

**Original article:**

## **Study of opportunistic parasitic infections in HIV/AIDS patients presenting with diarrhea & its co-relation with CD4 count**

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### **ABSTRACT**

**OBJECTIVE** – The present study was undertaken to detect opportunistic parasites in HIV Seropositive patients with diarrhoea and correlation with different levels of immunity (CD4 Count).

**MATERIALS AND METHODS** – The study was carried out at BLDEA's Sri B.M .Patil Medical College Hospital & Research Centre, Bijapur from DEC. 2011 to MAY 2013 among consecutively enrolled 110 HIV patients presenting with diarrhoea. Stool samples were examined for parasites by direct microscopy of feces i.e. Saline wet mount, Iodine wet mount, Lactophenol cotton blue mount, Modified Kinyoun's acid fast stain. CD4 counts of these patients was monitored and noted.

**RESULTS** – Opportunistic parasites were detected in 44.54% patients i.e. 49 of 110 patients studied. Majority of the study population belonged to the age group of 31 – 50 years (68.19%) with male preponderance (55.45%). Cryptosporidium was identified in maximum number of positive cases (32.73%) followed by mixed infection (9.09%) and Cyclospora infection (2.72%). Maximum patients which were positive for parasites had CD4 counts <100 i.e. 24 (50%), followed by CD4 counts 100 - 200 i.e. 22 (44.9%), whereas only 3 (6.1%) had CD4 counts 200 - 500. No pathogenic parasites were isolated in patients with CD4 counts > 500 cell/mm<sup>3</sup>.

**CONCLUSION** – The present study shows that diarrhoea in HIV/AIDS was mostly due to opportunistic coccidian parasites associated with low CD4 count. Thus Detection of etiologic pathogens might help clinicians to decide appropriate management strategies and reduce morbidity and mortality in such individuals.

**KEYWORDS:** Opportunistic parasites, CD4+ T cell count, HIV

### **INTRODUCTION**

Human immunodeficiency virus (HIV) infection has become a global epidemic. India has the third largest number of people living with HIV/AIDS. As per the 2015 HIV estimates, there were an estimated 21.17 lakh people living with HIV/AIDS in India with an adult prevalence of 0.26% in 2015.<sup>[1]</sup> HIV infection is marked by a progressive decrease in the number of circulating CD4 T-helper cells, which, over a period of years, leads to immunologic decline and death due to opportunistic infections and neoplasms. The spectrum of opportunistic infections differs from region to region.<sup>[2]</sup> Gastrointestinal infections are very common in patients with HIV infection or AIDS. Diarrhoea is a common clinical presentation of these infections. Reports indicate that diarrhoea occurs in 30-60 per cent of AIDS patients in developed countries and in about 90 per cent of AIDS patients in developing countries.<sup>[3]</sup> AIDS patients with chronic diarrhoea and occult enteropathogens often have greater mean weight loss and a significantly shorter survival, substantial work loss and a markedly decreased quality of life, such patients also frequently have annual health costs that are 50% higher than comparable patients without diarrheal symptoms.<sup>[4]</sup>

The etiologic spectrum of enteric pathogens causing diarrhoea includes bacteria, parasites, fungi and viruses,<sup>[5]</sup> though that of parasitic origin is prominent in patients with AIDS in developing countries. Of these, protozoan parasitic infections are the most serious ones causing severe morbidity and mortality.<sup>[6]</sup> The presence of opportunistic parasites *Cryptosporidium parvum*, *Cyclospora cayetanensis*, *Isospora belli* and *Microsporidia* are documented in patients with AIDS. Non opportunistic parasites such as *Entamoeba histolytica*, *Giardia lamblia*, *Ascaris lumbricoides*, *Strongyloides stercoralis*, *Blastocystis hominis* and *Ancylostoma duodenale* are frequently encountered in developing countries but are not currently considered opportunistic in AIDS patients.<sup>[7], [3]</sup>

There have been reports on frequency of various pathogens causing diarrhoea from different parts of India. . 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. However, there is a paucity of data on correlation of CD4+ T-cell counts and the etiology of diarrhoea among the HIV patients in this part of India. Thus, this study was conducted to identify the opportunistic parasitic infection in HIV/AIDS patients presenting with diarrhoea and to co-relate their presence with CD4+ T-cell counts.

### **MATERIAL AND METHODS**

A total of 110 patients of both sexes irrespective of age groups who were HIV seropositive with diarrhea attending BLDEA's Sri B.M. Patil Medical College Hospital & Research Centre, Bijapur from DEC 2011 to MAY 2013 were included in the study after the approval of institutional ethical committee and with the consent of the subjects. CD4 +T cell count was obtained from patients records.

The HIV serostatus of the patients was determined according to NACO guidelines. Strategy IIB & III were used for diagnosis of HIV infection. All patients were tested for HIV using three separate kits, commercially available ELISA/Rapid Tests (Genetic system, Bio-Rad Labs, USA and Tridot, J Mitra & Co, New Delhi), having different system in order to confirm the diagnosis.<sup>[8]</sup>

Diarrhoea was defined as 3 or more semi-liquid or liquid stools in twenty four hours. Diarrhoea associated with HIV infection may be acute or chronic. Acute diarrhoea is defined as diarrhoea of <14 days duration and chronic diarrhoea is defined as diarrhoea lasting for >14 days.<sup>[9]</sup> The stool samples were collected from patients who had not received Anti-parasitic medications, anti-diarrheal agents in the previous 2 weeks. The patients were asked to collect their stool sample preferably in the morning in sterile leak proof plastic containers with a wide mouth and a tight - fitting lid without any preservative. They were instructed to avoid contamination of the stool specimen with urine or water. The specimens were processed within 1-3 hours of collection. The specimens were examined by naked eye for Colour, Consistency, and Presence of blood, mucus, adult worms or segments of worms. Standard procedure for stool examination was carried out. Samples were examined microscopically directly and following concentration methods like formalin-ether concentration method and Sheather's sugar floatation technique<sup>[10] [11]</sup>. Direct microscopic examination of faeces in saline (0.85%) and 1:5 dilution of Lugol's iodine in distilled water was done to detect trophozoites, ova, cysts, larvae and oocysts. Ethanol fixed stool smears were stained with Modified Kinyoun's Acid-Fast Staining Method (Cold method) by using 1% H<sub>2</sub>SO<sub>4</sub> as decolourizer for detecting coccidian parasites (*Cryptosporidium*, *Cyclospora*, and *Isospora* species) in the faeces specimens.<sup>[12] [13]</sup>

Statistical analysis was done by using Graph Pad Prism version 6. Chi-square test was done to evaluate any correlation in HIV positives between parasitic infections and CD4 count. Observed differences in data were considered significant and noted in the text if P < 0.05 was obtained

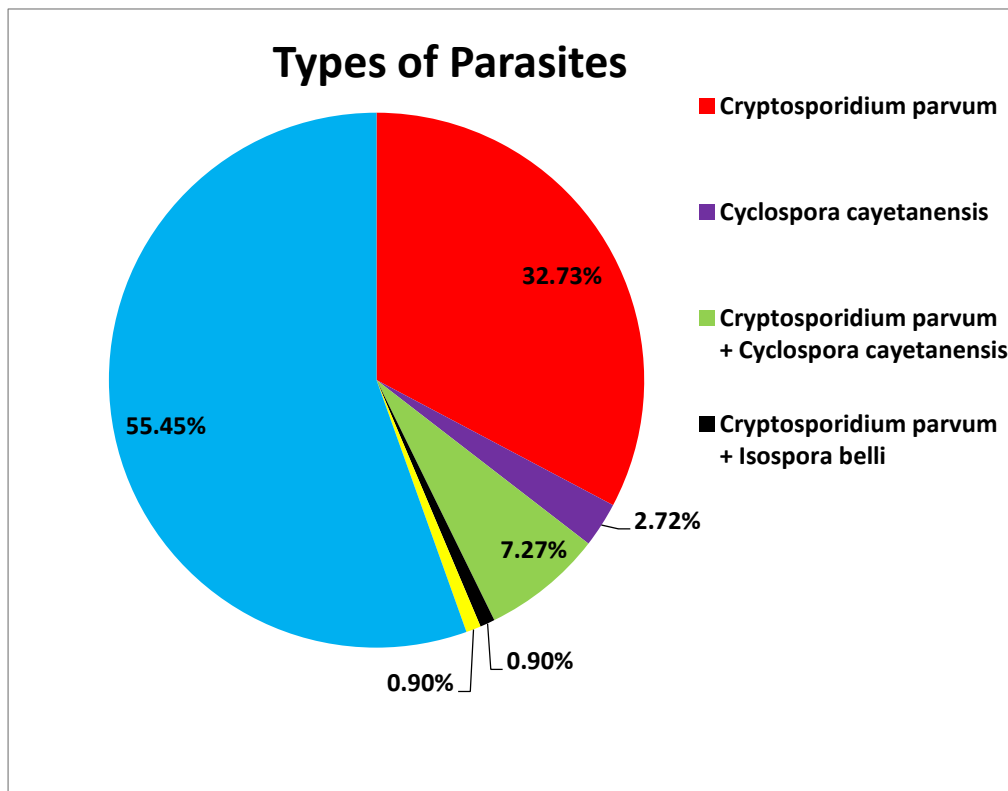
**RESULTS**

Out of 110 cases of HIV/AIDS studied male predominance was observed with number of male 61 (55.45%) followed by female population of 49 (44.55%). Male to Female Ratio is 1.2:1. The maximum number of ,i.e, 48 cases (43.64%) were in the age group of 31-40 years and least number of cases 3 each were seen in age groups <10 years & 11 – 20 years [Table 1].

**TABLE 1: Age and Sex Distribution**

Age (years)	Male		Female		Total	
	Number	Percentage	Number	Percentage	Number	Percentage
≤ 10	3	2.72 %	0	0 %	3	2.72 %
11 - 20	1	0.90 %	2	1.82 %	3	2.72 %
21 - 30	6	5.46 %	13	11.82 %	19	17.28 %
31 - 40	27	24.55 %	21	19.09 %	48	43.64 %
41 - 50	18	16.37 %	9	8.18 %	27	24.55 %
≥ 51	6	5.45 %	4	3.64 %	10	9.09 %
<b>Total</b>	<b>61</b>	<b>55.45 %</b>	<b>49</b>	<b>44.55 %</b>	<b>110</b>	<b>100 %</b>

Out of total 110 samples, 49 (44.54%) were positive for parasites and 61 were negative for parasites. Maximum among these were Cryptosporidium parvum positive 36 (32.73%) followed by Mixed infection 10 (9.09 %). Mixed infection of Cryptosporidium parvum with Cyclospora was seen in maximum samples 8 (7.27%) followed by 1 case each of Cyclospora with Isospora belli and Cryptosporidium parvum with Cyclospora and Isospora belli. [Figure 1]

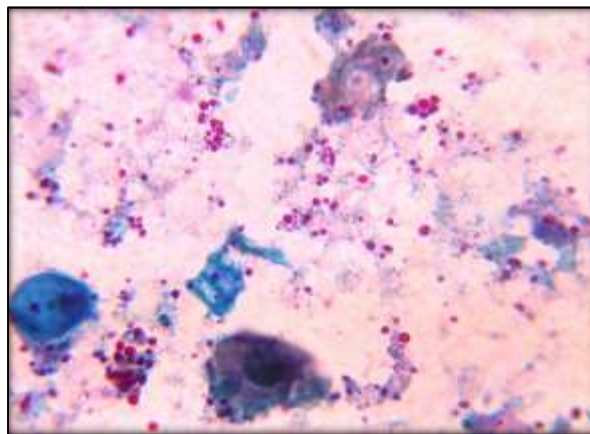


**FIGURE 1: Types of parasites identified in stool samples**

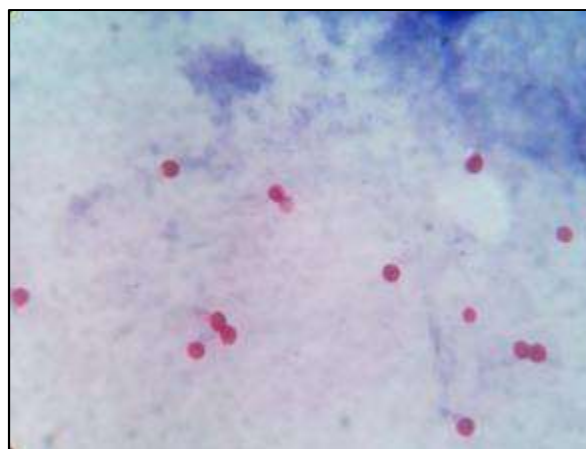
**TABLE 2: Correlation of CD4 counts and no. of cases positive for parasites.**

Parasites		CD4 counts ( Cells/mm <sup>3</sup> )			
		< 100	100 - 200	200- 500	>500
Cryptosporidium parvum		18	15	3	0
Cyclospora cayetanensis		0	3	0	0
Mixed infection	Cryptosporidium parvum + Cyclospora cayetanensis	5	3	0	0
	Cyclospora cayetanensis + Isospora belli	0	1	0	0
	Cryptosporidium parvum + Cyclospora cayetanensis + Isospora belli	1	0	0	0
<b>Total</b>		24	22	3	0

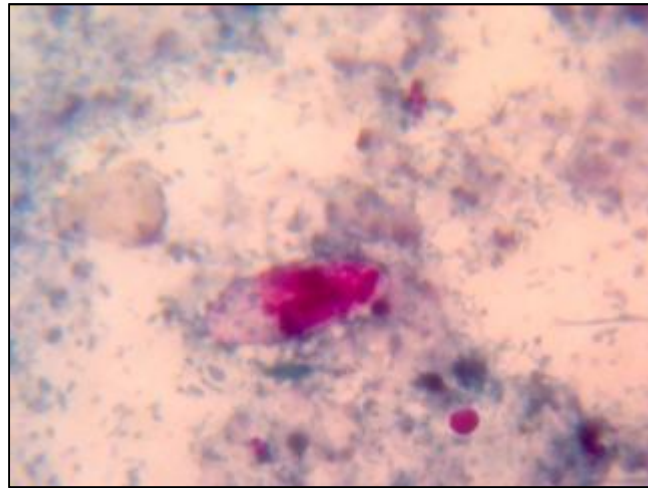
Based on CD4 counts Cryptosporidium parvum was the commonest parasite seen in 75% of patients with counts <100 cells/mm<sup>3</sup> and 68.19% in patients with counts 100 – 200. Whereas none of the cases of coccidian parasitic diarrhoea were observed in patients with CD counts >500.[Table 2]



**FIGURE 2: Cryptosporidium parvum in Modified Kinyoun's Acid-Fast Staining Method**



**FIGURE 3: Cyclospora cayetanensis Modified Kinyoun's Acid-Fast Staining Method**



**FIGURE 4: Mixed infection Modified Kinyoun's Acid-Fast Staining Method**

## DISCUSSION

Diarrhea is among the most frequent clinical symptoms in HIV-infected patients. With the emergence of AIDS, parasitic diarrhea has gained significance, as it is one of the important causes of morbidity and mortality. The line of treatment being different for diverse parasites necessitates a definitive diagnosis and study of the etiological agents causing diarrhea, especially when it can be fatal in this vulnerable group of individuals. This susceptibility to microbial infections can be related to the course of progression of HIV disease. As the CD4 count decreases, patients become susceptible to an increasing number of opportunistic parasitic infections, especially when the CD4 count decreases  $< 200 \text{ cells/mm}^3$ .

A total of 110 HIV seropositive patients were studied. Majority of the patients (68.19 %) belonged to the age group of 31- 50 years. Mean age in our study is 37.78 years  $\pm$  SD 11.13. Comparable age related data has been reported by other authors Beena et al 2009 <sup>[14]</sup> (78%) and Deorukhkar et al 2011 <sup>[15]</sup> (66.27 %). This reflects the increased positivity of HIV/AIDS in the sexually active age group as per the demographic distribution of the disease.

In this study there were 55.45 % of males (Mean age - 38.623  $\pm$  SD - 11.360, two tailed P Value  $< 0.0001$  - extremely significant) in comparison to 44.55 % of females (Mean age - 36.735  $\pm$  SD - 10.864, two tailed P Value  $< 0.0001$  - extremely significant). This male preponderance in the present study also coincides with the findings of other authors Beena et al <sup>[14]</sup> (58% male & 42% female), Raytekar et al <sup>[16]</sup> (55.9% Males , 44.01 % Females).

The overall identification of enteric parasitosis is 44.54% in this study. Nearly similar percentage of detection was seen in Saksirisant et al <sup>[17]</sup> where overall positive samples were 45.6% . Whereas Prasad et al<sup>[18]</sup> detected enteric pathogens in 73% of study group. In a study from Madurai, South India by Ramakrishnan et al <sup>[19]</sup> parasites were detected in 38.7% of cases.

**TABLE 3: Parasites identified**

Studies	Parasites Identified		
	Cr.	Cy.	Mixed
Mohandas et al 2002 <sup>[20]</sup>	10.8%	3.3%	-
Tuli et al 2008 <sup>[21]</sup>	39.8%	24.0 %	25.1%
Gupta et al 2008 <sup>[7]</sup>	29.2%	4.2%	-
Basak et al 2010 <sup>[22]</sup>	28.4 %	2 %	2 %
Sucilathangam et al 2011 <sup>[23]</sup>	36 %	3 %	9 %
Jha et al 2012 <sup>[24]</sup>	60.42%	2.9%	-
Vyas et al 2012 <sup>[25]</sup>	25.18%	8.65%	-
Saksirisantpant et al 2009 <sup>[17]</sup>	34.4%	1.1%	-
<b>Present Study</b>	<b>36 (32.73 %)</b>	<b>3 (2.72 %)</b>	<b>10 (9.09 %)</b>

In our study the maximum detected parasites i.e. 36 (32.73 %) were *Cryptosporidium parvum*, followed by *Cyclospora cayetanensis* 3 (2.72 %) and mixed infection 10 (9.09 %). Nearly similar percentage of detection was seen in Saksirisantpant et al <sup>[17]</sup> where 34.4% were *Cryptosporidium* and 1.1% were *Cyclospora* positive. Jha et al <sup>[24]</sup> showed *Cyclospora* positivity similar to that of our study i.e. 2.9% but *Cryptosporidium* (60.42%) was much higher compared to our studies. Several studies from India and other parts of the world also have reported *Cryptosporidium parvum* as the predominant pathogen. <sup>[20],[21],[24]</sup>

In this study we noticed a low level of *Isospora belli* and *Cyclospora*. As many of the study population might be under Trimethoprim - Sulphamethoxazole prophylaxis. Treatment with trimethoprim Sulphamethoxazole for other infections in AIDS is said to confer some protection against *Isospora belli* and *Cyclospora* <sup>[26]</sup> No isolation of helminths and trophozoites/oocysts of pathogenic protozoa was observed in this study as most of the patients with diarrhoea were on empirical anti-helminthic/anti-diarrhoeal treatment.

In our study mixed infection was 9.09% (10 Samples) out of which *Cryptosporidium parvum* with *Cyclospora* was seen in maximum samples 8 (7.27%) followed by 1 case each of *Cyclospora* with *Isospora belli* and *Cryptosporidium parvum* with *Cyclospora* and *Isospora belli*. In a case report by Chakrabarti et al <sup>[27]</sup> mixed infection of *Cryptosporidium parvum* with *Cyclospora* and *Isospora belli* was seen, while Basak et al <sup>[22]</sup> 2% of such cases were reported. In a study by Sucilathangam et al <sup>[23]</sup> mixed infection of *Cryptosporidium parvum* with *Cyclospora* was seen in 3 % of samples. Venkatesh et al <sup>[28]</sup> also reported co-infection of *Cyclospora* and *Isospora belli* in 1 patient.

In the present study maximum patients which were positive for parasites had CD4 counts <100 i.e. 24 (50%), followed by CD4 counts 100 - 200 i.e. 22 (44.9%), whereas only 3 (6.1%) have CD4 counts 200 - 500. No parasites were isolated in patients with CD4 counts > 500 cell/mm<sup>3</sup>. In study by Basak et al <sup>[22]</sup> correlation of CD4 count with percentage of parasite isolation was [CD4 <100 - (34.4%), 100 -200 (41.3%), 200-500 (17.2%)] similar to that of our study. Raytekar et al <sup>[16]</sup> shows intestinal parasites more commonly in patients with CD4 counts 200-499 cells/mm<sup>3</sup>.

In Present study based on CD4 counts *Cryptosporidium parvum* was the commonest parasite seen in 50% of patients with counts <100 cells/mm<sup>3</sup> and 41.4 % in patients with counts 100-200 cells/mm<sup>3</sup>. Patients with CD4 counts 200-500 cells/mm<sup>3</sup> showed 8.3% *Cryptosporidium parvum* and no parasite was seen in patients with

CD4 counts  $>500$  cells/mm<sup>3</sup>. In study by Sucilathangam et al [23] *Cryptosporidium parvum* (43%) was the predominant pathogen in patients with CD4 T-cell counts which was  $< 200$  cells/mm<sup>3</sup>. Raytekar et al [16] from Loni (Maharashtra), Kulkarni et al [3] from Pune and Vyas et al [25] stated that *Cryptosporidium parvum* was mainly associated with CD4 counts  $< 200$  cells/mm<sup>3</sup>.

In our study *Cyclospora* was identified only in patients with CD4 count 100 - 200 i.e. 3 (2.72%) and mixed infection was seen in 10 (9.09%) patients of which majority 6 (60%) were in patients with CD4 counts  $<100$  and remaining 4 (40%) in patients with CD4 counts 100-200. In study by Sucilathangam et al [23] all identification of *Cyclospora* and mixed infection was seen in patients with CD4 counts  $< 200$  cells/mm<sup>3</sup>. In study by Vyas et al [25] majority of *Cyclospora* identified i.e. 16.9% was seen in patients with CD4 count  $<200$  cells/mm<sup>3</sup>. In study by Gupta et al [7] mixed infection were seen in mean CD4 T cell count of 105.3 cells/mm<sup>3</sup>.

## CONCLUSION

Individuals with HIV/AIDS, because of their compromised immune status are at a higher risk of infections. Opportunistic enteric parasites affect the small intestine and produce overwhelming results with grave prognosis. As parasites cause prolonged, life-threatening diarrhoea in AIDS patients, identification of these opportunistic parasites at the earliest with simple techniques like wet mounts and Modified Kinyoun's acid fast staining could successfully identify a variety of enteric parasites and enable the clinician to give effective treatment and save the patient from increasing mortality.

## REFERANCES

1. Annual Report. National AIDS Control Organization, 2016 - 17.
2. Kumarasamy N, Solomon S, Flanigan TP, Hemalatha R, Thyagarajan S, Mayer KH. Natural history of human immunodeficiency virus disease in southern India. *Clinical Infectious Diseases*. 2003;36(1):79-85.
3. Kulkarni S, Kairon R, Sane S, Padmawar P, Kale V, Thakar M, et al. Opportunistic parasitic infections in HIV/AIDS patients presenting with diarrhoea by the level of immunosuppression. *The Indian journal of medical research*. 2009;130(1):63.
4. Lew EA, Poles MA, Dieterich DT. Diarrheal diseases associated with HIV infection. *Gastroenterology clinics of North America*. 1997;26(2):259-90.
5. Mitra AK, Hernandez CD, Hernandez CA, Siddiq Z. Management of diarrhoea in HIV-infected patients. *International journal of STD & AIDS*. 2001;12(10):630-9.
6. Kashyap B, Sinha S, Das S, Rustagi N, Jhamb R. Efficiency of diagnostic methods for correlation between prevalence of enteric protozoan parasites and HIV/AIDS status—an experience of a tertiary care hospital in East Delhi. *Journal of Parasitic Diseases*. 2010;34(2):63-7.
7. Gupta S, Narang S, Nunavath V, Singh S. Chronic diarrhoea in HIV patients: prevalence of coccidian parasites. *Indian journal of medical microbiology*. 2008;26(2):172.
8. The National AIDS Control Programme Phase III 2007. Available from: <http://siteresources.worldbank.org/INTHIVAIDS/Resources/375798-1151090631807/2693180-1151090665111/2693181-1155742859198/India20V3.pdf>.
9. Fauci AS. *Harrison's principles of internal medicine*: McGraw-Hill Medical New York; 2012.
10. Parija SC. *Textbook of medical Parasitology, Protozoology & Helminthology*. SciELO Brasil; 2008.
11. Winn WC, Koneman EW. *Koneman's color atlas and textbook of diagnostic microbiology*: Wolters Kluwer Health; 2006.

12. Garcia LS, Bruckner DA, Brewer TC, Shimizu RY. Techniques for the recovery and identification of *Cryptosporidium* oocysts from stool specimens. *Journal of clinical microbiology*. 1983;18(1):185-90.
13. Cheesbrough M. *District laboratory practice in tropical countries*: Cambridge university press; 2006.
14. Uppal B, Kashyap B, Bhalla P. Enteric pathogens in HIV/AIDS from a tertiary care hospital. *Indian journal of community medicine: official publication of Indian Association of Preventive & Social Medicine*. 2009;34(3):237.
15. Deorukhkar S, Katiyar R, Saini S, Siddiqui A. The Prevalence Of Intestinal Parasitic Infections In HIV Infected Patients In A Rural Tertiary Care Hospital Of Western Maharashtra (A 5 Year Study). *Journal of Clinical and Diagnostic Research*. 2011;5(2):210-2.
16. Raytekar NA, Deorukhkar SC, Saini S. INTESTINAL PARASITIC PREVALENCE IN HUMAN IMMUNO-DEFICIENT VIRUS (HIV) INFECTED PATIENTS WITH AND WITHOUT DIARRHOEA AND ITS ASSOCIATION WITH CD4 T CELLS COUNTS. *International Journal of Biomedical and Advance Research*. 2012;3(11):853-7.
17. Saksirisampant W, Prownobon J, Saksirisampant P, Mungthin M, Siripatanapibong S, Leelayoova S. Intestinal parasitic infections: prevalences in HIV/AIDS patients in a Thai AIDS-care centre. *Annals of tropical medicine and parasitology*. 2009;103(7):573-81.
18. Prasad K, Nag V, Dhole T, Ayyagari A. Identification of enteric pathogens in HIV-positive patients with diarrhoea in northern India. *Journal of Health, Population and Nutrition (JHPN)*. 2000;18(1):23-6.
19. 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. *Japanese journal of infectious diseases*. 2007;60(4):209.
20. Mohandas K, Sehgal R, Sud A, Malla N. Prevalence of intestinal parasitic pathogens in HIV-seropositive individuals in Northern India. *Japanese journal of infectious diseases*. 2002;55(3):83-4.
21. 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 gastroenterology*. 2008;8(1):36.
22. Basak S, Bose S, Mallick S, Ghosh A. Intestinal parasitic infections in HIV seropositive patients-A study. *Journal of Clinical and Diagnostic Research*. 2010:2433-7.
23. Sucilathangam G, Velvizhi G, Palaniappan T. The prevalence of coccidian parasites in and around tirunelveli in HIV positive individuals and its correlation with the CD4 count. *J Clin Diagn Res*. 2011;5(Suppl 6):1182-6.
24. Jha AK, Uppal B, Chadha S, Bhalla P, Ghosh R, Aggarwal P, et al. Clinical and Microbiological Profile of HIV/AIDS Cases with Diarrhea in North India. *Journal of pathogens*. 2012;2012.
25. Vyas N, Pathan N, Aziz A. Enteric pathogens in HIV-positive patients with diarrhoea and their correlation with CD4+ T-lymphocyte counts. *Tropical parasitology*. 2012;2(1):29.
26. Kumar SS, Ananthan S, Lakshmi P. Intestinal parasitic infection in HIV infected patients with diarrhoea in Chennai. *Indian Journal of Medical Microbiology*. 2002;20(2):88.
27. Chakrabarti P, Samantaray J, Malik S. Mixed infection with three intestinal coccidian parasites in an AIDS patient. *J Assoc Physicians India*. 2004;52:975.
28. Venkatesh Naik R Up, Lava R. Opportunistic intestinal parasitic infections in HIV seropositive patients. *International journal of pharmacy and biological sciences*. 2012;2(1):224-30. Epub 28-03-2012.