

## Short Communication

# Prevalence of Intestinal Parasitic Infestation in HIV/AIDS Patients with Diarrhea in Madurai City, South India

Kuppamuthu Ramakrishnan, Rajaiah Shenbagarathai<sup>1\*</sup>, Alagappan Uma<sup>2</sup>, Karuppusamy Kavitha<sup>3</sup>, Rathinasamy Rajendran<sup>4</sup> and Ponniah Thirumalaikolundusubramanian<sup>5</sup>

*Tuberculosis Research Centre; <sup>1</sup>Department of Biotechnology, Lady Doak College;*

*<sup>2</sup>Department of Microbiology, Medical College; <sup>3</sup>7th Day School;*

*<sup>4</sup>Centre for Research in Medical Entomology, Madurai; and*

*<sup>5</sup>Department of Internal Medicine, Madras Medical College, Chennai, India*

(Received November 13, 2006. Accepted April 2, 2007)

**SUMMARY:** The prevalence and pattern of parasitic infestation among 80 HIV/AIDS patients with diarrhea in Madurai, south India, was studied by microscopy. Eighty HIV-negative patients were used as controls. Intestinal parasites were detected in 31 HIV/AIDS patients (38.7%) and in 14 (17.5%) HIV-negative patients, a difference that was statistically significant ( $P < 0.05$ ). In HIV/AIDS patients with diarrhea, protozoa accounted for the majority of diarrhea cases (*Entamoeba* spp. 37.5%, *Cryptosporidium parvum* 28.7%). It is therefore suggested that enteric infections are more common in HIV-infected patients than in HIV-negative persons in south India, and this may be due to differences in immunological profile, susceptibility as well as factors related to sanitation and the environment.

Infections by opportunistic pathogens including various forms of intestinal parasitosis have been the hallmark of acquired immunodeficiency syndrome (AIDS) since the beginning of the epidemic. Diarrhea is a major gastrointestinal symptom in human immunodeficiency virus (HIV) infection affecting 90% of patients, and it becomes more frequent as immunodeficiency progresses. Diarrhea and weight loss are independent predictors of mortality (1).

The parasitic infestations are mainly due to unsanitary conditions. Several species of protozoa have been associated with acute and chronic diarrhea in HIV patients. These include *Cryptosporidium parvum*, *Isospora belli*, Microsporidia spp., *Giardia lamblia*, *Entamoeba histolytica*, *Cyclospora* spp., *Blastocystis hominis*, *Dientamoeba fragilis*, etc. (2). The present study was undertaken to determine the prevalence and pattern of parasitic infestation among chronic diarrheal patients with and without HIV/AIDS in Madurai, south India.

HIV/AIDS patients reporting to the Department of Sexually Transmitted Diseases (STD), Medicine and Thoracic Medicine in Govt Rajaji Hospital, Madurai, TamilNadu, south India, from 2003 to 2005 were included in the study. HIV infection was established by two different enzyme-linked immunosorbent assay (ELISA) tests, i.e., *Innotest*™ HIV<sub>1</sub>/HIV<sub>2</sub> (Innogenetics N.V., Ghent, Belgium) and Labsystems (Lab systems, Helsinki, Finland).

A total of 80 HIV/AIDS patients and 80 HIV-negative (home contacts of HIV/AIDS patients-without diarrhea) living in and around Madurai with chronic diarrhea with a duration of more than 1 month were selected. After written informed consent was obtained, fresh, unfixed stool samples were collected from the subjects during a visit to the respective departments of the hospital in a container with normal saline. The stool samples were examined for the presence of

trophozoites, oocysts, larvae and ova of intestinal parasites using normal saline and iodine preparations (3). A modified Ziehl-Neelsen (ZN) staining technique (4) was used to identify oocysts of *C. parvum* and also microsporidium. Formalin-ethyl acetate concentration of the samples was performed on fresh unpreserved stool samples to detect helminthic ova (centrifugation at  $500 \times g$  for 10 min). Sediment was examined using two 22-by-22 mm cover slip preparations (one with saline and one with iodine) (5). The saline and iodine preparations were observed under the microscope using both low and high power, and modified ZN stained by oil immersion was also observed to detect oocysts of *C. parvum* and *I. belli*. The Institutional Ethical Committee approved this project, and all the data were analyzed using simple descriptive statistics.

There were 58 males and 22 females in the HIV/AIDS group and 51 males and 29 females in the non-HIV group. Their ages ranged from 19 to 72 years, with a median age of 34 and a mean age of 36 years. In the HIV/AIDS-positive group, 31 subjects (38.7%), had one or multiple parasites and in the HIV-negative group 14 subjects (17.5%) had parasites, and the difference between them was statistically significant ( $P < 0.05$ ) (Table 1). The specieswise prevalence of intestinal parasites in the study group is shown in Table 2. Protozoan infection was more frequent than helminthes. In HIV/AIDS patients with diarrhea, *E. histolytica* infection was noted in 17.5%, while *C. parvum* oocyst was observed in 28.7%. The prevalence of these infestations was significantly higher than in the HIV-negative group (Table 2).

Table 1. Status of intestinal parasites among study population in Madurai

Study group	Single infection	Dual infection	Multiple infection	Total
AIDS-positive (%) <i>n</i> = 80	13 (16.2)	14 (17.5)	4 (5)	31 (38.7)
AIDS/HIV-negative (%) <i>n</i> = 80	4 (5)	5 (6.2)	5 (6.2)	14 (17.5)

\*Corresponding author: Mailing address: Department of Biotechnology, Lady Doak College, Madurai-625002, TamilNadu, south India. Tel: +91-452-2530527, Fax: +91-452-2530293, E-mail: shenbagarathai@rediffmail.com

Table 2. Specieswise prevalence of intestinal parasites among study group

Study group	Intestinal parasite						
	<i>Cryptosporidium parvum</i>	<i>Entamoeba histolytica</i>	<i>Entamoeba coli</i>	<i>Ascaris lumbricoides</i>	<i>Hymenolepis nana</i>	<i>Giardia lamblia</i>	<i>Isospora belli</i>
AIDS-positive (%) n = 80	23* (28.7)	14* (17.5)	16* (20)	6 (7.5)	1 (1.2)	3 (3.7)	1 (1.2)
AIDS/HIV-negative (%) n = 80	3 (3.7)	3 (3)	3 (3.7)	2 (2.5)	1 (1.2)	0	0

\*: Significant at AIDS positive cases by student *t* test ( $P < 0.05$ ).

Parasitic infestation among south Indian HIV/AIDS patients with chronic diarrhea was documented previously (6). Opportunistic intestinal parasitic infection should be suspected in any HIV-infected patient with advanced disease presenting with diarrhea. Kumar et al. (2002) have highlighted *C. parvum* as the predominant pathogen (11.8%) in HIV/AIDS cases with diarrhea (7). In the present study, *C. parvum* was isolated from 28.7% of established HIV/AIDS cases with chronic diarrhea, whereas it was found in 11.8% of cases at Chennai (7), 2.3% at Kolkatta (8), 11% at Mumbai (9) and 10% at Vellore (6). These variations may be due to the advanced stage of HIV and chronic diarrhea. *E. histolytica* (17.5) was the next most common parasite among these previous studies in contrast to the findings of the present study.

Intestinal parasites such as *C. parvum* and *I. belli* lead to increases in morbidity and mortality in HIV/AIDS cases (10). New diagnostic tests have shown a high prevalence of *E. histolytica* in some populations (11). Also, an increasing rate of HIV-seropositive status among amoebic liver abscess patients indicates that HIV-seropositive or AIDS patients are more susceptible to an invasive form of the disease (12). The clinical course and pattern of opportunistic infections (OIs) varies from patient to patient in different areas of India (13). No microsporidia infection was observed in our study using modified ZN staining technique. The highest risk of intestinal microsporidiosis has been established in those with a CD4 count less than 100 cells/mm<sup>3</sup>. Intestinal helminthes induce immunological alterations that favor the progression from HIV seroconversion to AIDS (14). Diarrhea was not noted in many of the HIV/AIDS cases referred for highly active antiretroviral therapy (HAART) in this center. Moreover, HAART has significantly improved the outcome of HIV/AIDS in terms of the prevention of OIs as well as in terms of mortality. Indian patients on HAART are experiencing a decrease in the number of OIs and in HIV-related morbidity and mortality (15).

The present study suggests that enteric infections in HIV-infected patients are different from those in HIV-uninfected persons in south India. This may be due to differences in immunological profile susceptibility as well as factors related to sanitation and the environment.

#### ACKNOWLEDGMENTS

The laboratory assistant Ms. R. Geetha, Mr. Kannan, Revised National

TB Control Programme Staff, Ms. Saraswathi, Lab. Technician, Department of Microbiology, Medical College, Madurai and the Statistic Assistant Mr. R. Parthasarathi, Department of Community Medicine, Medical College, Madurai, TamilNadu are gratefully acknowledged.

#### REFERENCES

1. Sharpstone, D., Neild, P., Crane, R., et al. (1999): Small intestinal transit, absorption and permeability in patients with AIDS with and without diarrhea. *Gut*, 45, 70-76.
2. Awole, M., Gebre-Selassie, S., Kassa, T., et al. (2003): Prevalence of intestinal parasites in HIV-infected adult patients in southwestern Ethiopia. *Ethiop. J. Health Dev.*, 17, 71-78.
3. Chessbrough, M. (1998): Parasitological tests. p. 184-235. In Chessbrough, M. (ed.), *District Laboratory Practice in Tropical Countries*. Tropical Health Technology, World Health Organization, Geneva.
4. Nagamani, K., Rajkumari, A. and Gyaneshwari (2001): Cryptosporidiosis in a tertiary care hospital in Andhra Pradesh. *Indian J. Med. Microbiol.*, 19, 215-216.
5. Susan, E.S., Clarisa, A.S., Yolanda, D., et al. (2001): Evaluation of the TriageMicro Parasite Panel for detection of *Giardia lamblia*, *Entamoeba histolytica*/*Entamoeba dispar*, and *Cryptosporidium parvum* in patient stool specimens. *J. Clin. Microbiol.*, 39, 332-334.
6. Mukhopadhyaya, A., Ramakrishna, B.S., Kang, G., et al. (1999): Enteric pathogens in south Indian HIV-infected patients with and without diarrhoea. *Indian J. Med. Res.*, 109, 85-89.
7. Kumar, S.S., Anandan, S. and Lakshmi, P. (2002): Intestinal parasitic infection in infected patients with diarrhoea in Chennai. *Indian J. Med. Microbiol.*, 20, 88-91.
8. Anup, P., Dipika, S., Kakali, M.D., et al. (2005): Asymptomatic cryptosporidiosis in a periurban slum setting in Kolkatta, India-a pilot study. *Jpn. J. Infect. Dis.*, 58, 110-111.
9. Chowdhary, A.S. and Joshi, M. (2002): Spectrum of parasitic infections in AIDS associated diarrhoea. Abstract no. 10953. 14th International Conference on AIDS. July 7-12, Barcelona, Spain.
10. Lindo, J.F., Dubon, J.M., Ager, A.L., et al. (1998): Intestinal parasitic infections in human immunodeficiency virus (HIV)-positive and HIV-negative individuals in San Pedro Sula, Honduras. *Am. J. Trop. Med. Hyg.*, 54, 431-435.
11. Stanley, S.L., Jr. (2003): Amoebiasis. *Lancet*, 361, 1025-1034.
12. Shamsuzzaman, S.M. and Hashiguchi, Y. (2002): Thoracic amoebiasis. *Clin. Chest Med.*, 23, 479-492.
13. Kumarasamy, N., Snigdha, V., Timothy, P.F., et al. (2005): Clinical profile of HIV in India. *Indian J. Med Res.*, 121, 377-394.
14. Harms, G. and Feldmeier, H. (2002): HIV infection and tropical parasitic disease-deleterious interactions in both directions. *Trop. Med. Inter. Health*, 7, 479-488.
15. Palella, F.J., Jr., Delaney, K.M., Moormam, A.C., et al. (1998): Declining morbidity and mortality among patient with advance human immunodeficiency virus infection. HIV out patients study investigators. *N. Engl. J. Med.*, 338, 853-860.