INTESTINAL PARASITES IN HUMAN IMMUNODEFICIENCY VIRUS INFECTED PATIENTS IN KATHMANDU, NEPAL

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There are already "concentrated" epidemics of HIV within the certain high-risk behavior groups in Nepal. The aim of this study is to highlight the intestinal parasites in the role of diarrhea in Human Immunodeficiency Virus infected patients who attended in Sukra Raj Tropical and Infectious Disease Hospital of Kathmandu, Nepal from May 1, 2003 to April 30, 2004. The totals of 86 stool samples were collected from 86 HIV patients once and they were examined by direct smear methods and modified Kinyoun acid-fast stain. Here, 18 females (78.3%) out of 23 HIV patients and 40 males (63.4%) out of 63 patients were found to be infected with intestinal parasites with the prevalence of 67.4%. Though in August and October 2003, 100% samples were positive, there was statistically no significant difference with months (χ^2=18.83, P>0.05). In this study, *Cyclospora* (19.8%), *Cryptosporidium* (14.0%), *Isospora* (3.5%), *Strongyloides* (10.5%), *Ascaris* (4.7%), *Giardia*(3.5%), *Hymenolepis nana* (2.3%), *Trichuris trichiura* (2.3%), *Entamoeba histolytica* (2.3%), Hookworm (2.3%) and *Enterobius* (2.3%) were reported with statistically significant (χ^2=18.3, P<0.05). The total prevalence of coccidian and non-coccidian parasites was 37.0% and 30.2% respectively with statistically significant (χ^2=15.51, P<0.05). The prevalence was found 100% in drivers, 80% in farmers, 100% in housewives and 48.9% in sex workers. Among 48 diarrheic patients, 36 (75.0%) showed infection. 17 (35.4%) was acute diarrheic among which 11(64.7%) was positive. Among 31 (64.6%) chronic diarrheic, 25 (80.6%) was positive. The control of intestinal parasites among HIV patients involves adequate treatment and proper health education, provision of adequate toilet facilities and pipe borne water and the study of molecular mechanism of opportunistic parasites.

Key words: HIV, Intestinal parasites, Nepal

INTRODUCTION

Though the absolute number of HIV/AIDS cases is still low, there are already "concentrated" epidemics within the certain high-risk behavior groups in Nepal. As of 2003, in Nepal, about 61,000 adults and children (ages 0-49 years), 60,000 adults (ages 15-49 years) and 16,000 women (ages 15-49 years) are estimated to be living with HIV/AIDS (UNAIDS, 2004). Nepal's National HIV/AIDS strategy (2002-2006) estimates that between 100,000 and 200,000 young adults will soon become infected, with an annual toll of up to 15,000 AIDS cases and death (http://www.unaids.org/wad2004/report.html).

Intestinal parasitic infections remain an important cause of morbidity and mortality in developing countries especially among pediatrics (Okpala, 1961; Awogun, 1984; WHO; 1981). They are frequently transmitted by unhygienic habit such as direct transfer of ova or cysts from anal region to mouth, eating with unwashed hands or eating and drinking of contaminated food and drink (Okpala, 1961).

Diarrhea is a common complication of HIV positive patients inducing weight loss and cachexia. It occurs in almost 90% of AIDS patients in developing countries (Mukhopadya et *al.* 1999). In HIV infected patients, progressive decline in their immunological responses makes them extremely susceptible to a variety of common and opportunistic infections. In

recent years, numerous studies have outlined the emergence of important gastrointestinal protozoa like *Microsporidia species, Cryptosporidium* species, *Isospora belli*, and *Cyclospora cayetanensis* (Mukhopadya *et al.* 1999, Cegieslki, 1994). Approximately 100 genera and 1000 species are currently recognized worldwide, and seven genera have been reported to cause disease in man. The aim of the present study is to highlight the intestinal parasites in those Human Immunodeficiency Virus infected patients who attended in Sukra Raj Tropical and Infectious Disease Hospital of Kathmandu valley, Nepal. This study is the first careful study of *Cyclospora cayetanensis* in Enzyme Linked Immunosorbent Assay (ELISA) positive tested HIV patients who visited this hospital from May 1, 2003 to April 30, 2004.

MATERIALS AND METHODS

The study was carried out on patients admitted to the Gastro Wards at Sukra Raj Tropical and Infectious Disease Hospital, Teku, Kathmandu, Nepal from May 1, 2003 to April 30, 2004. A total of 86 HIV positive patients were included in the study, these comprised of 48 HIV patients (age 16 to 55 years) who presented with diarrhea and 38 HIV patients (age 16 to 55 years) without diarrhea. HIV infection in patients was confirmed by ELISA adopted by the hospital authorities applying the diagnosis and definition of AIDS established by the Centers for Disease Control and Prevention. All the patients enrolled in the study were interviewed using a questionnaire to collect clinical data. The HIV symptomatic patients were categorized into two groups, that is, acute (<10 days) or chronic (11days-6 months) diarrhea.

The 86 stool samples were collected from 86 patients once and brought at laboratory and they were examined by direct smear methods (saline preparation and Iodine stain method). For the detection of coccidian parasites, modified Kinyoun acid-fast stain was used for staining. The stained smears were examined by direct microscope under magnification (400 X). Stool smears of *Isospora belli, Cyclospora cayetanensis* and *Cryptosporidium parvum* positive cases were sent to Laboratory of Tropical Disease Research Centre, Tripureshwor, Kathmandu, to confirm the diagnosis. These stool samples were preserved at 2.5% $K_2Cr_2O_7$ at refrigerator but were not allowed to freeze. The presence of other protozoan and helminth parasites was recorded. Diagnostic tests for bacteria or viral enteropathogen were not performed.

Data were edited as soon as possible to detect errors, and to make sure that data were accurate, uniform and well arranged. Data were analyzed by means of tables, diagrams and calculating the value of mean ($\overline{X}$), median (M_d), standard deviation (σ) and hypothesis testing. For statistical analysis, the chi-square (χ^2) test was used. Results were considered significant if P was less than 0.05 (at 95% confidence level).

RESULTS

A total of 86 stool samples of the patients of different age and sex groups were collected and examined. Among them, 31 HIV positive patients were infected with AIDS and chronic diarrhea, as defined by the Centers for Disease Control and Prevention [Atlanta, GA] (MMWR, 1985).

Among 86 HIV infected patients, the highest samples of the male (33, 52.4%), female (14, 60.9%), and male and female (47, 54.7%) were in the age groups of 26 to 35 years. Similarly, the highest prevalence of the intestinal parasites were {male (19, 47.5%), female (13, 72.2%) and males and females (32, 55.2%)} in the age groups of 26-35 yrs. The mean ($\overline{X}$), median (M_d) and standard deviation (σ) of the age groups of the patients were as follows: $\overline{X}$ =33.49 years, M_d = 32.31 years, σ = 7.52 years (HIV positive

total male and female), $\overline{X}$ =30.16 years, M_d = 30.15 years, σ=6.55 years (HIV positive females parasitic infected) and $\overline{X}$ =34.36 years, M_d=32.75 yrs, σ = 7.79 years HIV positive parasite infected patients).

Here, 18 females (78.3%) out of 23 HIV female patients and 40 males (63.4%) out of 63 patients were found to be infected with intestinal parasites. Hence prevalence of parasites was 67.4%.

Similarly, in August and October 2004, the highest prevalence (100%) was found, while, in May 2003, the lowest prevalence (20.0%) was found. In this study, the prevalence of intestinal parasites in drivers (100%, 9 out of 9), farmers (80%, 20 out of 25), housewives (100%, 7 out of 7) and sex workers (48.9%, 22 out of 45) was found.

In the present study, *Cyclospora cayetanensis, Cryptosporidium parvum, Isospora belli, Strongyloides stercoralis, Ascaris lumbricoides, Giardia lamblia, Hymenolepis nana, Trichuris trichiura, Entamoeba histolytica,* Hookworm *and Enterobius vermicularis* were reported. Among 58 positive samples, 34(58.6%) showed single infection and 24(41.4%) showed co-infection of intestinal parasites.

Among 48 diarrheic patients, 36 (75.0%) showed infection while 12 (25.0%) had no parasitic infection. There were 17 (35.4%) acute diarrheic patients among which 11 (64.7%) was infected with intestinal parasites. Among 31(64.6%) chronic diarrheic patients, 25 (80.6%) was infected with intestinal parasites.

There was statistically no significant difference of intestinal parasites among diarrheic and non-diarrheic patients in HIV infected patients (χ^2=3.84, P≤0.05). Similarly, there was statistically no significant difference of intestinal parasites among acute and chronic diarrheic patients (χ^2=1.49, P≤0.05).

Figure 1. Age and Sex-Wise Prevalence of Intestinal Parasites in HIV Infected Patients from Sukra Raj Tropical and Infectious Disease Hospital of Kathmandu, Nepal from May 1, 2003 to April 30, 2004.

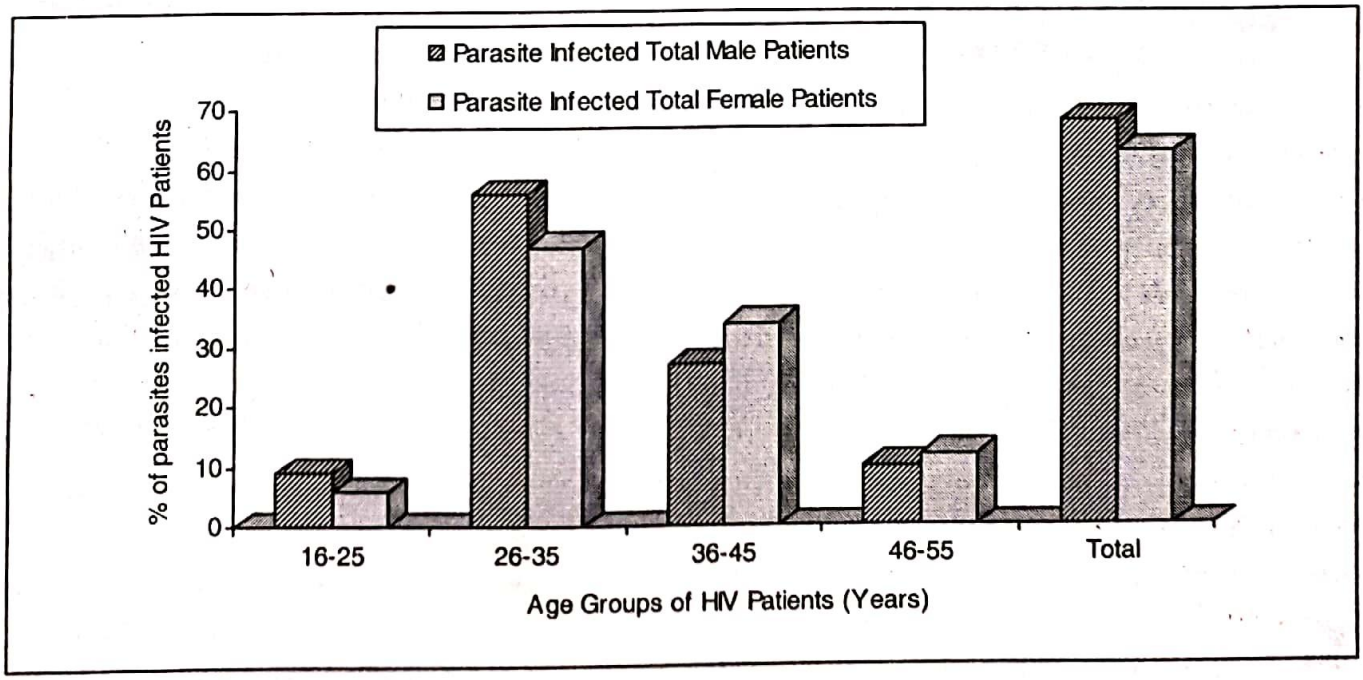
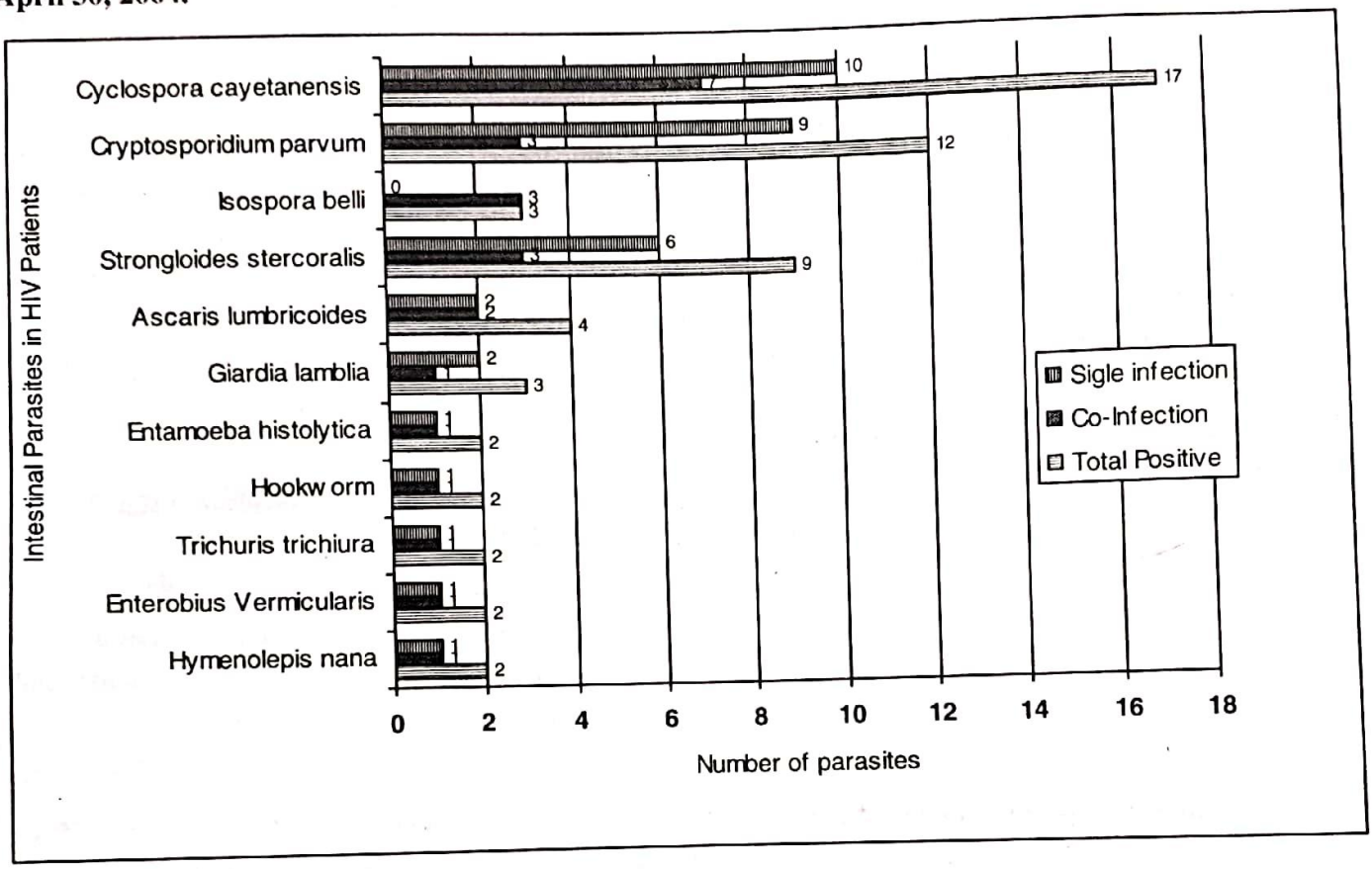


Figure 2. Distribution of Intestinal Parasites in the Fifty Eight Positive Samples of HIV Infected Patients from Sukra Raj Tropical and Infectious Disease Hospital of Kathmandu, Nepal from May 1, 2003 to April 30, 2004.



DISCUSSION

Diarrhea remains one of the most important health problems globally and a leading cause of morbidity and mortality in children, especially in developing countries. It accounts for over 50 million deaths (in all ages) worldwide and is ranked 3rd among diseases responsible for human mortality globally. This disease condition remains a major problem in the developing countries due mainly to poverty, characterized by the absence of potable drinking water, proper sanitary habits, absence of good fecal disposal system, poor hygienic practices by the impoverished citizens and over crowding (WHO, 1998). The association of protozoa pathogens with diarrhea has been on the increase (with the discovery of the *Cyclospora* in the mid and late 1990s), following the advent of the human immunodeficiency virus (HIV) infection and the Acquired Immunodeficiency Syndrome (AIDS) pandemic. Traditional diarrheal protozoa agents are *Giardia lamblia, Entamoeba histolytica Balantidium coli* and lately, coccidian agents like *Isospora belli, Cryptosporidium parvum, Microsporidia* and the newest one, *Cyclospora cayetanensis* (Wilson, 1999, Fryauff *et al.* 1999, Terra *et al.* 1998, Marshall *et al.* 1997, Clarke and McIntyre, 1996a)

Different factors contribute to the prevalence of intestinal parasites among a given population, the most important being environmental, parasitic and host factors (Chessbrough, 1998).

This is the first study of intestinal parasites in HIV patients conducted in Nepal. In the present study, the prevalence of intestinal parasites was 67.4% out of total 86 HIV infected patients. This prevalence is somewhat different from other study conducted in some other countries. The overall prevalence of 28.4% recorded in Nigeria in a study (Okodua *et al,* 2003), rate (33.6%) recorded by Agi P.I. (1995) in Sagbana community of the Niger-Delta, Nigeria and rate 44.8% among HIV infected patients reported by Awole *et al.* (2003) in Ethiopia are relatively low when compared to our present study. Awogun (1984) observed a prevalence rate of 70.8% in Ilorin, northern Nigeria which is similar to the present study. Similarly, the present result seems to be less than that of Oyerinde *et al.* (1979) who recorded prevalence rate of 89.5% in Lagos State, Nigeria.

The higher prevalence recorded among HIV seropositive patients could be as a result of low immune status of these patients. Immunocompromised individuals have been found to have a high infection rate of intestinal parasite (Chessbrough, 1998). The high rates of prevalence of intestinal parasites in HIV positive patients in our study obtained were because a) Measurements of oocysts, cysts and eggs were done with ocular micrometer to distinguish all primary parasites from the oocysts of coccidians (*Cyclospora cayetanensis*, *Cryptosporidium parvum* and *Isospora belli*). b) Wet mount by Iodine stain, acid–fast staining and bisporulation assay were done for all 86 stools samples of human immunodeficiency virus positive patients to diagnose the *Cyclospora cayetanensis* and *Cryptosporidium parvum.* c) No Coccidian positive patients were using trimethoprim-sulfamethoxazole during our study period.

In the present study, 47.5% males were infected with parasites in the 26-35 years age groups, but it was statistically no significant (χ^2=4.916, P>0.05). This result was due to low immune status of males accompanied by HIV infection. Similarly, 72.2% of females were highly infected in the 26-35 years age groups, but it was statistically no significant (χ^2=6.56, P>0.05). This high prevalence was obtained because they might have less active immune system due to reproductive age and loss of antibody through bleeding (menstruation) and HIV infection and other low risk factors like daily kitchen activities, usual contact with infected soil, water, foods and feces. There was statistically significant difference of the intestinal parasites among males and females according to age groups (χ^2=8.03, P<0.05). This might be due to the difference of immune status of each sex in different age-groups.

In the present study, there was statistically no significant difference of months and the intestinal parasites infection (χ^2=18.83, P>0.05). However, in August and October 2003, 100% positive cases were found. This may be due to low sample of stools or due to opportunistic infection of some coccidian parasites. Besides, it is difficult to explain the month wise distribution of intestinal parasites in HIV patients.

In the present study, there was significant difference of intestinal parasites and the occupation of the HIV patients (χ^2=16.54, P<0.05). Here, drivers and housewives were highly (100%) infected with intestinal parasites. This may be due to much carelessness during taking food and water and less active immune system of the drivers due to HIV infection and due to the daily kitchen activities, usual contact with infected soil, water, foods and feces and

low immunity of Nepalese housewives. Some parasites like Cyclospora *cayetanensis* is a soil borne (Bern *et al.* 1999, Koumans *et al.* 1998), waterborne (Huang *et al.* 1995, Rabold *et al.* 1994, Connor *et al.* 1993, Shlim *et al.* 1991) and food borne (Sherchand *et al.* 1999; 2001; 2003a; 2003b, Ortega *et al.* 1998, Graczyk *et al.* 1998, MMWR, 1997) and this parasite is prevalent in females (Sherchand *et al.* 1999; 2003a, Amin O.M., 1998).

There was significant difference of all parasites (χ^2=18.3, P<0.05) in the HIV patients. In the present study, *Cyclospora* (19.8%), *Cryptosporidium parvum* (14.0%), *Isospora belli* (3.5%), *Strongyloides stercoralis* (10.5%), *Ascaris lumbricoides* (4.7%), *Giardia lamblia* (3.5%), *Hymenolepis nana* (2.3%), *Trichuris trichiura* (2.3%), *Entamoeba histolytica* (2.3%), hookworm (2.3%) and *Enterobius vermicularis* (2.3%) were reported. So, the total prevalence of coccidian parasites and non-coccidian parasites were 37.0% and 30.2% respectively and the difference was statistically significant (χ^2=15.51, P<0.05). This proves that coccidian parasites are more prevalent than non-coccidian parasites in HIV patients because they may cause diarrhea in them in the absence of good immune status (Awole et al. 2003, Lindo, 1998, Pape *et al.* 1994). Since the beginning of the HIV epidemic in the world, opportunistic infections have been recognized as an important clinical complication (Zali *et al.* 2004, Vajpayee *et al.* 2003, Lebbad et al. 2001, Wiwanitkit, 2001). In particular, opportunistic protozoa play a major role as etiologic agents of chronic diarrhea in HIV-infected patients. Chronic diarrhea in such patients can profoundly compromise the absorptive function of the small intestine, and cause significant mortality (Escobedo and Nunez, 1999). The protozoan parasites, *Cryptosporidium parvum*, *Isospora belli*, *Cyclospora cayetanensis*, *Entamoeba histolytica*, and *Giardia lamblia* are the causative agents of diarrhea among this population (Mohandas *et al.* 2003).

In a study by Okodua M, (2003) *Ascaris lumbricoides* (20%), *Ancylostoma duodenale* (5.7%) *Entamoeba histolytica* (5.7%) *Entamoeba coli* (5.7%) *Cryptosporidium parvum* (5.7%) were detected more frequently. The present findings do not agree with those of Lindo *et al.* (1998) too who reported *Trichuris trichiura* (21.1%), Hookworm (17.3%), and *Strongyloides stercoralis* (7.7%) from stool samples of HIV infected individuals in San Pedro Sula, Honduras in Central America. The reason for these differences could be as a result of environmental and behavioral pattern of the people in these regions. The low prevalence of these nematodes in the present study may be due to the sandal wearing habit of some educated HIV patients in Nepal.

The high prevalence of *Strongyloides stercoralis* in this study suggests that this parasite can cause overwhelming infestation in HIV/AIDS which proves the study (Ambrioise-Thomas, 2001, Pollock, 1997). In the present study, 17 patients (19.8%) were found to be infected with *Cyclospora cayetanensis* and the prevalence 19.8% of *Cyclospora cayetanensis* observed in HIV patients was relatively low than reported from Guatemala (28%, Bern *et al.* 1999) and slightly higher than those reported in Haiti (14.0%, Verdier *et al.* 2000, 11.0%, Pape *et al.*1994) and much higher than those published from Peru (less than 1%, Cegielski *et al.*1999), from Tanzania (1%, Cegielski *et al.*1999), from Thailand (2.2%, Manatsathit *et al.*1996), from Honduras (6.8%, Girard de Kaminsky R , 1997), from Cuba (3%, Escobedo *et al.* 1999), from Egypt (4%, Abou el Naga IF, 1999), from Venezuela (9.8%, Chasin-Bonilla *et al.* 2001), India (0.7%, Kumar *et al.* 2002; 3.3%, Mohandas *et al.* 2002) and Ethiopia (3.7%, Awole *et al.* 2003).

Cryptosporidium parvum is among the opportunistic parasites commonly found in HIV patients; its detection in this study confirms this. In this study, the high prevalence of *Cryptosporidium parvum* (14.0%) as compared with report from other places like Cuba (11.9%, Escobedo *et al.*1999) and Ethiopia (11%, Awole *et al.* 2003) could be due to methodological differences. It is also possible that patients examined in this study have a high contact with carriers of *C. parvum* such as cattle which is more common in Nepal as an agricultural country.

The effect of intestinal parasites such as *Cryptosporidium parvum, Cyclospora cayetanensis, Isospora belli* and *Microsporidia* in HIV infected persons lead to increase in morbidity and mortality of such individuals (Lindo, 1998). The higher prevalence of *Cyclospora*, *Cryptosporidium* and *Strongyloides* in this study shows that a routine test of stool should be made to confirm these opportunistic parasites in HIV/ AIDS patients in Nepal. The control of intestinal parasite involves adequate treatment and proper health education, provision of adequate toilet facilities and pipe borne water so that the continually contaminating the environment with ova, cyst and larvae of parasite would be greatly reduced. The molecular mechanism of opportunistic parasites that cause diarrhea in the HIV/ AIDS patients should be studied further in Nepalese context.

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