Original Article

# Intestinal Protozoans in Adults with Diarrhea

## Muktikesh Dash, Sanghamitra Padhi, Pritilata Panda, Banojini Parida

*Department of Microbiology, Maharaja Krushna Chandra Gajapati Medical College and Hospital, Berhampur University, Odisha, India*

## Abstract

**Background:** Diarrhea is one of the most common presenting complaints in human immunodeficiency virus-infected individuals. **Aims:** The study was designed to determine the magnitude of opportunistic and nonopportunistic intestinal parasitic infections among diarrheal patients and association between CD4+ T-cell counts and human immunodeficiency virus (HIV)-infected intestinal parasites. **Materials and Methods:** A cross-sectional study was conducted among 207 enrolled diarrheal patients attending HIV integrated counseling and testing center from January 2012 to December 2012. Stool samples were subjected to special modified Ziehl-Neelsen and chromotrope staining method for detection of opportunistic protozoans. Blood samples were also collected from all study subjects for HIV testing and CD4+ T-cell counts were estimated by only in HIV-infected patients. **Results:** Intestinal parasitic pathogens were detected in 46.1% HIV-infected patients and the major pathogens were opportunistic protozoans 32.2% (37/115), most common being *Isospora belli* 16.5% (19/115) followed by *Cryptosporidium parvum* 12.2% (14/115). In HIV noninfected diarrheal patients, major pathogens detected were *Entamoeba histolytica/Entamoeba dispar* 8.7% (8/92) and *Ascaris lumbricoides* 3.3% (3/92). **Conclusions:** The opportunistic intestinal protozoans especially *I. belli* and *C. parvum* were most commonly isolated in HIV-infected patients with diarrhea. Majority of the infections occurred in patients when a CD4+ T-cell counts were less than 200 cells/μl.

**Keywords:** *Cryptosporidium parvum,* human immunodeficiency virus, intestinal protozoans, *isospora belli,* opportunistic

**Address for correspondence:** Dr. Muktikesh Dash, Department of Microbiology, Maharaja Krushna Chandra Gajapati Medical College and Hospital, Berhampur, Odisha - 760 004, India. E-mail: mukti_mic@yahoo.co.in

## Introduction

The global pandemic of human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) in its third decade has shown public health concerns especially infections by opportunistic pathogens including various forms of intestinal parasitosis. Diarrhea is one of the most common presenting complaints in HIV-infected individuals, reaching a rate up to 60% in developed countries and 90% in developing countries.[1] The infectious etiological agents include both opportunistic agents that consistently cause severe, often frequent or chronic diarrhea and nonopportunistic agents that usually cause acute, treatable gastro-intestinal

illness.[2] Many self-limiting and sporadic intestinal parasites have now become opportunistic parasites causing uncontrollable life threatening diarrhea among people living with HIV/AIDS.[3] Opportunistic intestinal protozoan parasites such as *Cryptosporidium parvum, Isospora belli, Cyclospora cayetanensis* and *Microsporidia* including *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis* have been reported in HIV infection.[4] Recent studies have also opined that mucosa dwelling parasites may benefit from HIV-induced pathological changes and reduced immune response due to HIV infection, which creates suitable environment for opportunistic intestinal parasites in HIV/AIDS patients.[3,4]

Intestinal parasites are frequently transmitted by low level of environmental and personal hygiene, contamination of food and drinking water and poor sanitary conditions in developing countries.[5] Proper investigation of the parasitic etiology of diarrhea leading to prompt and effective management can help in decreasing the morbidity and mortality in such patients.[6]

Only a few studies have been reported regarding the prevalence of intestinal parasites from eastern

part of India. Against the above background as well as the generated data that will improve the management of opportunistic infections in HIV/AIDS patients, this study is aimed to determine the prevalence of intestinal parasitic infections with special emphasis on opportunistic coccidian parasites among HIV-infected and HIV noninfected patients presenting with diarrhea as well as effect of CD4^{+} (cluster of differentiation 4) T-cell counts on prevalence of the disease among HIV-infected patients.

## Materials and Methods

### Study design and subjects

A cross-sectional study was carried out between January 2012 and December 2012 in a 900-bedded tertiary health institution, Odisha state with antiretroviral therapy (ART) facilities for HIV/AIDS management. This study was conducted after due approval of institutional ethical committee.

A total of 5973 subjects who attended integrated counseling and testing center (ICTC) were enrolled for HIV testing during study period and among them 372 suffered from diarrhea. Stool samples were collected from 207 consenting HIV-infected and HIV noninfected patients suffered from diarrhea were included in this study in order to determine the magnitude and prevalence of intestinal parasites among HIV-infected patients. Study patients were interviewed using a structured questionnaire and information was obtained on demographic characteristics, present and past history of diarrhea and antibiotic treatment. Diarrhea was defined as two or more liquid or three or more soft stools per day. Patients already received antiparasitics and antibiotics treatment and less than 18 years were excluded from the study. Verbal informed consent was obtained from all patients prior to collection of stool sample.

### Blood sample collection and processing, HIV serology, and CD4^{+} T cell count

All the ICTC attendees received relevant pretest counseling and written informed consent was obtained from each of them before HIV testing was carried out. Five milliliters venous blood sample was collected in a sterile plain container from all patients suffering from diarrhea who attended ICTC. Blood was allowed to clot for 30 minutes at room temperature (25–30 C) and serum was separated after centrifugation at low speed. The serum samples were then stored at 4 C and were tested within 48 hours.

HIV antibodies were tested by the three rapid tests protocol as per the guidelines laid down by the World Health Organization (WHO Testing strategy III) and National AIDS Control Organization, Government of India.[7] All positive test results were disclosed only after post test counseling of the patients. Antibodies to HIV (1 and 2) were tested initially with a SD BIOLINE HIV-1/2 3.0 rapid test [Standard Diagnostics, Inc. Korea]. The samples tested positive in the first method were subjected to tests with two different rapid tests, that is, PAREEKSHAK HIV 1/2 Triline Card Test [Bhat Bio-Tech India (P) Ltd., Bangalore, India] and PAREEKSHAK HIV 1/2 Rapid Test Kit (TRISPOT) [Bhat Bio-Tech India (P) Ltd., Bangalore, India]. The samples were considered as positive when found reactive by all three different methods. All tests were done according to manufacturer's instructions.

CD4^{+} T-lymphocytes count was carried out in HIV-infected patients by using BD FACS™ Calibour system (Becton Dickinson, Singapore). In brief, a three-color direct immunofluorescence reagent, that is, CD3 fluorescein isothiocyanate (FITC)/CD4 phycoerythrin (PE)/CD45 peridinin chlorophyll protein (PerCP) was used to identify and determine the absolute counts of helper T-lymphocyte (CD4^{+}) subsets in erythrocyte-lysed whole-blood.[8]

### Stool sample collection and examination

From consenting ICTC diarrheal attendees, single stool sample was collected for this study. All stool specimens were subjected to fresh direct wet mounts, formol-ether concentration technique, modified Ziehl–Neelsen stain and chromotrope stain for detection of intestinal parasites.

#### Direct wet mount

A direct saline and iodine wet mount of each sample was used to detect intestinal parasites microscopically. The wet mounts were examined under light microscope at ×100 and ×400 magnifications. A small portion of the stool samples were also preserved in 10% formalin for repeating the tests whenever required.

#### Formol ether concentration method

A portion of each fresh stool sample was taken and processed. Briefly, 1 g of stool was placed in a clear 15 ml conical centrifuge tube containing 7 ml formalin saline by using applicator stick. The resulting suspension was filtered through a sieve into another conical tube. After adding 3 ml of diethyl ether to the formalin solution, the content was centrifuged at 3200 rpm for 3 minutes. The supernatant was poured away and the tube was replaced in its rack. Finally smear was prepared from the sediment and observed under light microscope with a magnification of ×100 and ×400. Fresh stool specimens were used as saline wet mounts to detect motile trophozoites and concentrated stool specimens

were used as iodine wet mounts to detect ova, larva, and cysts.

### Modified Ziehl-Neelsen staining method
Thin smear was prepared directly from sediment of formol-ether concentrated stool and allowed to air dry. The slides were then fixed with methanol for 5 minutes and stained with carbol fuchsin for 30 minutes. After washing the slides in tap water, they were decolorized with acid alcohol (99 ml of 96% ethanol and 1 ml HCL) for 3 minutes and counterstained in methylene blue for 1 minute. The slides were then washed in tap water and observed for *Cryptosporidium parvum*, *Isospora belli*, and *Cyclospora cayetanensis* under light microscope with a magnification of 1000×.[9]

### Chromotrope staining method
A small part of 10% formalin fixed unconcentrated stool suspension was smeared on a glass slide. The thin smear was heat fixed on a slide warmer at 60 C until completely dry (5-10 minutes) followed by absolute methanol fixation for 5 minutes. The slides were stained with chromotrope for 90 minutes. After washing in tap water, they were destained with acid alcohol (995.5 ml of 90% ethanol and 4.5 ml glacial acetic acid) only for 1-3 seconds, then immediately rinsed with 95% ethanol by dipping. Subsequently the slides were changed twice in 100% ethanol for 3 minutes each and changed twice in xylene for 10 minutes each. The slides were then drained and mounted and observed for *Microsporidia* under light microscope with a magnification of 1000×.[9]

### Quality control
A control slide of *Cryptosporidium parvum, Isospora belli, Cyclospora cayetanensis*, and *Microsporidia* from a 10% preserved specimen were included along with each staining run.

### Statistical analysis
GraphPad Inc. statistical software (2236 Avenida de la Playa La Jolla, CA 92037 USA) was used for calculation of mean, median, standard deviation, range, and *P* value using Fisher's exact test. Statistical significance was defined when *P* value is less than 0.05.

## Results

A total of 207 diarrheal stool samples were examined for presence of intestinal parasites, 115 (55.6%) were collected from HIV-infected patients and the rest 92 (44.4%) were from HIV negative patients. The mean age of HIV-infected patients was 37.7 (9.7) (median 38, range 20-62 years) and HIV noninfected was 34.6 (12.8) (median 32, range 18–67 years).

Intestinal parasites were detected in 53 (46.1%) out of 115 samples from HIV-infected patients, whereas only 16 (17.4%) parasites were found from 92 HIV negative samples. Among HIV-infected patients, majority 32.2% (37 out of 115) had infected with opportunistic protozoans (Coccidia and Microsporidia) and the rest 16 (13.9%) suffered from nonopportunistic protozoans and nematodes [Table 1]. From 92 HIV negative stool samples, 16 (17.4%) were positive for intestinal parasites and only 1 (1.1%) had revealed opportunistic protozoan infection. The association of opportunistic protozoans with HIV-infected patients had shown statistically significant *P* value (*P* < 0.01).

Among HIV-infected patients, *I. belli* 16.5% (19/115) was most commonly detected parasites followed by *C. parvum* 12.2%, *E. histolytica/E. dispar* 7.8%, *Microsporidia* (2.6%), *S. stercoralis* (2.6%), and 0.9% each of *C. cayetanensis, A. duodenale*, and *G. lamblia*, respectively. From 16 (17.4%) parasite positive stool samples of 92 HIV-negative patients, *E. histolytica/E. dispar* was the common parasite (8.7%), followed by *A. lumbricoides* (3.3%), *A. duodenale* (2.2%), and 1.1% each of *E. vermicularis, T. trichiura*, and *C. parvum* respectively [Table 1].

CD4+ T-cell count was performed in all 115 HIV-infected patients. The prevalence of opportunistic intestinal protozoans among HIV-infected patients with CD4 counts <200 cells/μl and ≥ 200 cells/μl were 61.9% (26/42) and 15.1% (11/73), respectively. The CD4+ T-cell count was significantly associated with an increased prevalence of opportunistic intestinal protozoans in HIV-infected diarrheal patients (*P* < 0.001) [Table 2]. The mean CD4+ T-cell count among HIV-infected opportunistic protozoans was 135.2 (80.7) cells/μl (median 112, range 37–389 cells/μl).

The HIV-seropositive subjects were grouped by 18-27, 28-37, 38-47, and ≥ 48 years. The percentage of HIV-infected study participants who were males with diarrhea was more (61.7%, 71/115) than that of females (38.3%, 44/115). Similarly, the occurrence of opportunistic protozoans was higher in ≥ 48 years of age group followed by 38-47 years [Table 3].

## Discussion

HIV-infected individuals become susceptible to a variety of opportunistic parasite infections that occur with greater frequency and severity due to down regulation of immune system. Almost 80% of AIDS patients die from AIDS-related infections including intestinal parasites rather than HIV infection itself.[10] The Coccidian parasites (*C. parvum, I. belli* and *C. cayetanensis*) and Microsporidia (*Enterocytozoon bieneusi* and *Encephalitozoon intestinalis*) are the foremost among enteric parasites in

**Table 1: Distribution of different intestinal parasites detected from HIV-infected and HIV-negative diarrheal patients**

| Parasitic divisions | Parasites | Diarrheal patients (*n*=207) (%) | | Total | *P* value |
|---|---|---|---|---|---|
| | | No. of HIV-infected (*n*=115) | No. of HIV noninfected (*n*=92) | | |
| Intestinal Protozoa (Amoebas, Flagellates, Coccidia and Microsporidia) | *Entamoeba histolytica/ Entamoeba dispar* | 09 (7.8) | 08 (8.7) | 17 | 0.83 (NS) |
| | *Giardia lamblia* | 1 (0.9) | 0 | 1 | 0.37 (NS) |
| | *Cryptosporidium parvum* | 14 (12.2) | 1 (1.1) | 15 | <0.01 (S) |
| | *Isospora belli* | 19 (16.5) | 0 | 19 | <0.001 (S) |
| | *Cyclospora cayetanensis* | 1 (0.9) | 0 | 1 | 0.37 (NS) |
| | *Microsporidia* | 3 (2.6) | 0 | 3 | 0.34 (NS) |
| | Total Protozoa | 47 (40.9) | 9 (9.8) | 56 | <0.001 (S) |
| Intestinal nematodes | *Ascaris lumbricoides* | 2 (1.7) | 3 (3.3) | 5 | 0.81 (NS) |
| | *Ancylostoma duodenale* | 1 (0.9) | 2 (2.2) | 3 | 0.85 (NS) |
| | *Strongyloides stercoralis* | 3 (2.6) | 0 | 3 | 0.34 (NS) |
| | *Trichuris trichiura* | 0 | 1 (1.1) | 1 | 0.92 (NS) |
| | *Enterobius vermicularis* | 0 | 1 (1.1) | 1 | 0.92 (NS) |
| | Total Nematodes | 6 (5.2) | 7 (7.6) | 13 | |
| Total (Protozoa and nematodes) | | 53 (46.1) | 16 (17.4) | 69 | 0.71 (NS) |

$P<0.05$ (statistically significant); S: Statistically significant; NS: Not significant, HIV: human immunodeficiency virus

**Table 2: Correlation of CD4+ T-cell counts with opportunistic intestinal protozoans among HIV-infected diarrheal patients**

| CD4+T-lymphocytes (cells/μl) | Total no. of HIV-infected (*n*=115) | Total no. of HIV-infected positive with opportunistic protozoa (Coccidia and Microsporidia) (*n*=37) (%) | *P* value |
|---|---|---|---|
| <200 | 42 | 26 (61.9) | <0.001 (statistically significant) |
| ≥200 | 73 | 11 (15.1) | |

HIV: Human immunodeficiency virus; CD4: Cluster of differentiation 4

these patients.[2] Gastrointestinal parasitic infections largely present with diarrhea leading to life threatening complications.[11]

In this present study, enteric parasites were detected in 46.1% of HIV-infected patients with diarrhea. Various studies from India and other countries have reported low, similar, or higher prevalence of intestinal parasites, ranging from 15.3% to 77.14%.[12-17] The opportunistic protozoans (69.8%) predominated among all intestinal parasites with *I. belli* (51.3%) being the most commonly detected parasite followed by *C. parvum* (37.8%), Microsporidia (8.1%), and *C. cayetanensis* (2.8%). The overall high prevalence of 16.5% was shown by *I. belli* followed by *C. parvum* (12.2%), *E. histolytica/dispar* (7.8%), Microsporidia (2.6%), *S. stercoralis* (2.6%), *A. lumbricoides* (1.7%), and 0.9% each by *G. lamblia, C. cayetanensis and A. duodenale* in HIV-infected diarrheal patients. This present study

differed from most of the studies within and outside India where they have found out *C. parvum* was the most commonly prevalent protozoan.[12,13,15-28] Few studies within India have reported *I. belli* was the predominant opportunistic intestinal protozoan.[14,29] The reason for these large difference in prevalence of opportunistic intestinal infections that tend to vary from one locality to the other and from one country to the other depending on the level of contamination of water, foodstuff, and contact with animals, which are important factors in the dissemination of the parasites.[30] The variation in the prevalence of opportunistic intestinal protozoans in HIV diarrheal patients could also be related to the immune status of the HIV patients examined, the sensitivity of the diagnostic techniques used and the experience of the microscopist.[23]

HIV-seronegatives were predominantly infected by common intestinal parasites such as *E. histolytica/E.dispar* (8.7%) followed by *A. lumbricoides* (3.3%) and *A. duodenale* (2.2%). Single (1.1%) case of *C. parvum* was found in HIV noninfected diarrheal patient, which is in agreement with studies conducted by Ramakrishnan *et al.* and Mohandas *et al.* in India where they found out *C. parvum* in 3.7% and 2% cases, respectively.[5,18] Our study differed from the Gupta *et al.* study in India, the Akinbo *et al.* study in Nigeria, and the Alemu *et al.* study in Ethiopia where they did not find any opportunistic protozoans in control groups.[12,14,23]

The findings of Coccidia and Microsporidia among HIV-infected patients indicate that opportunistic parasites readily cause infection in immune-deficient individuals. In this present study, HIV-infected

**Table 3: Age and sex wise distribution of HIV-infected diarrheal patients**

| Age groups in years | Total no. of HIV-infected (*n*=115) | | | Total no. of HIV-infected positive with Coccidia and Microsporidia (*n*=37) | | |
|---|---|---|---|---|---|---|
| | Male | Female | Total (%) | Male | Female | Total (%) |
| 18-27 | 13 | 10 | 23 (20) | 3 | 2 | 5 (13.6) |
| 28-37 | 19 | 12 | 31 (26.9) | 6 | 3 | 9 (24.3) |
| 38-47 | 30 | 15 | 45 (39.2) | 12 | 4 | 16 (43.2) |
| ≥48 | 9 | 7 | 16 (13.9) | 4 | 3 | 7 (18.9) |
| Total (%) | 71 (61.7) | 44 (38.3) | 115 (100) | 25 (67.7) | 12 (32.3) | 37 (100) |

HIV: Human immunodeficiency virus

patients with CD4+ T-cell count <200 cells/µl are at an increased risk of acquiring opportunistic infections, which is similar to all previous studies.[5,12,14,15] Indeed, an immune-deficient state has been reported by other authors as risk factor for opportunistic protozoan infections.

Majority, that is, 67.7% of opportunistic intestinal parasites were detected in males in comparison to females (32.3%). This may be due to males utilizing more integrated counseling and testing services. They usually go undiagnosed for long periods and present late in the course of disease with severe persistent infections and low CD4+ T-cell counts.[14]

There were few limitations in this present study. The study was done on a small sample size as majority of the patients were referred from general practitioners and from secondary care centers, therefore they have already received some amount of antiparasitics and antibiotics. Also the single stool specimen processing might underestimate the actual parasite prevalence. As the etiology of diarrhea could not be determined in 53.9% of HIV-infected patients and 82.6% of HIV noninfected patients, comprehensive etiological studies are needed to cover bacterial, fungal, viral, and noninfective causes of diarrhea among patients.

## Conclusion

In conclusion, intestinal parasitic infections caused diarrhea in 46.1% of HIV-infected individuals. The opportunistic intestinal protozoans especially *I. belli* and *C. parvum* were most commonly isolated in HIV-infected patients with diarrhea. Majority of the infections occurred in patients when CD4+ T-cell counts were less than 200 cells/µl.

## References

1. Framm SR, Soave R. Agents of diarrhea. Med Clin North Am 1997;81:427-47.
2. Smith PD, Lane HC, Gill VJ, Manischewitz JF, Quinnan GV, Fauci AS, *et al*. Intestinal infections in patients with the acquired immunodeficiency syndrome (AIDS): Etiology and response to therapy. Ann Intern Med 1988;108:328-33.
3. Lindo JF, Dubon JM, Ager AL, de Gourville EM, Solo-Gabriele H, Klaskala WI, *et al*. Intestinal parasitic infections in HIV positive and HIV negative individuals in San Pedro Sula, Hunduras. Am J Trop Med Hyg 1998;58:431-5.
4. Goodgame RW. Understanding intestinal spore-forming protozoa: Cryptosporidia, Microsporidia, Isospora, Cyclospora. Ann Intern Med 1996;124:429-41.
5. Ramakrishnan K, Shenbagarathai R, Uma A, Kavitha K, Rajendran R, Thirumalaikolundusubramanian P. Prevalence of Intestinal Parasitic Infestation in HIV/AIDS Patients with Diarrhea in Madurai City, South India. Jpn J Infect Dis 2007;60:209-10.
6. Dwivedi KK, Prasad G, Saini S, Mahajan S, Lal S, Baveja UK. Enteric opportunistic parasites among HIV infected individuals: Associated risk factors and immune status. Jpn J Infect Dis 2007;60:76-81.
7. Laboratory diagnosis, biosafety and Quality control. Government of India: National AIDS Control Organization (NACO). c2007. (Accessed December 29, 2012, at http://www.nacoonline.org).
8. National AIDS Control Organization. National Guidelines for Enumeration of CD + T- Lymphocytes with single platform technology for initiation and monitoring of ART in HIV infected individuals. c2007. (Accessed August 2, 2012 at http://www.nacoonline.org/Quick_Links/Publication/Blood_Safety_Lab_Services/).
9. Centers for Disease Control and Prevention. DPDx: Laboratory Identification of Parasites of Public Health Concern. c2012. (Accessed January 16, 2013, at http://dpd.cdc.gov/dpdx/Default.htm).
10. Franzen C, Müller A. Cryptosporidial and Microsporidial-waterborne disease in immunocompromised host. Diagn Microbiol Infect Dis 1999;34:245-62.
11. Weber R, Bryan RT, Owen RL, Wilcox CM, Gorelkin L, Visvesvara GS. Improved light- microsporidial detection of Microsporidia spores in stool and duodenal aspirate. The Enteric Opportunistic Infections Working Group. N Engl J Med 1992;326:161-6.
12. Akinbo FO, Okaka CE, Omoregie R. Prevalence of intestinal parasitic infections among HIV patients in Benin City, Nigeria. Libyan J Med 2010;29:5.
13. Daryani A, Sharif M, Meigouni M, Mahmoudi FB, Rafiei A, Gohardehi Sh, *et al*. Prevalence of intestinal parasites and profile of CD+counts in HIV/AIDS people in north Iran, 2007-2008. Pak J Biol Sci 2009;12:1277-81.
14. Gupta S, Narang S, Nunavath V, Singh S. Chronic diarrhoea in HIV patients: Prevalence of coccidian parasites. Indian J Med Microbiol 2008;26:172-5.

15. Kulkarni SV, Kairon R, Sane SS, Padmawar PS, Kale VA, Thakar MR, *et al*. Opportunistic parasitic infections in HIV/AIDS patients presenting with diarrhoea by the level of immunesuppression. Indian J Med Res 2009;130:63-6.

16. Basak S, Bose S, Mallick SK, Ghosh AK. Intestinal parasitic infections in HIV seropositive patients – A study. J Clin Diagn Res 2010;4:2433-7.

17. Noor R, Saha SR, Rahman F, Munsi SK, Uddin MA, Rahman MM. Frequency of opportunistic and other intestinal parasitic infections in patients infected with human immunodeficiency virus in Bangladesh. Tzu Chi Med J 2012;24:191-5.

18. Mohandas, Sehgal R, Sud A, Malla N. Prevalence of intestinal parasitic pathogens in HIV-seropositive individuals in Northern India. Jpn J Infect Dis 2002;55:83-4.

19. Rao Ajjampur SS, Asirvatham JR, Muthusamy D, Gladstone BP, Abraham OC, Mathai D, *et al*. Clinical features and risk factors associated with cryptosporidiosis in HIV infected adults in India. Indian J Med Res 2007;126:553-7.

20. Tuli L, Gulati AK, Sundar S, Mohapatra TM. Correlation between CD4 counts of HIV patients and enteric protozoan in different seasons- An experience of a tertiary care hospital in Varanasi (India). BMC Gastroenterol 2008;8:36.

21. Vyas N, Pathan N, Aziz A. Enteric pathogens in HIV-positive patients with diarrhoea and their correlation with CD4+T-lymphocyte counts. Trop Parasitol 2012;2:29-34.

22. Ramana KV, Prakash K, Mohanty SK. A study of opportunistic parasitic infections and CD4 counts in HIV-seropositive individuals in Narketpalli, South India. Ann Trop Med Public Health 2010;3:49-52.

23. Alemu A, Shiferaw Y, Getnet G, Yalew A, Addis Z. Opportunistic and other intestinal parasites among HIV/AIDS patients attending Gambi higher clinic in Bahir Dar city, North West Ethiopia. Asian Pac J Trop Med 2011;4:661-5.

24. Minta DK, Dembélé M, Dolo A, Sidibé AT, Diarra AS, Konaté A, *et al*. Digestive parasitic diseases to HIV/AIDS infected patients of internal medicine and infectious diseases wards of the hospital du Point "G" Bamako – Mali. Mali Med 2007;22:33-6.

25. Kurniawan A, Karyadi T, Dwintasari SW, Sari IP, Yunihastuti E, Djauzi S, *et al*. Intestinal parasitic infections in HIV/AIDS patients presenting with diarrhea in Jakarta, Indonesia. Trans R Soc Trop Med Hyg 2009;103:892-8.

26. Saksirisampant W, Prownebon J, Saksirisampant P, Mungthin M, Siripatanapipong S, Leelayoova S. Intestinal parasitic infections: Prevalences in HIV/AIDS patients in a Thai AIDS-care centre. Ann Trop Med Parasitol 2009;103:573-81.

27. Stensvold CR, Nielsen SD, Badsberg JH, Engberg J, Friis-Møller N, Nielsen SS, *et al*. The prevalence and clinical significance of intestinal parasites in HIV-infected patients in Denmark. Scand J Infect Dis 2011;43:129-35.

28. Tian LG, Chen JX, Wang TP, Cheng GJ, Steinmann P, Wang FF, *et al*. Co-infection of HIV and intestinal parasites in rural area of China. Parasit Vectors 2012;5:36.

29. Vignesh R, Balakrishnan P, Shankar EM, Murugavel KG, Hanas S, Cecelia AJ, *et al*. High proportion of isosporiasis among HIV-infected patients with diarrhea in southern India. Am J Trop Med Hyg 2007;77:823-4.

30. Widmer G, Carraway M, Tzipori S. Waterborne Cryptosporidium: A perspective from the USA. Parasitol Today 1996;12:286-90.