

# Enteric Opportunistic Parasitic Infections Among HIV-Seropositive Patients in Kathmandu, Nepal

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## ABSTRACT

### Background

Enteric opportunistic parasitic infections are the major source of diarrheal disease in developing countries mainly in Human Immunodeficiency virus (HIV) infected patients.

### Objective

The study was to detect enteric parasites causing diarrhea and their association with immune status in HIV-seropositive patients.

### Methods

The present study was conducted in Dirgh-Jeevan Health Care Research Center and Tribhuvan University Teaching Hospital, Public Health Research Laboratory, Kathmandu, Nepal between June 2010 and May 2011 involving 146 Human Immunodeficiency virus (HIV) positive patients. Serostatus from these patients were detected by Enzyme Linked Immunosorbent assay. CD4+ T cell counts were done by flow cytometry. Stool was examined for enteric parasites by microscopy with special staining methods.

### Results

A total of 146 HIV sero-positive patients with and without diarrhea age between 20 to 45 years were included in the study. Of the 146 patients, the protozoan parasitic infection was found in 30.13% (44/146). Out of 146 patients, 78 had diarrhea in which parasitic infection was 39 (50%) and 7.35% (5/68) protozoal parasites positive cases did not have diarrhea. A significant difference ( $p < 0.05$ ) was observed in the level of infection of intestinal protozoan between the HIV seropositive with diarrhea and HIV-seropositive without diarrhea. Out of 43 patients whose CD4+ T cells were  $< 200/\mu\text{l}$ , 29 (67.4%) had opportunistic parasitic infection whereas out of 103 patients whose CD4+ T cells were  $\geq 200/\mu\text{l}$ , only 15 (14.56%) had opportunistic parasitic infection ( $P < 0.05$ ).

### Conclusion

Enteric opportunistic parasitic infections were detected in 30.1% among HIV-seropositive patients and low CD4+ T count indicated high enteric opportunistic infection. Early detection of enteric parasitic infections will help in the management and to improve the quality of life for HIV-infected individuals.

## KEYWORDS

Diarrhea, HIV, Opportunistic parasites

## INTRODUCTION

Enteric opportunistic parasitic infections are major source of diarrheal disease in developing countries mainly in HIV infected patients. The progressive decline and ultimate destruction of immune system functions, which are characteristic for AIDS, usually result in morbidity and ultimately death due to opportunistic bacterial, viral, fungi

and parasitic infections.<sup>1</sup> Gastrointestinal infections are very common in patients with HIV infection or AIDS.<sup>2</sup> Diarrhea is a common clinical presentation of these infections. Reports indicate that diarrhea occurs in 30-60 % of AIDS patients in developed countries and in about 90 per cent of AIDS patients in developing countries.<sup>3</sup> The presence of

opportunistic parasites *Cryptosporidium parvum*, *Isospora belli* and *Microsporidia* are documented in patients with AIDS.<sup>4,5</sup> Moreover, newly emerging coccidian parasites *Cyclospora cayetanensis* has been reported from HIV-AIDS patients with severe diarrhea in Nepal, India, Peru, Latin America, United States and Papua ne Guinea.<sup>6-9</sup> Non opportunistic parasites such as *Entamoeba histolytica*, *Giardia lamblia*, *Trichuris trichiura*, *Ascaris lumbricoides*, *Strongyloides stercoralis* and *Ancylostoma duodenale* are frequently encountered in developing countries but are not currently considered opportunistic in AIDS patients.<sup>10,11</sup> In immunocompromised patients, the intestinal opportunistic parasites probably play a major role in causing chronic diarrhea accompanied by weight loss.<sup>12</sup> The incidence and prevalence of infection with a particular enteric parasite in HIV/AIDS patients is likely to depend upon the endemicity of that particular parasite in the community.<sup>10</sup> *C. parvum*, *I. belli* and *E. histolytica* has been reported as the most frequently identified organisms in HIV infected individuals with diarrhea from India and other parts of the world.<sup>9,13-15</sup> HIV/ AIDS infection are rapidly causing a major threat in Nepal. But the study on enteric parasite in HIV/AIDS patients in Nepal is very scarce.<sup>6</sup> Hence the current study was conducted to determine the prevalence of enteric opportunistic parasitic infections among HIV-seropositive patients with and without diarrhea in Kathmandu, Nepal.

## METHODS

The study was carried out in Dirgh-Jeevan Health Care Research Center, Tripureswar and Tribhuvan University Teaching Hospital, Public Health Research Laboratory, Kathmandu, Nepal between June 2010 and May 2011. Ethical approval was taken from IRB prior to study. A total of 146 HIV seropositive patients with and without diarrhea participated in the study after giving consent and provided two consecutive stool samples. Before collecting the samples, patient information such as name, age, sex, occupation, clinical history as well as history of diarrhea, antibiotic and antiparasitic treatment history was obtained. Patients already on antiparasitic and antibiotic treatment were excluded from the study.

Blood samples were collected in plain and ethylenediaminetetraacetic acid (EDTA) vials with five ml each from all enrolled patients. Serum samples were used for HIV testing. HIV serostatus of the patients was determined by using commercially available ELISA antibody tests (Genetic system, Biorad Labs, USA and Tridot, J Mitra & Co., India). EDTA blood samples were used for CD4 cell counts and measured by using flow cytometry (Partec, GmbH, Germany). Briefly, 20 µl of CD4 PE antibody was placed in to a Partec test tube and 20 µl of well-mixed whole EDTA blood was added, mixed gently and incubated in the dark for 15 minutes at room temperature. The mixture was agitated during incubation every five minutes. Eight hundred microliters of CD4 buffer was added to the

mixture of antibody and sample and mixed gently. This was then plugged to the counter for counting.

Stool samples were collected in clean wide mouthed, leak proof plastic containers from each patient. Stool specimens were examined microscopically for ova, cysts, oocyst, or parasites, using normal saline and iodine mounts on grease-free slides. Following this, each fresh stool samples were preserved in 10% formal saline. The preserved samples were concentrated using formal-ether concentration methods and examined for, Oocyst of *Cryptosporidium spp*, *Isospora belli*, and *Cyclospora cayetanensis* were identified using modified Ziehl-Neelsen staining technique earlier described.<sup>9,16,17</sup> The data were analyzed using Chi square (x2) test and appropriate statistical software packages.

## RESULTS

The 146 HIV sero-positive patients with and without diarrhea included in the study were aged between 20 to 45 years. Of the 146 patients, the protozoan parasitic infection was found 30.13% (44/146). Of these 146 patients, 78 had diarrhea in which parasitic infection was 39 (50%) as shown in table 1. There was 7.35% (5/68) protozoal parasites positive of cases without diarrhea. A significant difference ( $p < 0.05$ ) was observed in the level of infection of intestinal protozoans between the HIV seropositive with diarrhea and HIV-seropositive without diarrhea. Although *Giardia lamblia* and *Entamoeba histolytica* are not considered as opportunistic pathogen it was included in the study because of increased prevalence of these parasites in developing countries.

**Table 1. Distribution of parasitic infection among HIV seropositive patients.**

Parasitic species	Cases with acute diarrhea n= 33 (22.6%)	Cases with chronic diarrhea n= 45 (30.8%)	Cases without diarrhea n= 68 (46.6%)	Total no. of cases n= 146
<b>Protozoal parasites:</b>				
<i>Giardia lamblia</i>	10	3	1	14
<i>Blastocystis hominis</i>	2	4	3	9
<i>Entamoeba histolytica</i>	2	5	1	8
<i>Cyclospora cayetanensis</i>	1	5	0	6
<i>Cryptosporidium parvum</i>	2	2	0	4
<i>Isospora belli</i>	1	2	0	3
Total	18	21	5	44(30.13%)

### Opportunistic parasites and CD4 count

In the study, out of 43 patients whose CD4+ T cells were  $< 200/\mu\text{l}$ , 29 (67.4%) had opportunistic parasitic infection whereas out of 103 patients whose CD4+ T cells were  $\geq 200/\mu\text{l}$

$\mu\text{l}$ , only 15 (14.56%) had opportunistic parasitic infection as depicted in table 2.

**Table 2. Opportunistic parasitic infections and CD4+ T count among HIV sero-positive patients.**

Characteristics	No. Of tested	No. of infection (%)
Gender (HIV patients):	Male: 61	26 (42.6)
	Female: 85	18 (21.2)
Clinical symptoms of HIV patients:	Diarrhea: 78	39 (50.00)
	Non-diarrhea: 68	5 (7.35)
CD4 Count (cells/ $\mu\text{l}$ ) of HIV sero-positive patients	< 200: 43	29 (67.44)
	> 200: 103	15 (14.56)

## DISCUSSION

Enteric parasitic infections still remains an important cause of morbidity and mortality in developing countries especially among HIV-infected persons with and without diarrhea.<sup>18</sup> The World Health Organization 2006 defines diarrhea wasting syndrome along with HIV-seropositive patients, the etiology of such diarrhea could either be parasites, bacteria, fungal, enteric virus or HIV itself.<sup>12, 19</sup>

In the present study the enteric parasites were detected in 30.13% from the samples with diarrhea and without diarrhea. There was significant difference the infection of opportunistic parasites among HIV- seropositive cases with diarrhea 50% (39/78) and without diarrhea 7.35% (5/68).

There are number of studies reported from Indian and other countries with the high prevalence of intestinal parasites 25 to 50 % which are near to our findings.<sup>20-22</sup> In the study 43 HIV seropositive patients had CD4 count less than 200 cell/ $\mu\text{l}$  with gastroenteritis parasitic infections and the infection of opportunistic parasites was 67.4% (29/43). Among there parasitic infections *Giardia lamblia* (32%) was predominant pathogens followed by *Blastocystis hominis*, *Entamoeba histolytica* (18%), *Cyclospora cayetanensis* (14%), *Cryptosporidium spp.* (9%) and *Isospora belli* (7%). Several studies from India and other parts of the world have reported the difference.<sup>23-25</sup> The prevalence of opportunistic parasite in patients with CD4 count less than or equal to 200 cell/ $\mu\text{l}$  was found in 14.5%. Cellular immunity is the major defense against intestinal parasitic infections, it is therefore, the reduction in CD4 count by the HIV predispose HIV infected patients to opportunistic intestinal persons to opportunistic infections.<sup>14,26</sup> In our study, CD4 count <200 cells/ $\mu\text{l}$  found a significantly higher prevalence of protozoan parasitic infections ( $p < 0.05$ ).

*C. parvum* is a major opportunistic parasitic infection found in other studies.<sup>4, 5, 27</sup> But our study showed low prevalence (9.1%). Similarly *C. cayetanensis* (13.6%) and *I. belli* (7%) were found in the study correlatives with the study done in India.<sup>27</sup> Occurrence of cryptosporidium in both diarrhea and non-diarrhea cases indicates high risk of infection of

this parasites in Nepal.

Detection rate of *Cyclospora* in this study was found to be 13.6% in HIV seropositive patients which does not correlates with the study in India (0.6%) and similar to other study (11%).<sup>27, 28</sup> *Isospora belli* was found (7%) to be predominant cause of morbidity in symptomatic acute and chronic diarrhea. There finding are parallel to those documented in similar studies conducted is different part of world in HIV infected patients.<sup>27,28,29</sup> *Isospora belli* infections are commonly seen in chronic diarrhoeal patients with HIV-AIDS in developing countries ranges 12-20% , *Entamoeba histolytica* was detected 18% in our study predominant cases with diarrhea should not neglected otherwise.<sup>7,11,22,23,25</sup> Difference in the incidence of intestinal protozoal parasitic infection reported by many researchers can be attributed to the difference in geographical distribution of parasites, sanitary practices, level of education, economic status, social behavior and different selection cases.<sup>14,24,29-31</sup> Although mixed infection is seen in HIV-AIDS patients but in our study we did not observed any such findings. The reason for the same could not ascertain. This could be attributed to the limited study sample and specified place of the country. Moreover, study report on opportunistic parasitic infection among HIV seropositive patients and load of CD4 count in Nepal are very scarce and there is no representative baseline information in the country.<sup>32</sup> Hence, it is important to investigate further to determine the rate of infection with enteric opportunistic parasites to determine the rate of infection with enteric opportunistic parasite in HIV-AIDS patients in other regions of Nepal which will provide the level of endemicity of the country. Skilled manpower and laboratory support required to investigate the carrier, latent and clinical infection. Stool sample examination with modified acid fast staining method as a concentration might help to investigate the existence of opportunistic parasitic infection in Nepal.

## CONCLUSION

The study indicated that enteric parasitic infection caused diarrhea (31.13%) of the HIV-seropositive patients. The majority of the infections in the patients with CD4 count < 200 cells/ $\mu\text{l}$  were due to enteric opportunistic parasitic infections. The current finding also highlights the importance of early detection of opportunistic parasitic infections among HIV-seropositive patients. This may help to improve the management and quality of life of HIV-infected individuals. Enteric parasites in order to avoid morbidity and mortality due to opportunistic pathogens.

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