Epidemiology of Cyclospora cayetanensis in HIV/AIDS patients in Kathmandu, Nepal

Ghimire TR[1], Mishra PN[2], Sherchan JB[3]
[1] Department of Biology, Bagmati Modern College, [2]Central Department of Zoology, Tribhuvan University, Kirtipur,
[3]Department of Microbiology and Parasitology, Tribhuvan University, Maharajgunj, Kathmandu, Nepal

ABSTRACT

Background: Intestinal parasitic infestation is one of the major health problems among HIV, a worldwide serious infection, in Nepal. *Cyclospora cayetanensis*, one of the opportunistic coccidia with limited epidemiological information, causes prolonged watery diarrhoea in these patients. The purpose of this study was to assess the epidemiological factors of *Cyclospora cayetanensis* in HIV/AIDS patients.

Methods: From May 2003 to April 2004, stool samples of HIV/AIDS patients and their family members, samples of water, soil, vegetables and human street faeces and animals' stools in and around their habitat were collected from various sites of Kathmandu and different laboratory methods were applied to record the intestinal parasites in this cross-sectional study.

Results: The prevalence of *Cyclospora* was 17.5 per cent and 26.1 per cent in males and females respectively without any statistically significant whereas, in monsoon and non-monsoon the infection was 32.1 per cent and 13.8 per cent respectively with statistically significant at 0.05 level (χ^2= 3.96, P=0.047). The *Cyclospora* was positive in soil, river, sewage, chicken, dogs, street human faeces and family members of HIV/AIDS patients. The patients who don't use soap for washing hands before meals [OR=4.76 (1.26<OR<19.50), P=0.008], the patients with usual contact with soil/fields [OR=3.55 (0.94<OR<14.45), P=0.034], the patients with daily kitchen activities [OR=2.20 (0.54<OR<8.68), P=0.29] and the patients who work as sex-work [OR=3.76 (1.00<OR<15.33), P=0.026] were more likely to be infected from *Cyclospora cayetanensis*.

Conclusions: *Cyclospora* causes chronic watery diarrhoea and loss of body weight in the absence of efficient immune system in AIDS patients. It is an opportunistic parasite in these patients and occurs with other pathogens such as *Stronayloides*, *Cryptosporidium*. Molecular diagnostic methods should be applied to identify the parasites in food, water, soil, sewage and street human faeces near the habitat of HIV/AIDS patients that will signify the epidemiological factors of cyclosporiasis.

Key words: cyclospora; epidemiology; Human Immuno Deficiency virus; Nepal

INTRODUCTION

Human Immunodeficiency Virus (HIV) infection, a worldwide infection, is a serious problem in the present day. One of the major health problems among HIV-infected patients is superimposed infection of virus, bacterium, parasite or fungus due to the defect of immunity. The risk for acquisition and the type of intestinal parasitic infection among persons with HIV infection is related to environmental sanitation, personal hygiene, diet

Correspondence: TR Ghimire, Department of Biology, Bagmati Modern College, Kathmandu, Nepal.

habit and internal defence mechanisms. *Cyclospora*, a newly recognized human protozoan parasite that causes prolonged watery diarrhoea in HIV/AIDS patients has limited epidemiological relevance especially in association with HIV infection.[1,2] Not clear risk factors of cyclosporiasis in these patients have been assessed much in the world. Food and water and sewage, soil and usual contact with animals are the risk factors for transmission of *Cyclospora* in different parts of the world.[3-13] The prevalence of the intestinal parasites in HIV/AIDS in this study was previously published in a journal and in this paper the epidemiological factors Cyclosporiasis have been assessed.[14] These factors include age and sex-wise distribution of *Cyclospora* infection among HIV patients, symptoms and pathogenicity caused by *Cyclospora*, seasonal pattern of *Cyclospora* infection, probable modes of transmission through soil, food, water and animals, and probable risk factors in Nepalese contexts.

METHODS

The study was cross-sectional descriptive type. In the first part of the study, we collected 86 stool samples from 86 HIV/AIDS patients from Sukra Raj Tropical and Infectious Disease Hospital, Teku from May 2003 to April 2004. Stool specimens were processed using a standard formalin-ethyl acetate concentration method and examined by two methods: direct light microscopy and stool smear stained with modified acid-fast stain and ocular micrometer method as previously described.[14,15]

In the second part of the study, we categorised three types of total 86 patients on the basis of laboratory findings and on the basis of some literatures of current definition given by Centres for Disease Control and Prevention (CDC), World Health Organisation (WHO) and National Recommended Case Definitions and Surveillance Standards, Ministry of Health, Epidemiology and Disease Control Division, Nepal. It included 55 HIV patients, 14 AIDS patients and 17 Cyclosporiasis AIDS patients. HIV patients meant those patients with HIV antibody detected by ELISA (Enzyme Linked Immunoabsorbent Assay) test; AIDS meant those patients with ELISA positive, chronic diarrhoea (≥1 month duration) and more than 10% weight loss; cyclosporiasis AIDS patients meant those AIDS patients whose stools contained *Cyclospora* oocysts.

From May 2003 to April 2004, samples of water, soil, green leafy vegetables, animals and humans faeces were collected every month from various sites of Kathmandu valley to determine the possible sources of infection. According to permanent and temporary addresses provided by these three types of patients and hospital authorities, we collected the samples from their nearest vegetable markets. Carrots and the leaves of cabbage, lettuce, cauliflower, green onion, radish, spinach, mustard leaves were washed in distilled water and the washings were kept in different test tubes. We sampled 25 washings of the total vegetables from different markets. Similarly, different numbers of water samples were taken from different water sources such as: sewage-9, pond-7, Dhungedhara-6, well-8, Bagmati and Bishnumati Rivers-12 and municipal tap-10.

We prepared 25 soil samples from their surroundings. We collected soil samples from five different sites in each house or room and mixed them in a beaker. We prepared the soil solution in water and filtered the soil through a thin cloth. Each water sample was kept in a test tube and prepared for centrifuge. We again collected 94 human stool samples found on the street around the habitat of the HIV/AIDS patients. These stools were proved to be those of humans on the basis of colour, odour, amount and the information provided by the people. According to the local people and street children, the stools were from the street children, homeless persons and travellers from outsides in the Kathmandu valley.

We also collected a total of 63 animal faecal samples from the owners or surroundings of the HIV/AIDS patients. We collected 11 stool samples from the family members of 3 HIV/AIDS patients living in Kathmandu valley.

We used laboratory of Central Department of Zoology, Tribhuvan University, Kirtipur and a private laboratory in Kathmandu for the whole research. The washings of vegetables, solution of soil and the water samples of different sources were centrifuged (at 3×1000 for 5 minutes, IEC Clinical Centrifuge, Made in USA 115 VAC, 50/60 Hz, Massachusetts) and examined microscopically. Animal and human faecal samples were examined by direct wet mount at 2.5 percent potassium dichromate solution. The recovery of oocysts of *Cyclospora* was confirmed by acid-fast staining and by using oculo-stage micrometer and bisporulation methods. For bisporulation assay, *Cyclospora* positive specimens were stored at an ambient temperature (approximately 23 degree centigrade) and were examined at regular intervals over a period of 2 weeks starting from the time of excretion.[14,15] We took photographs of the species of parasites found during research.

STATISTICAL ANALYSIS

We used Epi Info, Version 3.4, Epi Info (TM) 3.4.3, Database and Statistics Software for Public Health Professionals for the analysis of data. The results were analyzed by using tables, diagrams and associations were evaluated by means of statistical tools such as the prevalence rate, odds ratio (OR), relative risk (RR), Mantel-Haenszel Chi-square (x^2) test and p values. Fisher exact values (2-tailed) were used when required. The data were determined to be statistically significant at an alpha (α) level of less than 0.05.

Before collecting the stool samples from HIV/AIDS patients, we took permission first from the director of the hospital and then from the patients. We also collected the stool samples of their family members when they permitted us to do so. Some of the patients would not speak the truth regarding their behaviours/ living patterns. They thought that we were from project/ International Non-Governmental Organization and demanded money too. As we didn't comply, they didn't permit us to collect stool from their family members resulting in collection of very few samples. Our study is purely based on microscopic observations.

RESULTS

In the present study, among 63 males, 11 (17.5 per cent) were found to be infected with *Cyclospora* and among 23 females, 6 (26.1 per cent) were found to be infected. Males were found to be infected in all the age-groups from 16-25 years to 46-55 years; however, females were only infected from 16-25 years to 26-35 years age-groups. There was statistically insignificant of *Cyclospora* infection among age-groups of the males (x^2= 1.99, P=0.57). There was statistically insignificant of *Cyclospora* infection among age-groups of the females (x^2= 4.55, P=0.21). There was statistically insignificant of *Cyclospora* infection among the males and females (x^2= 0.78, P=0.37) [OR=0.60(0.17<OR<2.16), RR= 0.67(0.28<RR<1.60)] (Figure 1). The results can be seen in the figure 1.

Table 1 shows the relation of the symptoms and the presence of *Cyclospora* species and other parasites in 17 patients with HIV/AIDS. All 17 cyclosporiasis patients were suffering from chronic diarrhoea (duration ≥1 month and 4 times a day) and with more than 10 percentage weight loss. All positive patients were suffering from more than three symptoms. Two male patients of 37 and 42 years suffered from six types of symptoms. Out of 17 positive cases, 7 samples (41.2 per cent) were found to be co-infected with other intestinal parasites (*Cryptosporidium* in 4 samples, *Isospora belli* in 1 sample and *Strongyloides stercoralis* in 2 samples and *Giardia lamblia* and *Entamoeba histolytica* in 1 each sample). Maximum co-infection of *Cyclospora* (57.1 per cent) was found with *Cryptosporidium parvum*. The maximum co-infection of *Cyclospora* was found with the *Giardia lamblia*, *Entamoeba histolytica* and *Cryptosporidium parvum* in the 51 year-male patient.

The month wise prevalence of *Cyclospora*, as depicted in figure 2, in the 86 HIV/AIDS patients was as follows: May 2003: 20.0 percent (1 out of 5), June 2003: 18.2 per cent (2 out of 11), July 2003: 25.0 per cent (2 out of 8), August 2003: 40.0 per cent (2 out of 5), September 2003: 50.0 per cent (4 out of 8), October 2003:0.0 per cent (0 out of 2), November 2003:20.0 percent (1 out of 5), December 2003: 20.0 per cent (1 out of 5), January 2004: 25.0 per cent (1 out of 4), February 2004: 14.3 per cent (1 out of 7), March 2004: 14.3 per cent (1 out of 7) April 2004: 5.3 per cent (1 out of 19). The total prevalence of *Cyclospora* in the monsoon of Kathmandu (June 16- September 31 as per Central Department of Meteorology and Hydrology, Tribhuvan University, Kathmandu, Nepal, 2004) was 32.1 per cent (9 out of 28) and non-monsoon was 13.8 per cent (8 out of 58) which was found statistically significant difference at 0.05 level (Mantel-Haenszel x^2= 3.96, p=0.047). The *Cyclospora* infection is more likely prevalent in monsoon than in non-monsoon [OR= 2.96 (0.88<OR<10.09), RR= 2.33(1.01<RR<5.39)]

Table 2 shows the positive cases of *Cyclospora* in the laboratory diagnosis of different samples collected from the surroundings of HIV/AIDS patients. The *Cyclospora* positive cases were found in sewage (2 out of 9), river (1 out of 12) and soil (4 out of 45), chicken (2 out of 19), dogs (1 out of 13), human street faeces (6 out of 94), stools of family members of HIV/AIDS (2 out of 11). Among the 18 total positive samples found in this study, four were from the samples near the habitat of HIV positive patients, five from AIDS patients, and nine from cyclosporiasis AIDS patients. We detected ova of *Trichuris*, hookworm, *Ascaris*, *Taenia*, *Fasciola*, *Trichostrongylus*, unidentified Acanthocephalans, cysts of *Entamoeba*, *Giardia*, *Balantidium*, oocysts of *Cyclospora*, *Toxoplasma* and unidentified protozoan oocysts, larva of *Strongyloides*, hookworm and proglottids of *Taenia* and adult worms of and *Ascaris* from soil, sewage and human street faecal samples. In a sample of pond water, we examined *Cyclospora*-like organism (negative for bisporulation assay) and *Schistosoma*-like eggs concomitantly. The prevalence of one or more parasites in different samples was as follows: sewage (100 per cent, 39 out of 39), soil (80.0 per cent, 100 out of 125), street faeces (100 per cent, 94 out of 94), family members (63.6 per cent, 7 out of 11). We didn't record different parasites in animal faecal and water samples.

*Unsafe water source means public stand pipe, well, spring, water truck. Little safe water means municipal water piped into house or commercial bottled water.

**Untreated water means not commercially bottled or not boiled, chlorinated or filtered before drinking.

Table 3 shows the different risk factors of cyclosporiasis with the behaviours and clinical feature of HIV/AIDS patients as obtained by interview and hospital records. The risk factors include: not using toilet soap, not using soap before meals, open defaecation, using unsafe water sources, drinking untreated water, usual contact with animals, with soil/fields and with water, daily kitchen activities, consuming unwashed fruits and vegetables,

Table 1. Clinical features of 17 patients with chronic diarrhoea and more than 10 percent weight loss that passed *Cyclospora* oocysts. Kathmandu, Nepal from May 1, 2003 to April 30, 2004.

Case numbers	Age (years)	Sex	Other parasites	Symptoms
1.	16	Female	None	a, f, ap, n
2.	24	Female	None	a, f, ap
3.	25	Female	None	a, f, ap
4.	25	Male	None	a, ap, m, ebm
5	26	Male	None	a, f, ap, ebm
6.	29	Female	None	a, f, n
7.	29	Male	None	a, ap, ebm
8.	29	Male	Cp	a, f, ap, ebm
9.	31	Male	None	a, f, ap, ebm
10.	33	Male	None	a, f, ap, ebm, fv
11.	35	Female	Ib	al, f, m
12.	35	Female	Cp	al, f, m
13.	37	Male	None	a, ap, fv, al, m, n
14.	42	Male	Cp	a, f, ap, ebm, fv, n
15.	45	Male	Ss	a, f, ap, ebm
16.	47	Male	Ss	a, f, ap, ebm
17.	51	Male	Gl+Eh+Cp	a, f, ap, ebm, n

a: anorexia, al: allergy, ap: abdominal pain, ebm: explosive bowel movement, f: fatigue, fv: fever, m: myalgia, n: nausea, Cp: *Cryptosporidium parvum*, Ss: *Strongyloides stercoralis*, Ib: *Isospora belli*, Gl: *Giardia lamblia*, Eh: *Entamoeba histolytica*

Table 2. Laboratory results of *Cyclospora* in samples in and around the homes/rooms of HIV/AIDS patients, Kathamndu, Nepal.

Samples	Total samples	Numbers and Prevalence of Cyclospora	Numbers of Cyclospora samples and types of patients†
Soil	25	4 (16.0 per cent)	**1: H, 2: A, 1:AC**
All vegetables*	25	0 (0.0 per cent)	
Tap water	10	0 (0.0 per cent)	
Pond	7	0 (0.0 per cent)	
Tube well	8	0 (0.0 per cent)	
River	12	1 (6.7 per cent)	**1: H**
Dhunge dhara	6	0 (0.0 per cent)	
Sewage	9	2 (22.2 per cent)	**1: H, 1: AC**
Chicken	19	2 (10.9 per cent)	**2: AC**
Pigs	7	0 (0.0 per cent)	
Cats	3	0 (0.0 per cent)	
Dogs	13	1 (7.7 per cent)	**1: A**
Pigeon	4	0 (0.0 per cent)	
Goats	6	0 (0.0 per cent)	
Buffalos	5	0 (0.0 per cent)	
Cows	6	0 (0.0 per cent)	
Stools in street	94	6 (6.4 per cent)	**1: H, 2: A, 3: AC**
Stools‡	11	2 (18.2 per cent)	**2: AC**

*H: HIV Patients, A: AIDS patients, AC: Cyclosporiasis AIDS patients.
†Includes different numbers of samples from cabbage-6, Lettuce-4, Cauliflower-3, Green Onion-3, Radish-4, Spinach-2, Mustard Leaves-1 and Carrot-2.
‡Family members of the HIV/AIDS patients.

Table 3. Risk factors of cyclosporiasis according to the behaviours and clinical history of 17 Cyclospora infected AIDS patients and 69 Cyclospora non-infected HIV positive patients

Background characteristics of the patients	Total persons	Numbers and prevalence of *Cyclospora*	Statistical values (Cornfield 95% confidence limits for OR. Taylor Series 95% confidence limits for RR)	
Not using toilet soap	5	1 (20.0percent)	OR=1.02 (Cornfield limit invalid).	M-Hx^2=0.0
Using toilet soap	81	16 (19.8percent)	RR=1.01 (0.17<RR<6.17)	fp=1.0
Not using soap before meals	41	13 (31.7percent)	OR=4.76(1.26<OR<19.50).	M-Hx^2=6.96
Using soap before meals	45	4 (8.9percent)	RR=3.57(1.26<RR<10.07)	p=0.008
Open defecation	14	2 (14.3percent)	OR=0.63(0.09<OR<3.53)	fp= 0.73.
Toilet defecation	72	15 (20.8percent)	RR= 0.69(0.18<RR<2.67)	M-Hx2= 0.31
Using unsafe water sources*	33	7 (21.2percent)	OR=1.16(0.34<OR<3.85).	M-Hx2=0.07
Using safe water sources	53	10 (18.9percent)	RR=1.12(0.47<RR<2.66)	p=0.79
Drinking untreated water**	45	10 (22.2percent)	OR=1.39(0.42<OR<4.64).	M-Hx2=0.35
Drinking treated water	41	7 (17.1percent)	RR=1.30 (0.55<RR<3.10)	p=0.55
Usual contact with animals	75	8 (10.7percent)	OR=0.03(0.00<OR<0.17).	M=30.27.
No contact with animals	11	9 (81.8percent)	RR=0.13(0.06<RR<0.27)	fp=0.0000024
Usual contact with soil/fields	46	13 (28.3percent)	OR=3.55(0.94<OR<14.45).	M-Hx^2= 4.45.
Rare contact with soil/fields	40	4 (10.0percent)	RR= 2.83(1.00<RR<7.98)	p=0.034
Usual contact with water	32	3 (9.4percent)	OR=0.3(0.06<OR<1.25).	M-Hx^2=3.43.
Rare contact with water	54	14(25.9percent)	RR= 0.36(0.11<RR<1.16)	p=0.06
Daily kitchen activities	16	5 (31.3percent)	OR=2.20(0.54<OR<8.68).	M-Hx^2=1.62.
Rare kitchen activities	70	12 (17.1percent)	RR=1.82(0.75<RR<4.44)	fp= 0.29
Consuming unwashed fruits	26	2 (7.7percent)	OR=0.25(0.04<OR<1.30).	M-Hx^2=3.39.
Consuming washed fruits	60	15 (25.0percent)	RR=0.31(0.08<RR<1.25)	p=0.065
Consuming unwashed vegetables	31	6 (19.4percent)	OR=0.96(0.27<OR<3.28).	M-Hx^2=0.01.
Consuming washed vegetables	55	11 (20.0percent)	RR=0.97(0.40<RR<2.36)	p=0.94
Sex workers	45	13 (28.9percent)	OR=3.76(1.00<OR<15.33).	M-Hx^2=4.89.
Other (housewife, farmers)	41	4 (9.8percent)	RR=2.96(1.05<RR<8.36)	p=0.026
HIV with AIDS	31	17 (54.8percent)	OR=undefined	M-Hx^2=37.16
HIV only	55	0 (0.0percent)		p=0.000000

M-Hx^2= Mantel-Haenszel Chi-Square value, p=Probability value, fp=Fisher exact probability value

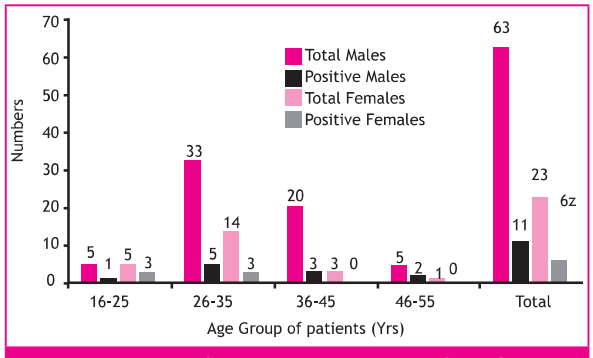

Figure 1. Age and sex-wise presence of *Cyclospora* oocysts, Kathmandu, Nepal from May 1, 2003 to April 30, 2004.

Figure 2. Month- and monsoon-wise prevalence of *Cyclospora* in the 86 HIV/AIDS patients in Kathmandu, Nepal from May 1, 2003 to April 2004.

occupation as sex work, and HIV infection followed by AIDS. The patients who don't use soap for hand washing before meals were 4.76 times more likely to be infected with *Cyclospora* than the patients who use soap for hand washing before meals [OR=4.76 (1.26<OR<19.50), p=0.008]. The patients with usual contact with soil/fields were 3.55 times more likely to be infected with *Cyclospora* than the patients with rare contact with soil/fields [OR=3.55 (0.94<OR<14.45), p=0.034].The patients with daily kitchen activities were 2.20 times more likely to be infected with *Cyclospora* than the patients with rare kitchen activities [OR=2.20(0.54<OR<8.68), pf= 0.291. The patients who work as sex workers were 3.76 times more likely to be infected with *Cyclospora* than the patients who work as housewives and farmers [OR=3.76(1.00<OR<15.33), p=0.026].

We followed a Cyclosporiasis female AIDS patient (case no.-12 in Table 1) who was suffering from intermittent diarrhoea for 4 months. She had a chicken that excreted *Cyclospora*-like organisms. She worked previously as a sex worker and currently she was working as a street vegetable vendor. She kept the vegetables for sale upon a thin plastic material on the ground in the nearest street side. She had usual contact with animals, water and soil. Though we found *Cryptosporidium* oocysts and some unidentified coccidia only from the vegetable samples, we detected multiple parasites including *Cyclospora* in the range of 6 oocysts per field of 400X from the stools and soils collected from the same site. We followed another cyclosporiasis male AIDS patient (case no.-15 in table 1) whose wife and daughter (neither of them are HIV infected) excreted *Cyclospora* oocysts. They had no gastrointestinal symptoms. But the patient was suffering from chronic diarrhoea for 6 months. He had a dog that also excreted *Cyclospora*-like oocysts. By profession he used to work as a sex worker but during the study, he was busy working in fields. He used to drink untreated water and water from the unsafe sources. We found *Cyclospora* positive stool in the nearest street and another positive case from the sewage water around his home.

DISCUSSION

Human Immunodeficiency Virus (HIV) infection is a world wide problem in the present day context with about 42 million people infected globally with about 60 thousand adults (ages 15-49) infected in Nepal.[16,17] Gastrointestinal involvement in HIV/AIDS is almost universal, and significant disease occurs in 50-90 percent of patients while diarrhoea can be a manifestation or a life threatening complication in HIV patients sometimes during the course of the disease.[18] The aetiology for such diarrhoea could either be parasitic, bacterial, fungal, enteric virus or HIV itself.[19] They are frequently transmitted by unhygienic habits such as eating with unwashed hands or consuming contaminated food and drink or activities which result in direct transfer of ova, cysts or oocysts from anal region to mouth.[20]

Cyclospora, one of the coccidian protozoa, has been reported with increasing frequency in North, Central, and South America, the Caribbean, Southeast Asia, Nepal, India, Bangladesh, Africa, Australia, England, Canada and Eastern Europe, Guatemala, Italy, China, Malaysia, Thailand, Brazil, Saudi Arabia.[21] The most highly endemic areas however are, in Peru[2], Haiti[22] and Nepal.[2,7,11,13-15,22]

Limited information is available on *Cyclospora cavetanensis* infection in patients with HIV: infection rates of 0.1 per cent in developed countries and less than 1 per cent to 19.8 per cent in developing countries have been reported.[14,15,21,23-27]

In this study, females are found to be more susceptible to *Cyclospora* than males. This is due to the less active immune system of the females followed by the loss of antibody through bleeding (menstruation), parturition and HIV infection with AIDS.

All 17 *Cyclospora* patients suffered more than 10 percent weight loss and chronic watery diarrhoea. The presence of opportunistic infective pathogens *Cryptosporidium*, *Isospora belli* and *Strongyloides stercoralis* in the stools of cyclosporiasis patients shows that these parasites are significantly more frequent in the low immunity group with diarrhoea.[28,29] Here, ten AIDS patients had *Cyclospora* as the sole parasite, which can describe this coccidian as a cause of chronic watery diarrhoea in the absence of efficient immune system in these patients.[18,20] *Cyclospora* can be considered as an opportunistic infection in the presence of HIV infection because it preceded the development of AIDS in 54.8 per cent patients (17 cyclosporiasis cases out of 31 AIDS patients) which is 37 per cent in another study.[25]

The highest prevalence of *Cyclospora* in September, 2003 and the zero prevalence October, 2003 are due to the small size of the samples. The results might be explained on the basis of the late start of monsoon in 2003 in Kathmandu. The occurrence of *Cyclospora* can be seen as related to the rain only during monsoon but not during other seasons. The prevalence obtained in different months in the study does not synchronize with the rainfall. The seasonality of *Cyclospora* in HIV patients is not uniform among Guatemala, Peru, Indonesia and Nepal and defies easy explanation.[25,31]

The detection of *Cyclospora* and other intestinal parasites in sewage and river water, soil and human faeces in street proves that water is an important vehicle for transmission of these parasites. The water and sewage contaminated

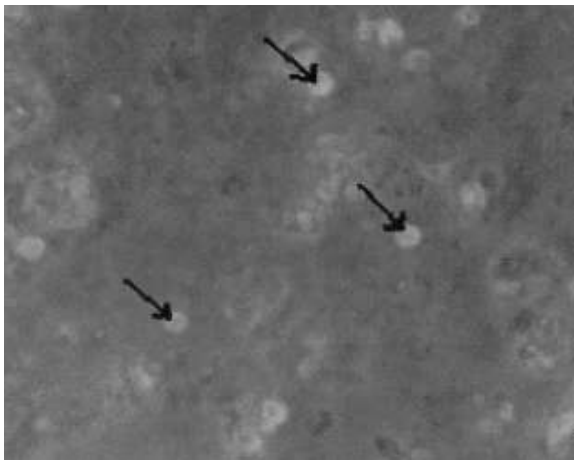

Figure 1. *Cryptosporidium* spp. at X 800 (Phase Contrast Microscope)

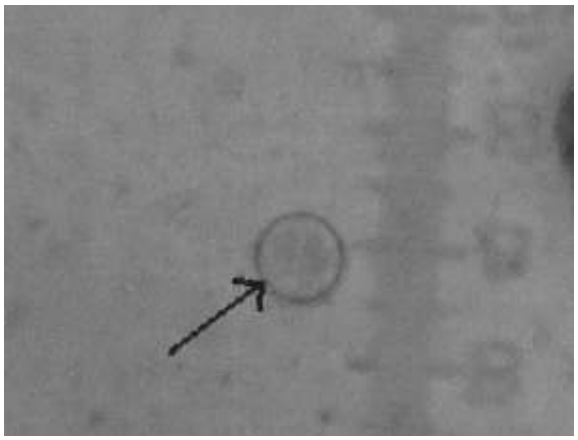

Figure 2. Entamoeba coli at X 800 (Iodine stain)

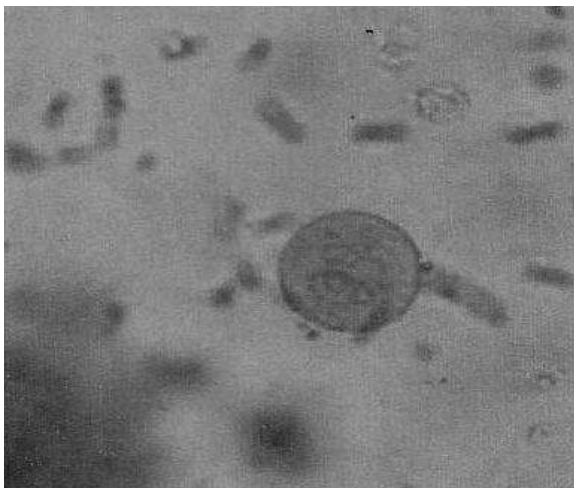

Figure 3. *Entamoeba histolytica* at X 800 (Iodine stain)

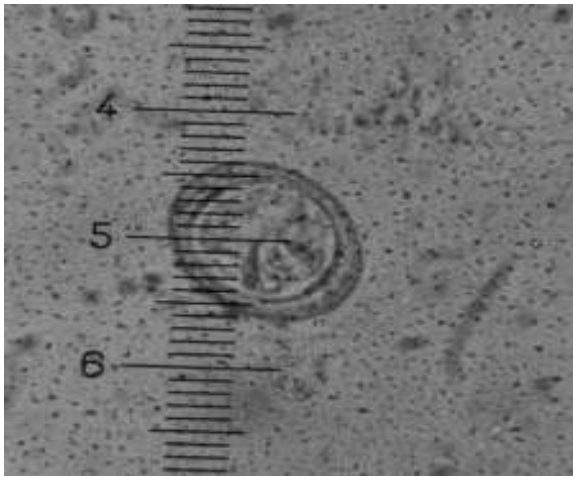

Figure 4. *Hymenolepis nana* at X 400 (Wet Mount)

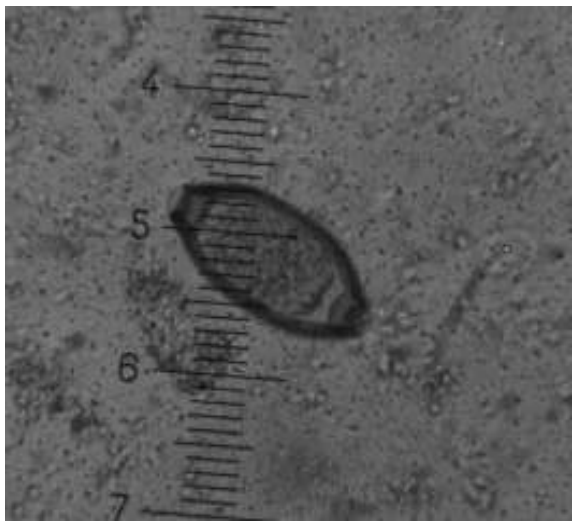

Figure 5. *Trichuris trichiura* at X 400 (Wet Mount)

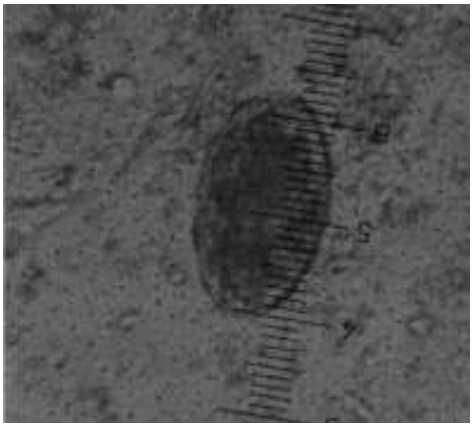

Figure 6. Egg of unknown helminth at X 400 (Wet Mount)

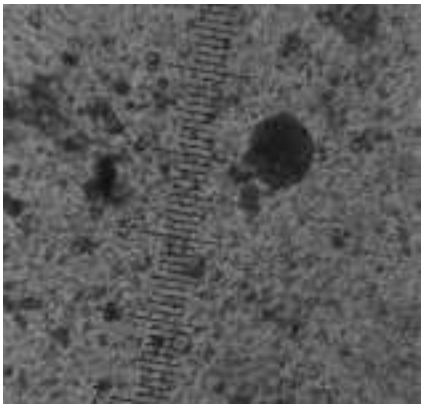

Figure 7. *Cyclospora* at X 400 (Wet Mount)

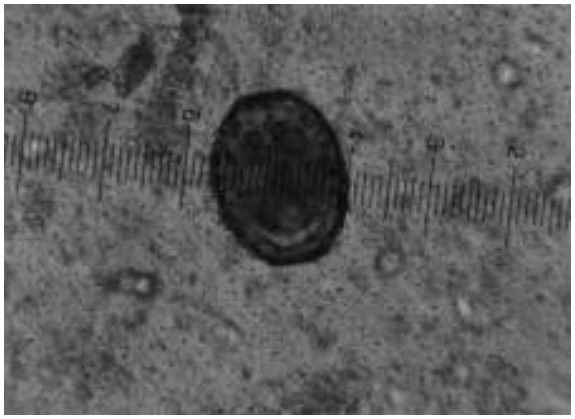

Figure 9. *Ascaris lumbricoides* at X 400 (Wet Mount)

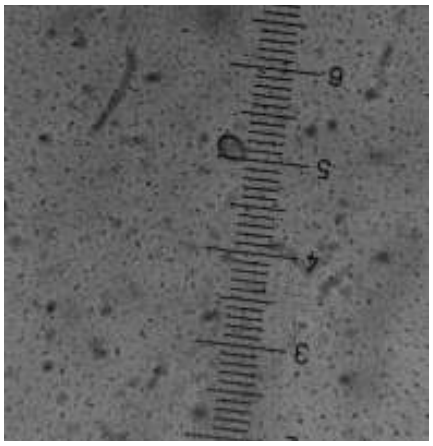

Figure 8. *Giardia lamblia* at X 400 (Wet Mount)

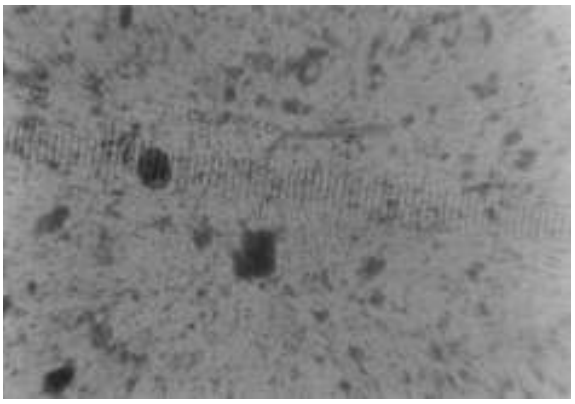

Figure 10. *Strongyloides stercoralis* larva at X 100 (Wet Mount)

with *Cyclospora* have previously been identified as risk factors for infection in multiple countries during both outbreak and non-outbreak situation.[5-10,32] One of the most important river and sewage pollution is from the contact of waste disposal deposits in Nepal.[13] The street children and homeless persons have low socioeconomic status and they dispose the faeces near the road side or along the river side at nighttimes and contaminate these sources. The presence of *Cyclospora* in the previously described samples shows that these people are the good carriers of the faecalborne, waterborne and soilborne coccidia.

Our results showing the absence of *Cyclospora* in food might be due to low sensitivity of the diagnostic tools applied in our study. Food is easily contaminated and provides parasites an optimal environment for survival prior to host ingestion. The following are the possible epidemiological routes of foodborne transmission of other intestinal parasites: in rainy season, the seepage of water from the distributed pipe sources or rain water may contaminate vegetables either when they are openly kept on the ground for selling or in the fields just before harvesting, fertilization of plants with human waste or indirectly via contaminated water used for crop irrigation and to wash products. Washing vegetables does not completely remove *Cyclospora*, *Cryptosporidium*[4] and other parasites.

The *Cyclospora* positive results from fresh stools of chicken and dog suggest that either these animals might act as paratenic hosts for this coccidian or the presence of oocysts might be due to cross-contamination human and animal stools through seepage of water. There are also reports of this parasite in the dogs and poultry.[13,32,33]. But we couldn't identify whether this *Cyclospora* species represents *Cyclospora cayetanensis*[34] or an undescribed species of coccidia.

The presence of maximum *Cyclospora* samples in the family members of cyclosporiasis AIDS patients can be explained on the basis of transmission due to close contact with risk factors such as infected patients or carelessness in handling and cross-connection of contaminated food, soil, faeces and water.

The infection of *Cyclospora* is directly related to the behaviours and clinical history of the HIV/AIDS patients. The factors such as not using soap for washing before meals, usual contact with soil/fields, daily kitchen activities and sexwork as occupation satisfy as risk for the *Cyclospora* transmission. *Cyclospora* is a finger-borne parasite. It is more prevalent in the people with the usual contact with soil or fields, where there is probability of contamination of soil in fingers. Similarly, due to daily kitchen activities, there is usual contamination of polluted water and food. Most of the sex workers in Nepal are HIV infected and are more easily contaminated with *Cyclospora* like coccidia due to actively involvement in sexual and other activities such as eating, drinking, sleeping in groups. It requires days or weeks for *Cyclospora* oocysts to be infected and there will be low chance of sporulated oocysts transmission by a single unusual sexual behaviour such as oro-anal sex. However, there will be probability of oocysts sporulation outside the body near the anal region at favourable environment in the presence of poor hygiene and regular oro-anal sexual behaviour may be the route of infection in sexworkers. Though statistically not determined, HIV people characterized by AIDS were infected by 54.8 per cent with *Cyclospora* proving AIDS to be an important risk factor of cyclosporiasis [OR=undefined, Mantel-Haenszel x^2=37.16, p=0.000000]. So, these characters are the risk factors of cyclosporiasis in these patients in Nepal. It is not easy to explain other factors as risk for the *Cyclospora* transmission due to the variation of Cornfield Limits, Taylor series, chi-square values and odds ratios and relative risks.

Epidemiologically, *Cyclospora* causes chronic watery diarrhoea in the absence of efficient immune system in AIDS patients. It is an opportunistic parasite in these patients and occurs with other pathogens such as *Strongyloides*, *Cryptosporidium*. The study of *Cyclospora* transmitting routes (street children and homeless persons in Kathmandu valley defecate near road and riverside from where *Cyclospora* washed by rain water moves into the food items) remains incomplete due to low sensitivity test. Appropriate molecular diagnostic methods should be applied to diagnose the parasites in food, water, soil, sewage and street human faeces near the HIV/AIDS patients so that the cross connection of *Cyclospora* can be confirmed among these patients. These diagnostic tools should also be applied to perform the in-ovo and in-vivo study in some animals like chicken, dogs to understand the life cycle of these parasites in cyclosporiasis HIV/AIDS patients. Besides, concerned authorities should encourage and support the family members of HIV/AIDS patients, street children and homeless persons to examine their stools in a good laboratory and to treat them with free antiparasitic drugs.

ACKNOWLEDGEMENT

We acknowledged Dr. Ram Baran Yadav, former health minister of Government of Nepal, for his idea of laboratory tools and manuscript preparation and also to Dr. Shiva Shankar Jha, Director, Sukra Raj Tropical and Infectious Disease Hospital for giving us the permission to collect the samples in the hospital. We also like to thank Dr. Ananda Ballav Joshi, Associate Professor, Institute of Medicine, Tribhuvan University Teaching Hospital, Maharajguni, Kathmandu for his best guidance for the whole research. Dr. Tej Kumar Shrestha, Professor, Central Department of Zoology, Tribhuvan University, Kirtipur, Nepal, for providing necessary laboratory facilities for his help during sample collection, processing, examination and interview with HIV/AIDS patients and Mrs. Shailee Singh Rathour, Nepal Health Research Council, Ram Shah Path, Kathmandu for her supports during manuscript preparation. We are grateful to authorities of Nepal Academy of Science and Technology, Satdobato, Lalitpur and Central Department of Zoology, Tribhuvan University, Kathmandu for providing dissertation grant partly to complete this research.

REFERENCES

1. Tarimo DS, Killewo JZJ, Mnjas IN, Msamanga GI. Prevalence of intestinal parasites in patient with enteropathic AIDS in North-Eastern Tanzania. E Afr Med J. 1996; 73: 397-9.
2. Ortega YR, Sterling CR, Gilman RH, Cama VH, Diaz F. Cyclospora species-a new protozoan pathogen of humans. N Eng J Med. 1993; 328:1308-12.
3. Connor BA, Shlim DR, Scholes IV, Rayburn JL, Reidy J, Raiah R. Pathogenic changes in the small bowel in nine patients with diarrhoea associated with a coccidia-like body. Ann Intern Med. 1993; 119: 377-82.
4. Ortega YR, Roxas CR, Gilman RH et al. Isolation of Cryptosporidium parvum and Cyclospora cavetanensis from vegetables collected in markets of an endemic region in Peru. Am J Trop Med Hyg. 1997; 57: 683-6.
5. Rabold JG, Hoge CW, Shlim DR. Cyclospora outbreak associated with chlorinated drinking water. Lancet. 1994; 344: 1360-1.
6. Bern C, Hernandez B, Lopez MB et al. Epidemiologic studies of Cyclospora cavetanensis in Guatemala. Emerg Infect Dis. 1999; 5: 766-74.
7. Hoge CW, Shlim DR, Rajah R et al. Epidemiology of diarrhoeal illness associated with coccidian-like organism among travellers and foreign residents in Nepal. Lancet. 1993; 341: 1175-9.
8. Hale D, Aldeen W, Carroll K. Diarrhoea associated with cyanobacteria-like bodies in an immunocompetent host: an unusual epidemiological source. JAMA. 1994; 271:144-5.
9. Ooi WW, Zimmerman SK, Needham CA. Cyclospora species as a Gastrointestinal Pathogen in immunocompetent Hosts. J Clin Microbiol. 1995; 33: 1267-9.

10. Wurtz R. Cyclospora: a newly identified intestinal pathogen of humans. Clin Infect Dis. 1994; 18: 620-3.

11. Ghimire TR. Mishra PN. Intestinal parasites and haemoglobin concentration in the people of two different areas of Nepal. J Nep Health Res Counc. 2005; 3: 1-7.

12. Koumans EHA. Katz ID. Malecki MI et al. An outbreak of cyclosporiasis in Florida in 1995: a harbinger of multistage outbreaks in 1996 and 1997. Am J Trop Med Hygiene. 1998; 59: 235-42.

13. Sherchand JB. Cross JH. Emerging Pathogen Cyclospora cayetanensis in Nepal. Southeast Asian J Trop Med Public Health. 2001; 32: 143-50.

14. Ghimire TR. Mishra PN. Intestinal parasites in the Human Immunodeficiency Virus Infected patients in Kathmandu, Nepal. The Nep J Zool. 2006; 1: 9-19.

15. Eberhard ML. Pieniazek NJ. Arrowood MJ. Laboratory diagnosis of Cyclospora infections. Arch Pathol Lab Med. 1997; 121: 792-7.

16. World Health Organisation (WHO). Joint United Nations Programme on HIV/AIDS (UNAIDS). AIDS epidemic update: UNAIDS/WHO. 2002; 2 – 41.

17. Regional and world data for this indicator are from the UNAIDS AIDS Epidemic Update December 2004. http://www.unaids.org/wad2004/report.html, assessed: January, 2005.

18. Awole M. Gebre-Selassie S. Kassa T. Kibru G. Prevalence of intestinal parasites in HIV-infected adult patients in south-western Ethiopia. Ethiop J Health Dev 2003; 17: 71 – 78.

19. Soave R. Framm SR. Agents of diarrhoea. Med Clin North Am. 1997; 81: 427-47.

20. Okpala I. A survey of the incidence of intestinal parasites among Government Workers in Lagos, Nigeria. West Afr Med J. 1961; 10: 148 –57.

21. Ortega YR. Sterling CR. Gilman RH. Cyclospora cayetanensis. Adv Parasitol. 1998; 40: 400-18.

22. Eberhard ML. Nace EK. Freeman AR. Cyclospora cayetanensis in domestic animals in an endemic area in Haiti. J Parasitol. 1999; 85: 562-3.

23. Long EG. Ebrahimzadeh A. White EH. Swisher B. Callaway CS. Algs associated with diarrhoea in patients with Acquired Immunodeficiency Syndrome and in travellers. J Clin Microbiol. 1990; 28: 1101-4.

24. Wurtz RM. Kocka FE. Peters CS. Weldon-Linne CM. Kuritza A. Yungbluth P. Clinical characteristics of seven cases of diarrhoea associated with a novel acid-fast organism in the stool. Clin Infect Dis. 1993; 16: 136-8.

25. Pape JW. Verdier R-I. Boncy M. Boncy J. Johnson WD Jr. Cyclospora infection in adults infected with HIV: clinical manifestations, treatments and prophylaxis. Ann Int Med. 1994; 121: 654-7.

26. Soave R. Cyclospora: an overview. Clin Infect Dis. 1996; 23: 429-37.

27. Cegielski JP. Ortega YR. McKee S et al. Cryptosporidium, Enterocytozoon and Cyclospora Infections in Pediatric and Adult Patients with Diarrhoea in Tanzania. Clin Infect Dis. 1999; 28: 314-21.

28. Wiwanitkit V. Intestinal parasitic infections in Thai HIV-infected patients with different immunity status. Bio Med Central Gastroenterol. 2001; 1: 3.

29. Chacin-Bonilla L. Estevez J. Monsalve F. Quijada L. Cyclospora cayetanensis Infections among diarrhoeal patients from Venezuela. Am J Trop Med Hyg. 2001; 65: 351-4.

30. Kumar SS. Ananthan S. Saravanan P. Role of coccidian parasites in causation of diarrhoea in HIV infected patients in Chennai. Ind J of Med Research. 2002; 116: 85-89.

31. Herwaldt BL. Cyclospora cayetanensis: A review focusing on the outbreaks of cyclosporiasis in the 1990's. Clin Infect Dis. 2000; 31: 1040-57.

32. Yai LE. Bauab AR. Hirschfield MP. do Loiveira ML. Damkaceno IT. The first two cases of Cyclospora in dogs, Sao Paulo, Brazil. Rev Int Med Trop Sao Paulo. 1997; 39: 177-9.

33. Garcia-Lopez HL. Rodriguez-Tovar LE. Medina-de la Garza CE. Identification of Cyclospora in poultry [letter]. Emerg Inf Dis. 1996; 2: 356-7.

34. Ortega YR. Gilman RH. Sterling CR. A new coccidian parasite (Apicomplexa: Eimeriidae) from humans. J Parasitol. 1994; 80: 625-9.