.5.1 Color

Based on the color, the stool specimen were categorized into two groups i.e. Normal color of stool (yellowish brown) and abnormal color of stool (muddy, black, pale and etc).

4.5.2 Consistency

Based on consistency, stool specimens were categorized as formed, semi-formed and loose. The trophozoites are usually found in the soft or loose stools whereas the protozoal cysts are usually found in formed and semi-formed stool. Helminthic eggs and larva are found in any type of stool specimen.

4.5.3 Blood and mucus

The stool specimens were observed whether it contains blood and mucus or not. Blood and mucus may be found in stool from patients with amoebic dysentery, intestinal schistosomiasis, invasive balantidiasis and in severe *T. trichiura* infections. Other non parasitic conditions in which blood and mucus may be found include bacillary dysentery, *Campylobacter* enteritis, ulcerative colitis, intestinal tumor and haemorrhoids.

4.5.4 Adult worms and segments

The stool specimens were observed whether it contains adult worms and segments or not. The adult worms of *A. lumbricoides* and *E. vermicularis* are often seen in the specimen. Tapeworm segments may be occasionally seen in stool specimens.

4.6 Microscopic examination

This is required for the detection and identification of protozoal cysts, oocysts, trophozoites and helminthic eggs or larva. Liquid specimen should be examined within 30 minutes of collection. Semi-formed or soft specimen should be examined within 1 hour of collection. Formed specimen should be examined on the day of collection.

Microscopical examination was done by saline and iodine wet mount and modified acid fast stain. The slides were observed under low power followed by high power and in case of acid fast stained smears by oil immersion. Parasites were identified by their morphology, motility and staining characteristics.

4.6.1 Saline wet mount

It is used to detect helminthic eggs and larva, protozoal cysts and trophozoites. A drop of normal saline was taken on a clean glass slide, a pinch of stool was mixed with it, and the mixture was covered with a cover slip and observed under microscope.

4.6.2 Iodine preparation

It is mainly used for detecting protozoal cysts, however, helminthic eggs are also stained and can be detected. Iodine stained cysts show pale refractile nuclei, yellowish cytoplasm and brown glycogen material. The trophozoites are killed hence their motility is lost in iodine mount.

A drop of 5 times diluted Lugol's iodine was taken on a slide and a pinch of stool was mixed with it. The preparation was covered with a cover slip and examined under low power and confirmed under high power.

4.6.3 Modified acid fast (Ziehl-Neelsen) staining

It is required for accurate identification of the oocysts of *C. parvum*, *I. belli, C. cayetanensis* and the spore of *Microsporidium*. The oocysts are acid fast and stained red or pink against a blue background stained with methylene blue. Both hot and cold methods of staining can be used with equal sensitivity .This study followed the cold Kinyoun methods as follows.

- A fecal smear was made on a glass slide and dried in air.
- The smear was fixed in absolute methanol for 3 minutes.
- The slide was flooded with carbol fuschin for 15-20 minutes and then washed with tap water.
- The smear was then decolorized with 1% sulfuric acid for 15-20 seconds
- The smear was washed with tap water and then counterstained with 0.5% methylene blue for 1 minute.
- The slide was washed with tap water, air dried and examined under 40X followed by oil immersion.

4.7 Concentration of parasites

The stool specimen was subjected to concentration when the direct smear revealed no parasites. This concentrates the eggs, larva and cyst when they are present in small numbers and increases the sensitivity of microscopic examination. Trophozoites are destroyed in the process. There are various floatation and sedimentation techniques of concentration but we had used the following techniques.

4.7.1 Formalin-ether sedimentation technique

This is the most sensitive method of concentrating cysts, eggs and larva without distortion of their morphology. It takes a short time and the chance of error is minimal. The technique is as follows.

- About 1 gm of feces was emulsified in about 4 ml of 10% formalin in a test tube.
- A further 3-4 ml of 10% formalin was added and the tube was capped and shaken well.

- The suspension was sieved through double gauze in a funnel into a 15 ml centrifuge tube.
- 3-4 ml of diethyl ether was added and the tube was vigorously shaken for 5 minutes.
- The tube was centrifuged immediately at3000rpm for 5 minutes.
- The layer of fecal debris formed between diethyl ether and formalin was remoed along the side of the tube with a glass rod.
- The supernatant layers of liquid were discarded and the sediment was examined by saline and iodine wet mounts.

4.7.2 Sucrose floatation technique

This technique concentrates the oocysts of *C. parvum, I. belli* and *C. cayetanensis*. This was performed as mentioned below.

- Using a wooden applicator stick, about 1 gm of stool was mixed with 5 ml of 10% formalin in a test tube.
- The suspension was filtered through three layers of cotton gauze in a test tube.
- The 3/4th of the tube was filled with the saturated sucrose solution.
- The tube was centrifuged at 2000rpm for 10 minutes.
- The material on the top of the tube was picked up with the loop and made a smear on the slide.
- The modified acid fast staining was performed and observed under 40X followed by oil immersion.

CHAPTER-V

RESULT

A total 196 stool samples were studied during the period of June to November 2005. The samples had been collected from different rehabilitation centers from Kathmandu Valley as well as from the community. In case of high risk group subjects the samples had been collected from commercial sex workers and highway drivers and conductors from outside the valley. Single stool specimens were processed of each study subjects. Among them, 112 were HIV seropositive whereas 84 were high risk group for HIV infection but their serostatus were not known. The overall prevalence of parasitic infection was found to be 35.7% (70/196) (26.7% among HIV/AIDS subjects and 47.6% among high risk group population).

5.1 Color of the stool

Out of 196, samples with normal color had positive rate of 28.3% (28/99) whereas it was 43.3% (42/97) with abnormal color.

Table 1. Distribution of parasitic infections based on stool color

S.N	Color of stool	Total tested	Parasite positive	Percentage
1	Normal color of stool	99	28	28.3
2	Abnormal color of stool	97	42	43.3
	Total	196	70	

5.2 Consistency of stool

In this study, 61 formed, 115 semi-formed and 20 loose stool samples were examined respectively. The parasitic infections were diagnosed in 70.0% of the loose stools, 33.0% in semi-formed stools and 29.5% in formed stools. The study found association of parasitic infections with the loose consistency of the stool samples.

Table 2. Distribution of parasitic infections based on stool consistency

S.N	Consistency of stool	Total tested	Parasite positive	Percentage	P-value
1	Formed	61	18	29.5	P<0.05
2	Semi-formed	115	38	33.0	
3	Loose	20	14	70.0	
	Total	196	70		

Based on whether the stool samples were mucoid or non mucoid, a total 65 mucoid stools and 131 non mucoid stools were examined respectively. The study found positive rate of 44.6% and 31.3% among mucoid and non-mucoid stool samples respectively. The result was not significant statistically.

Table 3. Distribution of parasitic infections based on stool consistency

S.N	Presence or absence of mucous	Total tested	Parasite positive	Percentage	P-value
1	Mucoid	65	29	44.6	P>0.05
2	Non-mucoid	131	41	31.3	
	Total	196	70		

5.3 Age and genderwise distribution of infection among HIV seropositive subjects

In this group, a total 63 (56.25%) male and 49 (43.75%) female were studied. Of the total, 1 was of the age group below 20 years, 100 were of the age group between 20-40 years and 11 were of the age group above 40 years. The prevalence of parasitic infections among the male was found to be 25.40% whereas in female the prevalence was found to be 28.60%. There was no significant difference of parasitic infections in both the population groups. The highest prevalence of infections was seen in the age group of 20-40 years 15 (26.8%) in case of male population and above 40 years 3 (60%) in case of female population though the result was not significant statistically.

Table 4. Age and genderwise distribution of infection among HIV seropositive individuals

S.N	Age group	Male				Female			P-value
		Total	Para+	%	P-value	Total	Para+	%	
1	<20 years	1	0	0	P>0.05	0	0	0	P>0.05
2	20-39 yrs	56	15	26.8		44	11	25	
3	>40 years	6	1	16.7		5	3	60	
	Total	63	16	25.4		49	14	28.6	

5.4 Age and genderwise distribution of infection among high risk group population

In this group, a total 43 (51.20%) males and 41 (48.80%) females were studied. The prevalence of infection in case of male was found to be 46.50% whereas in female the prevalence was found to be 48.80%, though the result was not significant statistically. The prevalence of infection in both male and female was found highest in the age group of 20-40 years though the result was not significant statistically.

Table 5. Age and genderwise distribution of infection among high risk group population

S.N	Age group	Male				Female			
		Total	Para+	%	P-value	Total	Para+	%	P-value
1	<20 years	12	5	41.7	P>0.05	7	3	42.8	P>0.05
2	20-39 yrs	31	15	48.4		34	17	50	
	Total	43	20	46.5		41	20	48.8	

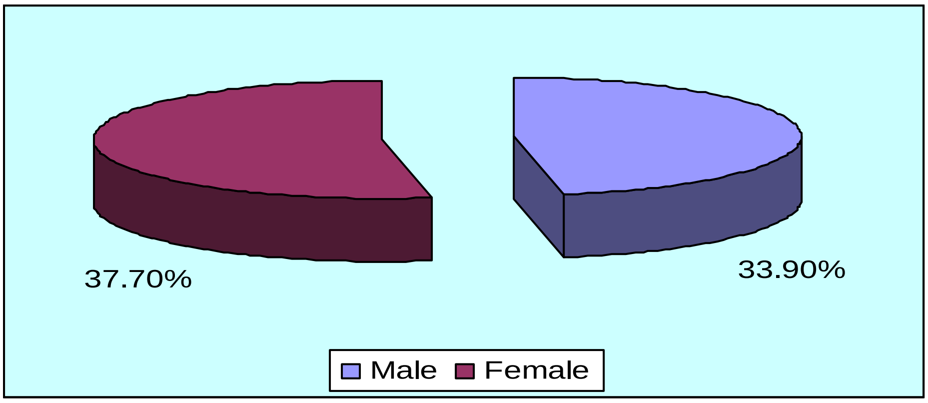

Fig 1. Genderwise distribution of parasitic infection

5.5 Ethnicwise distribution of infection

Out of 196 study population, 83 (42.3%) were *Indo-Aryans* and 113 (57.7%) were of *Tibeto-Burmans* origin. Among *Indo-Aryans*, 25 (30.1%) were found to be infected with parasites and 45 (39.8%) of *Tibeto-Burmans* were found to be infected with parasites though the result was not significant.

Table 6. Ethnicwise distribution of infection

S.N	Ethnic group	Tested	Parasite positive	Percentage	P-value
1	*Indo-Aryans*	83	25	30.1	P>0.05
2	*Tibeto-Burmans*	113	45	39.8	
	Total	196	70		

5.6 Parasitic infection among HIV seropositive subjects

Among total infected (30/112), 73.30% of the subjects were found to be infected with single parasite and 26.70% of the subjects were found to be infected with multiple parasites. The prevalence of monoparasitism and multiparasitism were 19.60% and 7.10% respectively.

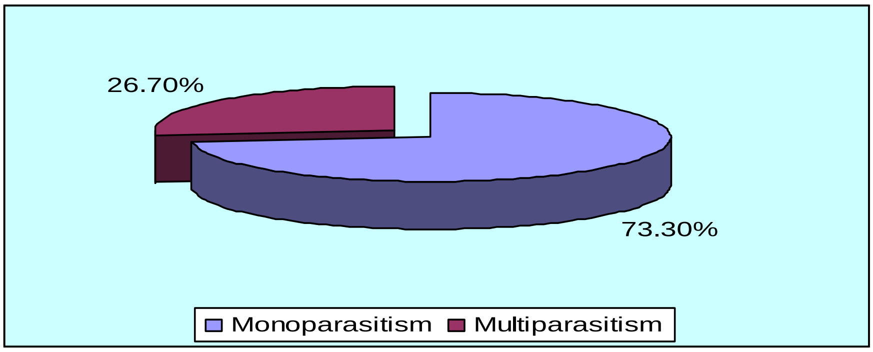

Fig 2. Monoparasitic and multiparasitic infections among HIV seropositive subjects

5.7 Parasitic infection among high risk group population

Among total infection (40/84), 90.00% of the subjects were found to be infected with single parasite and 10.00% of the subjects were found to be infected with multiple parasites. The prevalence of monoparasitism and multiparasitism were 42.80% and 4.80% respectively.

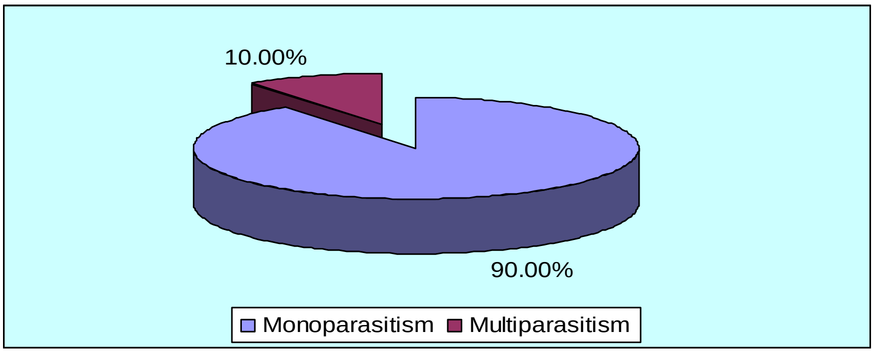

Fig 3. Monoparasitic and multiparasitic infections among high risk group population

5.8 Distribution of infection based on GIT symptoms

Out of 196 study population, 95 (48.4%) were symptomatic and 101 (51.6%) were non symptomatic. Among symptomatic cases 63 (66.3%) were infected with parasites whereas, 7 (6.9%) asymptomatic cases were found to be infected with the parasites. This study found association of parasitic infections with the gastrointestinal tract symptoms.

Table 7. Distribution of parasitic infections based on GIT symptoms

S.N	GIT Symptoms	Tested	Parasite positive	Percentage	P value
1	Symptomatic	95	63	66.3	P<0.05
2	Asymptomatic	101	7	6.9	
	Total	196	70		

5.9 Parasites detected among HIV seropositive subjects

In this group, among 30 infected subjects, 19 (63.3%) were infected with *Trichuris trichiura*, 6 (20%) were infected with *A. lumbricoides*, 4 (13.3%) were infected with Hookworm, 3 (10%) were infected with *E. histolytica*, 2 (6.7%) were infected with *C. parvum*, 2 (6.7%) were infected with *G. lamblia*, 1 (3.3%) were infected with *H. nana* and 1 (3.3%) were infected with *B. hominis.*

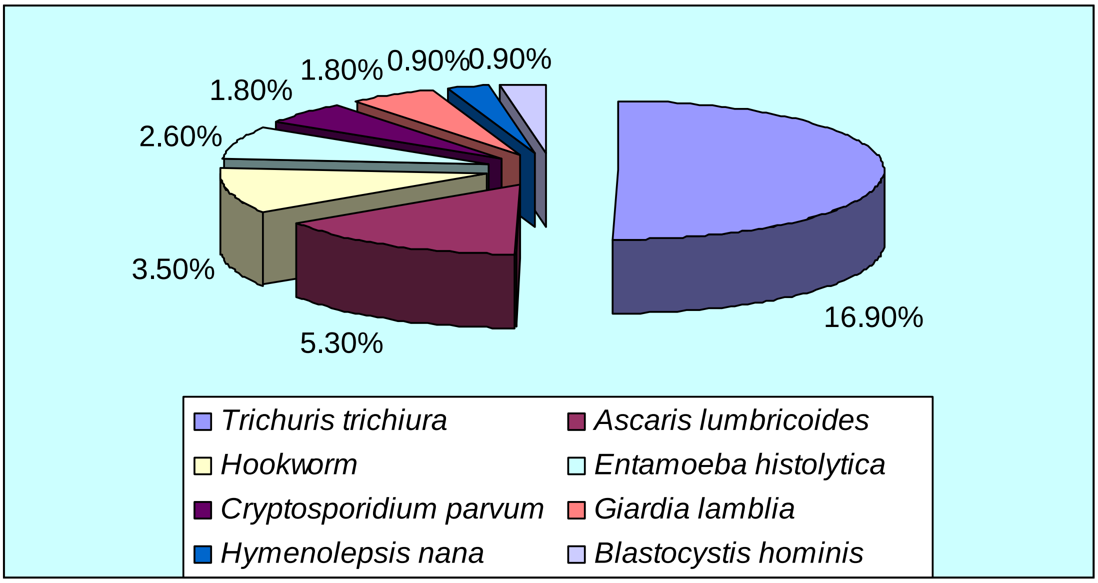

Fig 4. Parasites detected among HIV seropositive subjects

5.10 Parasites detected among high risk group population

In this group, among 40 infected subjects 25 (32.5%) were infected with hookworm, 13 (32.5%) were infected with *T. trichiura*, 5 (12.5%) were infected with *A. lumbricoides* and 1 (2.5%) was infected with *E. histolytica.*

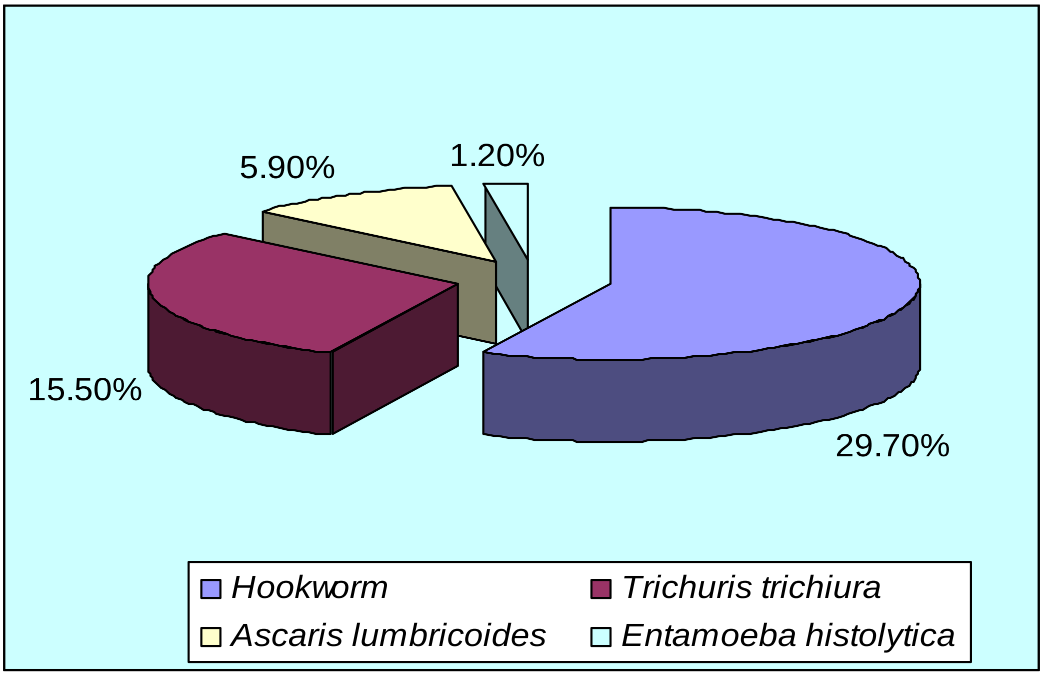

Fig 5. Parasites detected among high risk group population

5.11 Pattern of infection among HIV seropositive subjects

In this group, of the total infected, 23 (76.7%) had helminthic infections, 5 (16.7%) had protozoal infections and 2 (6.7%) had mixed infections. The overall prevalence of the helminthic infection was found to be 20.5% and the prevalence of protozoal and mixed infections were found to be 4.5% and 1.7% respectively.

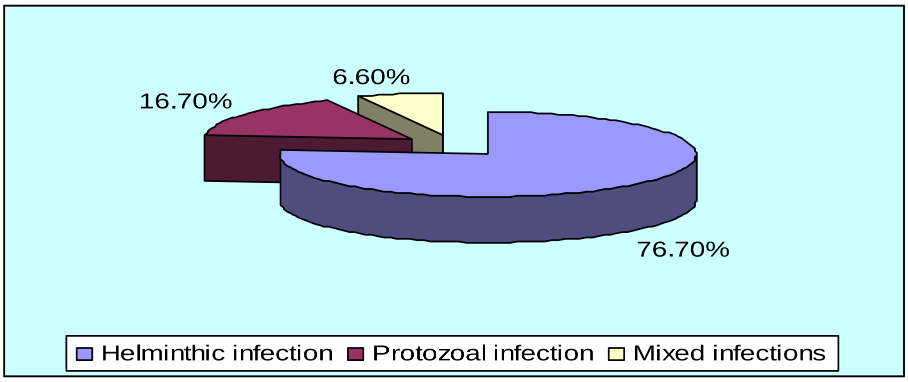

Fig 6. Pattern of infection among HIVseropositive subjects

5.12 High risk group population

In this group, of the total infected 39 (97.5%) had helminthic infection and 1 (2.5%) had protozoal infection. The overall prevalence of the helminthic and protozoal infections were found to be 46.4% and 1.2% respectively.

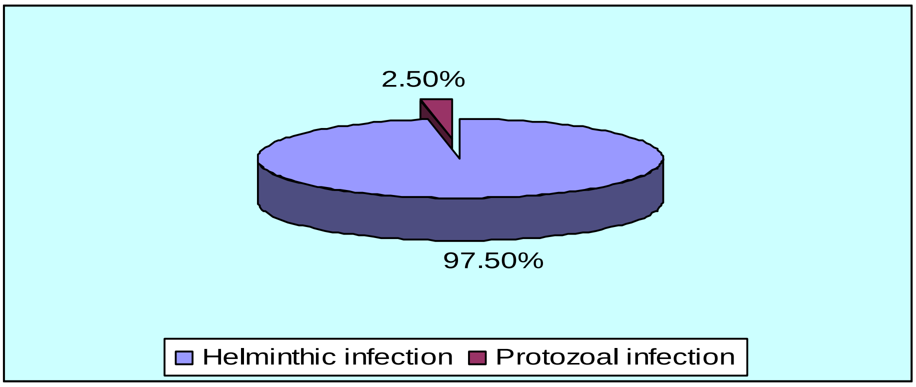

Fig 7. Pattern of infection among high risk group population

5.13 Dwelling status of HIV seropositive subjects

In this group, the present study had studied 65 (58%) subjects who were living in the different rehabilitation centers in Kathmandu and its outskirts and 47 (42%) not living in the rehabilitation centers. Among rehabilitation dwellers 12 (18.5%) were found to be infected with different intestinal parasites constituting the overall prevalence of 10.7% and among those who were living in the community, 38.3% were found to be infected with parasites constituting the overall prevalence of 16%. This study found association of parasitic infections with the dwelling status of the study population.

Table 8. Dwelling status and parasitic infections among HIV seropositive subjects

S.N.	Dwelling status	Total	Percentage	Parasite+	Percentage	Total %	P value
1	Living in rehabilitation center	65	58%	12	18.5%	10.7%	P<0.05
2	Not Living in rehabilitation center	47	42%	18	38.3%	16.0%	
3	Total	112		30		26.7%	

CHAPTER-VI
DISCUSSION AND CONCLUSION

6.1 Discussion

The overall prevalence of the parasitic infections was found 35.7% (26.7% among HIV seropositive subjects and 47.6% among high risk group population). However, the prevalence of parasitic infections among HIV/AIDS subjects ranges from 18.4% to 76.2% in different parts of the world (Awole *et al*, 2003; Gomez Morales *et al*, 1995; Guk *et al*, 2005; Hunter *et al*, 1992; Modjarrad *et al*, 2005; Mohandas *et al*, 2002; Okodua *et al*, 2003; Tamiro *et al*, 1996; Zali *et al*, 2004). In Nepal, the prevalence of the parasitic infections in such population was found 30% (Sapkota *et al*, 2004). The finding of the present study was in agreement with the findings of Guk *et al* (2005), Modjarrad *et al* (2005), Mohandas *et al* (2002), Okodua *et al* (2002), Sapkota *et al* (2004) and Tamiro *et al* (1996) and lower than the findings of Awole *et al* (2002), Gomez Morales *et al* (1995) and Hunter *et al* (1992). The finding was lower than in comparison to those studies. In comparison, the lower prevalence of intestinal parasitic infections among HIV/AIDS subjects could be correlated to the relatively high level of immune status among the study population, and the fact that diarrhoea and intestinal parasitic infections were strongly associated with lower CD_4 counts (Brink *et al*, 2002). Moreover, only one sample was taken from each patient, and it had been shown that second specimes yield more pathogen detections. In addition, incorporation of more sophisticated methods of diagnosis including jejunal fluid examination and biopsy increase the total yield of pathogens (Mukhopadhya *et al*, 1999). However, the immune status of the study population was not elucidated. The finding was higher in comparison to the finding of Zali *et al* (2004) in Iran. In addition to impaired defense mechanism in HIV/AIDS patients itself, poor hygiene and poverty might be important factors behind the burden of gastrointestinal infection in Nepalese HIV/AIDS patients. The increase in rainfall, temperature and relative humidity are contributing factors that help in rapid contamination and transmission of organisms in large dosages. Several other parameters including lack of health education, illiteracy, unsafe drinking water, lack of sanitation and hygienic knowledge, undernutrition, overcrowding, superstition, widespread faecal contamination of the environment etc. are associated with risk of diarrhoea (WHO Report on Diarrhoeal Diseases, 1994).

The reported prevalence of intestinal parasitosis in Nepal varied considerably from one study to another (Ishiyama *et al*, 2001; Gianotti, 1990; Nepal and Palfy, 1980; Ono *et al*, 2001; Rai and Gurung, 1986; Rai *et al*, 1995, 2001, 2002; Reily, 1980; Sherchand *et al*, 1996; Takemasa *et al*, 2004) with nearly 100% in some rural areas (Estevez *et al*, 1983; Nepal and Palfy, 1980; Rai and Gurung, 1986; Reily, 1980). Most of the studies were focused on school children rather than the adult population eventhough, Sherchand *et al* (1996), reported higher prevalence of the parasitic infections among adults with abdominal discomfort. Developing countries face economical, sociological and medical burdens due to the increase of rural migrants to urban areas with deterioration of already poor general public health services. In this context overcrowding and insufficient health care promote the spread of intestinal parasites (Crompton and Savioli, 1993).

Present study revealed the prevalence of parasitic infections as 25.4% and 28.6% among male and female population respectively in case of HIV/AIDS subjects and 46.5% and 48.8% among male and female respectively in case of high risk group population. Though the result showed the higher prevalence among female population, it was not significant statistically. The finding of the present study was in agreement with the findings of Hailemariam *et al* (2004), Okodua *et al* (2003) and Sapkota *et al* (2004). Similarly, the higher prevalence of the parasitic infections were seen in the age group of 20-39 years among male and above 40 years among female population in case of HIV/AIDS subjects respectively, though the result was not significant statistically. The result was in agreement with the findings of Awole *et al* (2003), Cruz *et al* (1996), Hailemariam *et al* (2004), Okodua *et al* (2003) and Sapkota *et al* (2004). In Nepal, around 80% of the total HIV positive are in the age group of 20-39 years (NCASC, 2006). The factors considered as major contributors for rapid spread of HIV and other infections in the country include poverty and the mobility of this economically productive age group for the search of temporary jobs and low level of awareness on HIV/AIDS and the parasitic infections. This study included those HIV seropositive individuals who had the prior history of injecting drug use, commercial sex workers, clients of commercial sex workers and the housewives. Majority of the sample population were of this age group, so the prevalence was also found higher. The prevalence of parasitic infections among female was higher in the age group above 40 years. This unusual finding could be due to the low number of the sample from this age group.

In both the study population, the prevalence of parasitic infections were higher among the *Tibeto-Burmans* group, though the result was not significant statistically. The ethnicwise distribution of parasitic infections among the HIV/AIDS individuals in Nepal have not assessed yet so the findings of the present study was not able to compare with other findings. However, conflicting results had been reported by many investigators among HIV seronegative individuals in Nepal (Ishiyama *et al*, 2001; Rai *et al*, 2002; Rai *et al*, 2005; Sharma *et al*, 2004).

The prevalence of the multiple parasitic infections were found to be higher among the HIV/AIDS subjects in comparison to the high risk group population though the result was not significant statistically. Similar results had been reported by Awole *et al* (2003), Cotte *et al* (1993), Feitosa *et al* (2001) and Hailemariam *et al* (2004). The strong evidence had not been suggested but it indicated the facilitated establishment of parasites in immunocompromised patients (Hailemariam *et al*, 2004). Some reports from Nepal (Estevez *et al*, 1983; Rai *et al*, 2001) and elsewhere in the world (Kasuya *et al*, 1989; Rajeswari *et al*, 1994) had shown high levels of multiple parasitic infections. This was a clear indication of large numbers of various species of parasites in the local community (Sharma *et al*, 2004).

Present study found 66.3% of the symptomatic cases, infected with the parasites. The result was statistically significant. Present study took into account the GIT symptoms such as diarrhoea, vomiting, nausea, anorexia and steatorrhoea. Similar results had been reported by other investigators elsewhere in the world (Awole *et al*, 2003; Gomez Morales *et al*, 1995; Guk *et al*, 2005; Hunter *et al*, 1992; Modjarrad *et al*, 2005; Mohandas *et al*, 2002; Okodua *et al*, 2003; Tamiro *et al*, 1996; Zali *et al*, 2004). Diarrhoea is a common complication of HIV infection, 30-90% of patients with AIDS suffer from diarrhoea at some points of their illness and the detection of aetiologic agents vary from 40 to 83% (Prasad *et al*, 2000).The findings of the present study was in agreement with the findings of Sherchand *et al* (1996), from Nepal who reported the highest prevalence of parasitic infections among children and adults with abdominal discomforts.

Present study found the prevalence of 20.5%, 4.4% and 1.8% helminthic, protozoal and mixed infections respectively among HIV/AIDS individuals. This study revealed that helminthic infections were more common than the protozoal infections. Similar results had been reported elsewhere in the world (Awole *et al*, 2003; Fontanet *et al*, 2000; Hailemariam *et al*, 2004; Kaminasky *et al*, 2004; Modjarrad *et al*, 2005; Okodua *et al*, 2003; Wiwanitkit *et al*, 2001). The finding of the present study was not in agreement with the findings of most investigators who reported the highest prevalence of the protozoal infections among HIV/AIDS patients (Arinas-Pinto *et al*, 2003; Guk *et al*, 2003; Mohandas *et al*, 2002; Prasad *et al*, 2000; Sapkota *et al*, 2004; Zali *et al*, 2004). In Nepal, the annual rate of the soil transmitted helminthiasis ranged from 18% to 36.6%. The annual incidence decreased every successive calendar year in both adults and children, irrespective of sex (Rai *et al*, 1994). Due to the contamination of environment (soil/water), over 60% Nepalese are infected with some kinds of intestinal parasites (Rai, 2005). All helminths stimulate strong immune responses (Andreassen**,** 1997; Ishikawa *et al*, 1998; Loukas and Prociv, 2001; Malhotra *et al*, 1997; Soboslay *et al*, 1997; van Dam *et al*, 1996; White *et al*, 1997). Although these responses are useful for diagnosing infection, they frequently appear not to be protective. Moreover, damage also occurs indirectly as a result of the host defense mechanisms (Jusot *et al*, 1996; Stadecker *et al*, 1998). Immune-mediated inflammatory changes occur in the skin, lungs, liver, intestine, central nervous system, and eyes as worms migrate through these organs. Systemic changes such as eosinophilia, edema, and joint pain reflect local allergic responses to the parasites. The fact that many worms are extremely long-lived means that many inflammatory changes become irreversible, producing functional changes in tissues. All helminths release relatively large amounts of antigenic materials, and this voluminous production may divert immune responses or even locally exhaust the immune potential.

People infected with helminths will be immune activated and therefore more prone to become infected with HIV; furthermore, once these individuals have been infected with HIV, the infection will progress faster, and therefore eradication of worms from dually infected individuals will ameliorate HIV progression. As helminth infection is activating the immune system, it will cause an increase in the plasma HIV viral load (VL) and thereby will also affect HIV transmission. In addition, since the chronic helminthic infection skews the immune response profile toward a T-helper type 2 (TH_2) profile and also leads to hyporesponsiveness and anergy, it will affect the ability of the host to generate potent and protective immune responses when vaccinated against HIV (Bentwich *et al*, 1995, 1997, 1998, 1999; Borkow *et al*, 2000; Borkow *et al*, 2002).

The prevalence of the parasitic infection was found lower among those HIV infected population who used to dwell at different rehabilitation centres of the Kathmandu Valley in comparison with those who did not. The result was found statistically significant. This study had not found such investigations in Nepal and elsewhere so far the literature was concerned. The lower prevalence recorded in this study might be due to awareness, improvement of environmental sanitation and regular monitoring of the HIV infected population by the physician. These facilities were not seen among those who did not dwell at the rehabilitation centers and in contrast faced the problem of stigma and social discrimination. They had faced the economic problem and even could not buy the medicine for the treatment even though they were known about the infections.

The present study detected 8 different parasites among the HIV/AIDS individuals and 4 parasites among the high risk group population. The helminthic parasite *T. trichiura* and hookworm were most prominent among HIV infected and high risk group population respectively.

Among HIV/AIDS individuals, the overall prevalence of *T. trichiura* was found 16.9% constituting the most prominent helminth in this group of population. Higher prevalence of *T. trichiura* was reported by Kaminsky *et al* (2004) from Honduras. The reported prevalence of *T. trichiura* among HIV/AIDS individuals in Nepal and elsewhere in the world varied from 2.7% to 44.3% (Awole *et al*, 2003; Dias *et al*, 1988; Hailemariam *et al*, 2004; Kaminsky *et al*, 2004; Okudua *et al*, 2003; Sapkota *et al*, 2004). The finding was in agreement with the finding of Awole *et al* (2003) from Southwestern Ethiopia, higher than the finding of Okudua *et al* (2003) from Nigeria, Hailemariam *et al* (2004) from Ethiopia and Sapkota *et al* (2004) from Nepal, and lower than the finding of Kaminsky *et al* (2004) from Honduras. The prevalence of *T. trichiura* in Nepal ranges from less than 5% to as high as 94.5% in a backward community in Bhaktapur district. In another area of the same district, the incidence was 40%. A lower prevalence of trichuriasis has been reported in American Peace Corps Volunteers (3%). In Terai area, the incidence ranges from 6.3% to 11% whereas in rural hilly areas less than 5% to 35.9% (Rai, 2005). This could be the reflection of the poor environmental sanitation and personal hygienic practices, which emphasizes the need for intervention measures at the community level to reduce the risk factors of acquiring intestinal parasites.

In Hondurus Kaminsky *et al* (2004), reported that the mean eosinophil percents for participants who were co-infected with soil-transmitted helminths was consistently and significantly higher than in those who were not infected with these types of parasites. These results are consistent with studies that show that infection with intestinal helminths results in immune responses involving cytokines produced by T-helper cell type 2, with IgE production, eosinophilia and mastocytosis (Baum *et al*, 2003; Bentwich *et al*, 1996; Bundy *et al*, 2000; Kalinkowich *et al*, 1998). The results showed a high prevalence and a great variety of helminths as single or mixed infections. They suggested high human fecal contamination and frequent exposures. Because primary parasitic infections were generally associated with low mortality rate, control programs were usually given low priority. The newly emerging relationship of helminthic infections to HIV, however, and its potential impact on HIV progression, especially in countries where co-infection with HIV is a growing reality justify efforts for strengthening such programs, and increasing measures to prevent and remedy conditions that foster transmission of parasitic infections.

On the other hand, and from a practical point of view, it is interesting to realize that in the absence of a copro-parasitological study, the finding of high eosinophil blood count should suggest to perform fecal examinations and, eventually, administer antihelminthic treatment.

The prevalence of trichuriasis was not consistent among the study population. The variation in the prevalence might be due to the geographical variation in the distribution of parasites and the type of soil found in that region and the high level of awareness among the population from where the samples had been collected. According to their demographic status, the physician used to visit them once a week and they were taking antihelminthic drug after check up. However, this parasite can escape the single dose of the antihelminths and require second for complete dose (Rai *et al*, 1998; Uga *et al*, 2004).

This study revealed the prevalence of ascariasis as 5.3% and 5.9% among HIV positive subjects and high risk group population respectively. The reported prevalence of ascariasis varied from 11.2 to 52.7% elsewhere in the world (Awole *et al,* 2004; Hailemariam *et al*, 2004; Kaminsky *et al*, 2004; Modjarrad *et al*, 2005; Okodua *et al*, 2003). However, in Nepal investigator did not report the occurance of *A. lumbricoides* among HIV infected population. Rai (2005), had reported *A. lumbricoides* as the most common parasite in Nepal. The prevalence of *A. lumbricoides* infection among Nepalese ranges from 8.5 to 90% and countryside being the most vulnerable areas. He reported that during the period of 1979 to 1995 more than half of the population in Nepal were infected with *Ascaris*. However, only 2% of American Peace Corps

Volunteers in Nepal had ascariasis indicating the positive impact of education and awareness.

Present study revealed the lower prevalence of ascariasis in comparison to the other studies (Hailemariam *et al*, 2004; Kaminsky *et al*, 2004; Modjarrad *et al*, 2005; Okodua *et al*, 2003). The result was in agreement with the finding of Awole *et al* (2003) from Southwestern Ethiopia and that reported by Rai (2005), from Nepal. In Nepal, the prevalence of such soil transmitted helminthics is decreasing day by day with the increase in the level of awareness among the people who used to dwell in the urban areas. The present study had collected most of the sample from rehabilitation centre of the Kathmandu Valley. The HIV infected people living in such places had high level of awareness as they were used to visit by the clinician once a week. They had better sanitary conditions in comparison to the other parts of the country. This study had collected single stool specimen for the investigation. This might be the reason of low prevalence. The geographical variation of the parasites might be another factor for the lower prevalence.

This study revealed the prevalence of 3.6% and 29.7% of hookworm infection among HIV seropositive subjects and high-risk group population respectively. The reported prevalence of hookworm infection among HIV/AIDS individuals in Nepal and elsewhere in the world varied from 0.8 to 39.2% (Awole *et al*, 2003; Hailemariam *et al*, 2004; Kaminsky *et al*, 2004; Modjarrad *et al*, 2005; Mohandas *et al*, 2002; Okodua *et al*, 2003; Sapkota *et al*, 2004). Hookworm is one of the commonest soil transmitted helminthes in Nepal. Rai (2005), reported the prevalence of 7% to over 80% in certain communities of Nepal. In Southern Nepal, the incidence ranges 11% to 29%. In Kathmandu Valley it ranged from 7% to 57.6%. Highest prevalence had been observed in hilly populations.

The finding of this study was in agreement with the finding of Hailemariam *et al* (2004) from Ethiopia and Sapkota *et al* (2004) from Nepal. Previous report did not show any interaction between HIV and helminthic infections (Awole *et al*, 2003). However the newly emerging relationship of helminthic infections to HIV and its potential impact on HIV progression, especially in countries where co-infection with HIV is a growing reality justify efforts for strengthening such programs and increasing measures to prevent and remedy conditions that foster transmission of parasitic infections (Kaminsky *et al*, 2004). The prevalence rate among the high risk group population was greater than the HIV infected population. This reflected the marked geographical variation, low level of awareness, provision of poor sanitary condition and high mobility (highway truckers and prostitutes) of the study population. As the sample had been collected from the rural area of Nepal, the health service provided by the government was not so satisfactory and this might be one factor for the higher prevalence.

This study revealed the prevalence of 2.7% and 1.2% of *E. histolytica* infection among HIV seropositive and high risk group population respectively. The reported prevalence of *E. histolytica* among HIV/AIDS individuals in Nepal and elsewhere in the world varied from 1.7 to 11.5% (Awole *et al*, 2003; Esfandiari *et al*, 1995; Hailemariam *et al*, 2004; Mohandas *et al*, 2002; Okodua *et al*, 2003; Prasad *et al*, 2000; Sapkota *et al*, 2004). The finding was in agreement with the finding of Mohandas *et al* (2002) from Northern India. It was somewhat lower in comparison with the previous study done by Sapkota *et al* (2004), in Nepal. The lower prevalence might be due to the study of single stool specimen and the use of traditional method of detecting cyst and trophozoite of the parasite. Incorporation of more sophisticated methods of diagnosis including jejunal fluid examination and biopsy increase the total yield of the pathogen (Mukhopadhya *et al*, 1999). Although *E. histolytica* was not classified under opportunistic parasites, it was significant compared to CD_4 counts (Sandraei *et al*, 2005). Similar result had been reported from Argentina and Brazil (Mendez *et al*, 1994; Moura *et al*, 1989). The protozoan infections such as *G. lamblia* and *E. histolytica* have not been found to be opportunistic in HIV infected patients because there is no evidence for an increased prevalence of these parasitic infections in HIV patients despite the fact that important immune defenses against them may be expected to be deranged by HIV infection. Hence, exposure to *G. lamblia* and *E. histolytica* are likely to occur independently of HIV infection, but heavier parasite loads may accumulate as well as experience delayed clearance of the parasite in individuals with concurrent HIV induced immunosuppression (Awole *et al*, 2003). However, in Nepal, Rai (2005), reported that *E histolytica* ranks second among the intestinal protozoan parasites. The reported incidence of intestinal amoebic infections ranged from less than 3 to 28.8%. In a hospital based study the year to year incidence ranged from 1.9% to 14.6%. Serological study revealed 24.6% of Nepalese have anti-amoebic antibody.

This study showed the prevalence of *Cryptosporidium* infection as 1.8% among HIV seropositive subjects whereas there was no infection among high risk group population. The reported prevalence of cryptosporidiosis among HIV/AIDS individuals in Nepal and elsewhere in the world varied from 1.2 to 11% (Awole *et al*, 2003; Ghimire *et al*, 2004; Guk *et al*, 2005; Mohandas *et al*, 2003; Okodua *et al*, 2003; Prasad *et al*, 2000; Ratnam *et al*, 1985; Sapkota *et al*, 2004; Uga *et al*, 1998; Zali *et al*, 2004). The incidence of *Cryptosporidium* in Nepal ranged from less than 1.0 to 10.4% in diarrhoeal stool samples (Dhakal *et al*, 2004; Rai, 2005; Sherchand *et al*, 1996). In a community in Southern Nepal, *Cryptosporidium* oocysts had been found in 4.4% (Rai, 2005).

The finding of the present study was in agreement with the finding of Zali *et al* (2004). The prevalence was lower in comparison with the previous study done by Dhakal *et al* (2004), Ghimire *et al* (2004), Sapkota *et al* (2004) and Sherchand *et al* (1996) from Nepal. The diarrhogenic cases in this study were very few and present study had examined the single stool specimen from the study population. This might be the reason of low prevalence. Moreover, oocyst excretion is usually low and variable hence multiple examinations and prior concentrations of stool specimens might be necessary (Awole *et al*, 2003). *C. parvum* infection alone had been associated with low CD_4 counts (Brink *et al*, 2002). This study had not collected the patients' CD_4 profile and include HIV positive rather than whether they developed the late sequlae or not. However, the number of AIDS patients with the complaint of diarrhoea were lower than in other studies done elsewhere in the world (Awole *et al*, 2003; Ghimire *et al*, 2004; Guk *et al*, 2005; Mohandas *et al*, 2003; Okodua *et al*, 2003; Prasad *et al*, 2000; Ratnam *et al*, 1985; Sapkota *et al*, 2004; Uga *et al*, 1998; Zali *et al*, 2004). The higher detection rate in other studies might be due to the use of highly sensitive methods of diagnosis.

Present study have found the prevalence of *G. lamblia* as 1.8% among HIV seropositive individuals and nil among high risk group population. The reported prevalence of giardiasis varied from 1.5 to 55% in Nepal and elsewhere in the world (Awole *et al*, 2003; Cotte *et al*, 1993; Guk *et al*, 2005; Hailemariam *et al*, 2004; Mohandas *et al*, 2002; Okodua *et al*, 2003; Sapkota *et al*, 2004; Zali *et al*, 2004). The finding was in agreement with the finding of Awole *et al* (2003) from Southwestern Ethiopia and Guk *et al* (2005) from South Korea but lower in comparison with studies in different other countries. It did not occur in greater frequencies in HIV positive patients than in HIV negative individuals (Mohandas *et al*, 2002). Reports from other countries demonstrated that the prevalence of these pathogens were not influenced by moderate reduction of cell mediated immunity (Brandonosio *et al*, 1999; Gomez Morales *et al*, 1995). However, Feitosa *et al* (2001), reported that giardiasis had been found in patients with immunological dysfunction other than HIV infection.

In Nepal, Rai (2005), reported *G. lamblia* as commonest intestinal protozoan parasite in Nepal. Symptomatic infections were mainly associated with diarrhoea and steatorrhoea. As shown by the literature published from Nepal during 1979 to 1995, *G. lamblia* constitutes most common intestinal protozoa infecting man. Similar finding was observed in a ten years study conducted in hospital population with a year to year incidence ranging from 5.2% to 26.4%. In terai area, incidence of *G. lamblia* was less than 14.0% whereas in hilly areas, it was less than 10.0%. In Kathmandu Valley, the incidence had been reported to be as high as 28.8%.

Present study found the prevalence of *B. hominis* as 0.9% among HIV seropositive individuals and nil among high risk group population. The reported prevalence elsewhere in the world vary from 2.1 to 14.1% (Awole *et al*, 2003; Hailemariam *et al*, 2004; Mohandas *et al*, 2002; Prasad *et al*, 2000; Zali *et al*, 2004). Few reports from Nepal had reported *B. hominis*. The reported incidence ranged from less than 1.0% to

24.9%. Recently, in a remote hilly region in Far-western Region, it was found to be 1.3%. The prevalence of *B. hominis* in Kathmandu Valley in patients with diarrhoea and school attending children were 2.0% and 7.9% respectively. However, one report showed no association between *B. hominis* infection and diarrhoea in travelers (Rai, 2005).

Present study had found a lower prevalence of *B. hominis* in comparison with studies in other countries. In Nepal, no previous reports were found to assess the prevalence of *B. hominis* among HIV/AIDS individuals. However, the finding was in agreement with the overall prevalence as reported by Rai (2005) from Nepal. *Blastocystis* spp. was found to be significantly higher in HIV/AIDS patients than in the control population (Hailemariam *et al*, 2004). Conflicting evidence exists as to whether *B. hominis* should also be considered as a significant cause of AIDS associated diarrhoea (Albrecht *et al*, 1995). Usually, the presence of more than 5 parasites/HPF of stool specimen was used as a criterion for positive reporting. Although the role of *B. hominis* as an emerging pathogen remains unresolved, the number of parasites excreted in stool might possibly be an indicator of its pathogenic role in HIV related diarrhoea (Prasad *et al*, 2000).

Present study had found the prevalence of *H. nana* as 0.9 % among HIV seropositive individuals and nil among high risk group population. Similar finding was reported by Awole *et al* (2003) from Southwestern Ethiopia. *H. nana* is a commonest tapeworm infecting man in Nepal. It was reported to be less than five percent; 3.3% in Southern Nepal and 4.9% in school children in Kathmandu Valley. Also a ten years study conducted in hospital populations had shown an incidence of less than five percent throughout the study period except in the very first year. Eggs of *H. nana* and *H. diminuta* had also been found in soil samples studied in Kathmandu. Very rarely, despite of therapeutic intervention, persistent hymenolepiasis for over three years had also been observed (Rai, 2005).

6.2 Conclusion

It may be concluded that in Nepalese HIV infected patients, both intestinal helminthic and protozoal parasitic infections are still highly prevalent. Similarly, the higher prevalence of multiple parasitic infections among such population indicates the severe conditions. The high prevalence of helminthic parasites among such population indicates the adequate treatment and health education, provision of adequate toilet facilities and pipe borne water so that the continually contaminating the environment with ova and larvae of parasite would be greatly reduced. Finally, in the management of HIV infected patients in Nepal with or without diarrhoeal symptoms, stool examination is still a useful investigation.

CHAPTER-VII

SUMMARY AND RECOMMENDATION

7.1 Summary

1. One hundred and ninety six stool samples were collected from 112 HIV seropositive and 84 high risk group population for HIV infection from different rehabilitation centers of Kathmandu Valley, its outskirts and from Kavrepalanchowk District. Among them 106 were male and 96 were female.

2. 70 (35.7%) were parasite positive (26.7% HIV seropositive and 47.6% high risk group population).

3. The prevalence of parasitic infection was higher among *Tibeto-Burmans* in comparison to *Indo-Aryans* ethnic group.

4. Multiple parasitic infection was higher among HIV infected subjects in comparison to high risk group population.

5. 66.3% of symptomatic cases were found to harbour parasites.

6. *Trichuris trichiura* was the commonest helminths among HIV infected subjects and hookworm was commonest among high risk group population.

7. The prevalence of parasitic infection was lower on those HIV-infected who used to dwell at the rehabilitation centers in comparison to those who didn't.

7.2 Recommendation

1. It is recommended to include HIV- negative control group of the same age group and of the same settings. This will reflect the real scenario and actual effect of such parasitic infection among HIV/AIDS patients.

2. It is recommended to include sensitive diagnostic tools for the diagnosis of opportunistic pathogens such as *Cryptosporidium* spp., *Microsporidium* spp. etc.

3. It is recommended to study the parasites as well as bacterial, viral, fungal gastrointestinal tract infections among HIV/AIDS patients.

4. It is recommended to include more subjects from the community which will reflect the accurate prevalence of parasitic infections among such populations.

5. Study throughout a year would give seasonal variation of infection.

6. Our data support the value of standard fecal examination in HIV-infected patients even is the absence of diarrhoea.

7. The intestinal parasites in HIV infected persons lead to increase in morbidity and mortality of such individual. So, it is strongly recommended to follow the control measures such as appropriate education, deworming programme, incorporation of poverty alleviation techniques and effective sanitation and supply of clear water.

8. It is recommended for HIV infected subjects to monitor their CD_4 counts regularly.

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APPENDIX-I

COMPOSITION AND PREPARATION OF REAGENTS

Carbol Fuchsin

Ingredients	amount
Basic fuchsin	4.0 gms
Phenol	8.0ml
Alcohol 95%	20.0 ml
DW	100.0ml

Basic duchsin is mixed with alcohol in a bottle.

DW is added slowly with constant shaking.

Phenol (melted in a water bath at 56^0C) is added with a pipette.

Formalin (10%)

Conc. formaldehyde (40%)	25ml
Normal saline (0.85%)	75ml

1% HCl in Methanol (1% acid alcohol)

First 3% solution was prepared as follows:

Absolute methanol or ethanol	680ml
DW	290ml
Conc. HCl	30ml

Then 1% solution was prepared by mixing 30ml of 3% solution with 60ml DW.

5% H_2SO_4

95 ml of DW was taken in a beaker.

5ml of conc. H_2SO_4 was added slowly along the side of the beaker with a pipette.

Lugol's Iodine

Stock solution

Ingredients	amount
I_2 crystals	5.0gms
KI	10.0gms
DW	100.0ml

KI was dissolved in DW and I_2 crystals were added slowly.

The solution was filtered and kept in a stoppered Amber-color bottle.

The solution deteriotes quickly hence should be prepared every 2 weeks.

Working solution (5 times diluted)

2ml stock solution+ 8ml DW

0.5% Malachite green

Malachite green	0.5gm
DW	100ml

Physiological saline

NaCl	0.85gm
DW	100ml

Sucrose solution

Sucrose crystals	500gms
Phenol	6.5gms
DW	320ml

APPENDIX-II

STATISTICAL ANALYSIS

Association of GIT symptoms among HIV+ and high risk group population

GIT symptoms	Parasite positive	Parasite negative	Total
Symptomatic	63	32	95
Asymptomatic	7	94	101
Total	70	126	196

Test statistics is $\chi 2$

H_0 : There is no significant association of GIT symptoms among HIV+ and high risk group population.

H_1: There is significant association of GIT symptoms among HIV+ and high risk group population.

$$\chi 2 = \frac{(ad - bc)^2 \ N}{(a \Gamma b) (b \Gamma c) (a \Gamma c) (b \Gamma d)}$$

We found χ^2 cal=75.2. Thus χ^2 cal > χ^2 at $\Im$ = 0.05, df = i.e. 3.841

Hence H_0 = is rejected and H_1 is accepted i.e. there is Association of intestinal parasitic infection according to gender

Association of Gender with parasitic infections

	Male	Female	Total
Intestinal parasite +ve	36	34	70
Intestinal parasite -ve	70	56	125
Total	106	90	196

Test statistics is χ^2

H_0 : There is no significant association of parasitics infection with gender.

H_1: There is significant association of parasitics infection with gender.

$$\chi^2 = \frac{(ad - bc)^2 \ N}{(a \Gamma b)(b \Gamma c)(a \Gamma c)(b \Gamma d)}$$

We found χ^2 cal=0.31. Thus χ^2 cal $<\chi^2$ at $\Im = 0.05$, df = i.e. 3.841

Hence H_0 = is accepted and H_1 is rejected i.e. there is no association of intestinal parasitic infection according to gender

APPENDIX-III

NATIONAL HIV/AIDS SCENARIO

Ministry of Health and Population

National Center for AIDS and STD Control

Teku, Kathmandu

Cumulative HIV and AIDS Situation of Nepal

As of June 30, 2006

Condition	Male	Female	Total	New cases in June 2006
HIV Positives (Including AIDS)	5012	1978	6990	340
AIDS (OUT of total HIV)	787	198	1085	38

Cumulative HIV infection by sub-group and sex

Sub-groups	Male	Female	Total	New cases in June 2006
Sex workers (SW)		619	619	1
Clients of SWs/STD	3394	103	3497	124
Housewives		1145	1145	95
Blood or organ recipients	8	2	10	0
Injecting Drug Use	1474	24	1498**	86
Children	136	85	221	34
Total	5012	1978	6990	340

Cumulative HIV infection by age group

Age group	Male	Female	Total	New cases in June 2006
0-4 Years	57	37	94	13
5-9 Years	65	41	106	17
10-14 Years	25	15	40	3
15-19 Years	194	196	390	5
20-24 Years	847	416	1263	37
25-29 Years	1237	512	1749	84
30-39 Years	1988	573	2561	136
40-49 Years	505	159	664	36
50- above	94	29	123	9
Total	5012	1978	6990	340

*Death- 336 (New death cases in June 2006- 14)

**Mode of Transmission- IDU or Sexual

Data include reports from sentinel surveillance sites and voluntary confidential testing centers.

APPENDIX-IV

QUESTIONNAIRE

Name of the institute:

Address:

Date:

Patients name/code:

Sex:

Marital status:

Height:

Weight:

Complaints (symptoms if any):

First diagnosis of HIV:

Stage of HIV related syndrome:

Any secondary/opportunistic infection diagnosed:

Medication :

Date and time of collection of sample:

Results of stool examination:

Macroscopic examination

Color

Consistency

Blood and mucous

Adult worms and segments

Microscopic examination:

Saline wet mount:

Iodine wet mount:

Kinyoun modified ZN staining:

Photograph 1: An egg of *Trichuris trichiura* (Saline wet mount, 40x)

Photograph 2: An egg of hookworm (Saline wet mount, 40x)

Photograph 3: An egg of *Hymenolepis nana* (Saline wet mount, 40x)

Photograph 4: A cyst of *Entamoeba histolytica* (Iodine preparation, 40x)

Photograph 5: A cyst of *Giardia lamblia* (Iodine preparation, 40x)

Photograph 6: Sample processing of at the Laboratory