

# Identification of Enteric Pathogens in HIV-positive Patients with Diarrhoea in Northern India

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## ABSTRACT

Enteric pathogens associated with chronic diarrhoea in HIV-positive patients were studied. The study was conducted during January 1995-December 1998. Stool specimens from all diarrhoea patients (n=26) were examined microscopically for ova and parasites using wet preparations and stained smears. Stool samples from diarrhoea patients were also cultured on appropriate media to isolate enteric bacterial pathogens. Of the 59 patients, 26 (44%) had prolonged diarrhoea for more than 4 weeks. Enteric pathogens were detected in 19 (73%) of the 26 patients: 17 patients harboured a single pathogen, and 2 patients had mixed pathogens. The detection rate of emerging parasites, including *Isospora*, *Cryptosporidium*, *Blastocystis hominis*, and *Strongyloides stercoralis* as a single agent, was significantly higher than conventional pathogens (50% vs 19.2%;  $p < 0.05$ ). Only one patient harboured both conventional and emerging pathogens (*Entamoeba histolytica* and *Cryptosporidium*). *Isospora belli* was detected in 8 (31%) of the 26 diarrhoea patients: in 7 (27%) patients as a single agent and in one patient with *S. stercoralis*. *Cryptosporidium* was identified in 3 (11%) diarrhoea patients: in 2 (8%) patients as a single agent and in one patient with *E. histolytica*, followed by *B. hominis* in 2 (8%) patients. *E. histolytica* was most commonly isolated (3/26; 11.5%), followed by *Giardia lamblia*, enteropathogenic *Escherichia coli*, and *Campylobacter jejuni* (one patient each). Parasitic pathogens were frequently associated with HIV-positive patients with diarrhoea in northern India. *I. belli* was the most frequent parasite isolated, followed by *Cryptosporidium*. Stools of all HIV-positive patients with diarrhoea should thoroughly be investigated to identify aetiological agents for proper management.

**Key words:** Enteropathogens; *Isospora*; *Cryptosporidium*; *Blastocystis hominis*; Diarrhoeal diseases; Acquired immunodeficiency syndrome; HIV

## INTRODUCTION

Infections of the gastrointestinal tract are common in patients with acquired immunodeficiency syndrome (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 (1,2). The impact of

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some relatively new spore/oocyst-forming intestinal protozoa, such as *Cryptosporidium*, *Cyclospora*, *Microsporidia*, and *Isospora*, on patients with AIDS is severe (3). Infections caused by these parasites cannot be differentiated clinically unless specific faecal examination is carried out. The isolation rates of intestinal parasites in patients with AIDS and chronic diarrhoea vary from 40% to 83% (4,5), and the parasitic agents also differ markedly from region to region (6).

The number of HIV-positive patients is increasing in India, and till date, data on the prevalence of diarrhoea and the spectrum of pathogens responsible for enteric infections in HIV-positive patients are lacking. The study

was, therefore, carried out to identify enteric parasites in HIV-positive patients with chronic diarrhoea.

## METHODS AND MATERIALS

### Study population

During January 1995-December 1998, 59 HIV-positive patients with fatigue, unexplained fever, persistent diarrhoea, marked weight loss, oral candidiasis, tuberculosis, generalized lymphadenopathy, etc. (7), and with clinical evidence of infectious complication(s) were referred to our department for diagnosis of infectious agent(s). The presence of HIV in these patients was confirmed at our centre by at least two different immunoassays and/or western blot (WHO strategy II, 1993) (8). Of them, 26 patients experienced prolonged diarrhoea (>3 loose stools/day for more than 4 weeks). Stool specimens from these patients were investigated to identify enteric parasites.

### Collection and processing of specimens

Three consecutive freshly-voided stool specimens from each patient were collected in a clean wide mouth container, and were processed for isolating enteric pathogens as follows within 2 hours of their collection:

**Isolation of parasitic pathogens:** Iodine and saline wet mount preparations were made from both unconcentrated and formol-ether-concentrated stool specimens, and were examined under the microscope. To detect *Cryptosporidium* and *Cyclospora*, stool specimens were stained by modified acid-fast stain, following a standard protocol (9). Faecal specimens, collected from 8 patients only during 1998, were examined for *Microsporidia* using modified trichrome stain (10).

**Isolation of bacterial pathogens:** All stool samples were routinely cultured on MacConkey, sorbital MacConkey (SMAC), deoxycholate citrate, thiosulphate citrate bile sucrose, and Campy-BAP agar media to isolate known bacterial enteropathogens as described earlier (11). Routine enrichment of all specimens was done in tetrathionate broth for *Salmonella* species. Bacterial pathogens were identified, following the conventional methods (12). All D-sorbital-negative *Escherichia coli* on SMAC agar were tested for agglutination with O157 antiserum (Denka Seiken Co., Japan). Almost pure or predominant growth of *E. coli*, in absence of any other known pathogens, was tested for enterotoxin production by VET-RPLA (Denka Seiken Co., Japan), slide agglutination, using enteropathogenic *E. coli* (EPEC) antisera of Welcome Diagnostics, and adherence to HEP-2 cells (13).

No attempt was made to isolate viruses.

**Statistical analysis:** Z test for proportion was used for data analysis.

## RESULTS

Of the 59 HIV-positive patients with AIDS, 26 (44%) presented with chronic diarrhoea for more than 4 weeks. Of these 26 patients, 20 (77%) were males and 6 (23%) females. Their age ranged from 6 to 60 years with the mean age of 32 years.

The aetiologic agents were identified in 19 (73%) of the 26 patients—17 (65%) harboured a single pathogen, while mixed pathogens were detected in 2 patients. Emerging enteric parasites and conventional protozoal/bacterial pathogens were detected in 13 (50%) and 5 (19%) patients respectively as a single agent (table). The remaining one patient harboured both emerging (*Cryptosporidium*) and conventional (*Entamoeba histolytica*) pathogens. The difference between the two groups of patients harbouring emerging and conventional pathogens was found to be statistically significant ( $p < 0.05$ ), and the value ( $p < 0.05$ ) remained unchanged when the data of the sole patient with both emerging and conventional pathogens were considered either as mutually exclusive or overlapping category. Of the emerging parasites, *Isospora belli* was most frequently detected (in 7 patients as a single agent and in one patient in association with *Strongyloides stercoralis*), followed by *Cryptosporidium* (in 3 patients as a single agent and in one patient with *E. histolytica*) and *Blastocystis hominis* (in 2 patients). *E. histolytica* was the most frequent conventional pathogen (3/26; 11.5%), followed by *Giardia lamblia*, enteropathogenic *E. coli* (EPEC), and *Campylobacter jejuni* in one patient each. EPEC was confirmed by agglutination with group-specific antisera and localized adherence pattern to HEP-2 cells.

**Table.** Spectrum of pathogens isolated from 26 patients with HIV-related diarrhoea

Enteric pathogen	No. detected	%
Emerging pathogens (n=13; 50%)*		
<i>Isospora belli</i>	7	26.9
<i>Cryptosporidium</i>	3	11.5
<i>Blastocystis hominis</i> (>10/HPF)	2	7.7
<i>Isospora belli</i> + <i>Strongyloides stercoralis</i>	1	3.8
Conventional pathogens (n=5; 19.2%)*		
<i>Entamoeba histolytica</i>	2	7.7
<i>Giardia lamblia</i>	1	3.8
Enteropathogenic <i>Escherichia coli</i>	1	3.8
<i>Campylobacter jejuni</i>	1	3.8
Conventional + emerging pathogens		
<i>E. histolytica</i> + <i>Cryptosporidium</i>	1	3.8
Total	19	73.1

\*Emerging vs conventional pathogens in HIV-related diarrhoea ( $p < 0.05$ )

## DISCUSSION

Diarrhoea is a common complication of HIV infection; 30-90% of patients with AIDS suffer from diarrhoea at some points of their illness (1,2), and detection of aetiologic agent(s) varies from 40% to 83% (4,5). In the present study, 44% of the HIV-positive patients with AIDS had a history of chronic diarrhoea, and the aetiologic agents were detected in 73% of them. A recent report of the National AIDS Control Organization, New Delhi, India, showed that 47% of AIDS patients in 1997 experienced chronic diarrhoea, but the spectrums of pathogens involved were not thoroughly investigated (14). Until recently, more frequently-associated parasites in diarrhoea were: *G. lamblia*, *E. histolytica*, *Balantidium coli*, etc. Since the onset of AIDS epidemic, the numbers of parasitic pathogens recognized and the frequencies with which they are encountered in clinical practice have increased. These parasites can cause self-limiting diarrhoea of short duration in healthy individuals, but in the immunocompromised host, including AIDS patients, the diarrhoea is usually chronic and, sometimes, life-threatening (15).

In the present study, detection of emerging parasites in HIV-related diarrhoea was significantly higher compared to conventional pathogens (table). *I. belli* (27%) was the most frequently-encountered pathogen in our patients, followed by *Cryptosporidium* (11%) and *B. hominis* (8%) as single pathogens. Till date, such a high prevalence of *Isospora* has not been reported in India, where the number of HIV-positive patients is increasing day by day. No *Microsporidia* and *Cyclospora* were detected in our study. Since stool specimens from only 8 patients were examined for *Microsporidia*, it is difficult to comment on its prevalence in our patients. Thorough investigations on a large number of patients are required to know the exact role of *Microsporidia* in HIV-related diarrhoea in India. *Cryptosporidium* was found in 10-20% of patients with AIDS worldwide (16), and the prevalence of *Microsporidia* ranged from 3% to 50% (15,18). *Isospora* is infrequently associated with diarrhoea due to AIDS in the USA and Europe (about 2%), but is commonly isolated in patients with AIDS and chronic diarrhoea in Brazil (9.9%), Zaire (12%), Zambia (16%), and Haiti (12%) (15). *Cyclospora* is again common in Haiti (11%), but only rarely detected in US and Tanzanian patients with AIDS and chronic diarrhoea (<1%) (15,17). In a recent study of 22 HIV-infected patients with diarrhoea in Thailand, *Microsporidia* was the most common pathogen (27% of cases), followed by *Cryptosporidium* (9%) and *Isospora* (4.5%) (19). Studies from various parts of the world show contrasting prevalence rates with marked geographical variations. This emphasizes the need for thorough investigations of these patients to identify pathogens for proper management.

Conflicting evidence exists as to whether *B. hominis* should also be considered as a significant cause of AIDS-associated diarrhoea (20). Usually, the presence of more than 5 parasites/HPF of stool specimen is used as a criterion for positive reporting. Although the role of *B. hominis* as an emerging pathogen remains unresolved, we detected >10/HPF of *B. hominis* (vacuolar form) in 2 of our patients as a single pathogen. The number of parasites excreted in stool might possibly be an indicator of its pathogenic role in HIV-related diarrhoea.

The present study has shown that *I. belli* has been found to be the most important emerging pathogen in HIV-positive patients with diarrhoea in northern India. Simple stool examination can identify enteric parasites in the majority of patients.

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