DOI: 10.7860/NJLM/2022/49133.2563

Original Article

Microbiology Section

Evaluation of Diagnostic Techniques in Detection of Enteric Coccidian Parasites in Patients with HIV

AMIYABALA SAHOO[1], RAKESH KUMAR MAHAJAN[2]

ABSTRACT

Introduction: Diarrhoea is one of the commonest opportunistic infections seen in the course of the HIV disease and is a cause of considerable morbidity and mortality. Protozoan intestinal infections constitute one of the most important ailments affecting these immune deficient groups.

Aim: To evaluate modified acid-fast staining technique with modified safranin technique in detection of enteric coccidian parasites and its correlation with Cluster of Differentiation (CD4) cell counts.

Materials and Methods: A prospective cross-sectional study was done for a period of 16 months (November 2015 to March 2017) was conducted in 200 Human Immunodeficiency Virus (HIV) positive patients with diarrhoea in ABVIMS and Dr. RML hospital, Delhi, India. Three consecutive early morning stool samples (3-5 gm/3-5 mL) along with 3 mL venous blood in Becton Dickinson Ethylenediamine Tetra-Acetic Acid (BD EDTA) vial for CD4 cell estimation were collected. The smears were subjected to Kinyuon method and Modified Safranin Technique. Enzyme Linked Immunosorbent Assay (ELISA) for detection of *Cryptosporidium* antigen was also performed. All statistical analysis was performed using STATA version 16.1 software.

Results: A total of 58 (29%) enteric coccidian parasites were detected. *Isospora belli* was the most common parasite in HIV positive patients followed by *Cryptosporidium* spp. The maximum parasitic isolation was in the patients with CD4 cell counts below 250 cells/µL.

Conclusion: Routine screening of stool samples of HIV positive patients with diarrhoea should be undertaken for enteric coccidian parasites to prevent delay in diagnosis and prevention of morbidity and mortality associated with these infections.

Keywords: Diagnosis, Diarrhoea, Enzyme linked immunosorbent assay, Human immunodeficiency virus

INTRODUCTION

Opportunistic infections have been recognised as an important pathological phenomenon hardwired to outcome of HIV infection. Gastrointestinal Tract (GIT) infections are the most common opportunistic infections. Diarrhoea is one of the most prevalent complications associated with HIV infection, and it is a significant cause of morbidity and mortality. It has been estimated that 30% patients with Acquired Immune Deficiency Syndrome (AIDS) in United States of America (USA) and 63-93% patients in developing countries like India suffer from diarrhoeal illness [1]. The aetiological spectrum of enteric pathogens causing diarrhoea includes parasites, bacteria, fungi, and viruses [2]. Protozoan intestinal infections constitute one of the most serious illness affecting these immunocompromised individuals. In healthy people, these parasites can induce self-limiting diarrhoea that lasts just a few days, but in immunocompromised people, such as HIV positive people, the diarrhoea is typically persistent, intractable, and often life-threatening [3]. The severity of enteric protozoan parasite infection is determined by absolute CD4 T cell counts, with lower counts indicating more serious disease and a higher chance of disseminated infection [4].

As most of the protozoan infections are treatable, it is important that an early and accurate diagnosis be made and current practices of empirical treatment are replaced with the targeted drug intervention on the basis of lab confirmed diagnosis. Thus, this study was planned to evaluate the staining methods and an ELISA to accurately identify the enteric protozoan parasites and also to elucidate the association between CD4 T cell counts and infection with protozoan parasites.

MATERIALS AND METHODS

A prospective cross-sectional study was conducted in a total of 200 HIV positive patients admitted in the wards of department of medicine or attending the Antiretroviral Therapy (ART) clinic were investigated for enteric coccidian parasites. This study was conducted during the period between November 2015 to March 2017 in ABVIMS and Dr. RML Hospital, New Delhi, India, after approval from the Institutional Ethics Committee (12/2015/IEC/PGIMER/RML4912). The patients were instructed to collect three consecutive early morning stool samples (3-5 gm/3-5 mL) in a clean wide mouthed plastic container with screw capped lid as the shedding of the enteric coccidian parasites in stool is intermittent. Informed consent was obtained from the patients. Based on the prevalence of enteric coccidian parasites in HIV positive patients (18.4-70%) and allowable error 10% for a two-tailed study with a=0.05 and power 80%, the sample size was found to be 200 [5-7].

Inclusion criteria: The HIV seropositive patients of age more than 18 years presented with diarrhoea.

Exclusion criteria

- HIV seropositive patients with age less than 18 years.
- HIV seropositive patients on cotrimoxazole, antiprotozoals or antimotility drugs in the preceding two weeks.

Study Procedure

Stool processing and examination: The colour and consistency of the specimen was noted. Stool samples were processed immediately. If there was a delay in the processing of the samples, they were preserved at 4°C. The samples were divided into two parts. The first part was concentrated by formol ether technique from which wet mount and smears were prepared for staining by modified Kinyoun and hot safranin technique. The second part of the unconcentrated stool samples fixed with 10% formalin was subjected to sandwich ELISA for *Cryptosporidium parvum* antigen detection.

Formalin ether concentration method [8]

A portion (5 mL) of each fresh stool sample was taken and mixed with 10% formalin to bring the volume in the centrifuge tube to 15 mL. It was centrifuged at 500×g for 10 minutes.

- The supernatant was discarded. 10 mL of 10% formalin was added to the sediment and mixed thoroughly.
- 4 mL of ethyl acetate was added and shaken vigorously in an inverted position for 30 seconds after putting a stopper.
- Centrifuge at 500×g for 10 minutes.
- Free the plug of debris from the top of the tube by ringing the sides with an applicator stick. The top layer of supernatant was added.
- Few drops of 10% formalin were added to resuspend the concentrated specimen from which smears and wet mount were prepared.

Wet mount of stool: The sediment obtained after formalin ether concentration method was observed under low power (10X) of light microscope and all suspicious findings were confirmed under high power (40X) [8].

Staining methods: The smears were subjected to two staining techniques as per standard procedures

Modified acid-fast staining (Kinyuon method): The smear was fixed with absolute methanol and stained with Kinyoun's carbol fuschin. Destaining was done by 1% acid alcohol and the smear was counterstained by malachite green [9].

Modified safranin technique (hot method) staining procedure: 1% acid alcohol was used for fixation. Safranin was used for staining and malachite green as counterstain [9].

ELISA for detection of *Cryptosporidium* antigen: *Cryptosporidium* antigen was detected by using commercially available ELISA kit CoproELISATM *Cryptosporidium* manufactured by Savyon Diagnostics Ltd., Israel. The test was performed as per kit literature.

CD4 cell estimation: Three mL venous blood was collected in BD EDTA vial from the patient. CD4 estimation was done on Fluorescence-Activated Single Cell Sorting (FACS) Calibur (Becton Dickinson) flow cytometer as per manufacturer's guidelines. A 20 µl of BD Tritest CD3/CD4/CD45 reagent was taken in a BD Trucount tube. 50 µL of well-mixed anticoagulated whole blood was added to this. The tube was capped and vortexed gently to mix. It was then incubated for 15 minutes in dark after which 450 µL 1X BD FACS lysing solution was added. The mixture was again vortexed to mix and incubated for another 15 minutes. It was again vortexed and loaded into loader racks for analysis by BD FACS Calibur. CD4 counts are expressed in cells/µL were obtained for all the HIV seropositive patients.

STATISTICAL ANALYSIS

Based on the prevalence of enteric coccidian parasites in HIV positive patients (18.4-70%) and allowable error 10% for a two-tailed study with a=0.05 and power 80%, the sample size was found to be 200 [5-7]. All statistical analysis was performed using STATA version 16.1 software (College station, Texas, USA). Comparison of proportions was done by fisher exact test of significance. Sensitivity, specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) was calculated. Correlation between various tests was determined with Bonferroni correction. The alpha error was set at 0.5 two-sided and power at 0.8 ($p \leq 0.05$ is considered to be significant). All the tests were applied at 95% significance level.

RESULTS

Out of 200 patients a total of 58 enteric coccidian parasites were detected in 48 males and 10 females. Detection of enteric coccidian parasites was maximum in the age group 31-50 years i.e., in 20 male patients and eight in female patients while 18 were detected in males in 18-30 age group. Out of 58, 32 (55.17%) were *Isospora* spp., 18 (31.03%) were *Cryptosporidium* spp. and 8 (13.80%) were *Cyclospora* spp. *Isospora* spp. was the most common enteric coccidian parasite to be detected followed by *Cryptosporidium* spp. and *Cyclospora* spp. [Table/Fig-1].

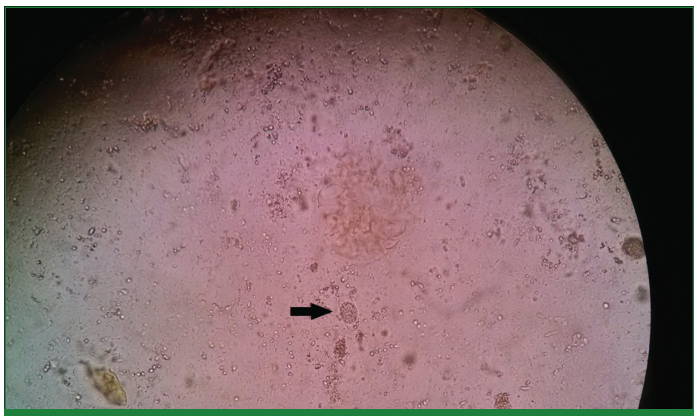
Enteric coccidian parasites	18-30 years		31-50 years		>50 years		Total
	Female	Male	Female	Male	Female	Male	
Cryptosporidium spp.	0	2	2	10	2	2	18 (31.03%)
Cyclospora spp.	0	4	0	4	0	0	8 (13.80%)
Isospora spp.	0	12	6	6	0	8	32 (55.17%)
Total	0	18	8	20	2	10	58

[Table/Fig-1]: Distribution of age in relation to gender for enteric coccidian parasite positive cases.

Cryptosporidium spp. was detected in 18 stool samples of HIV positive patients presenting with diarrhoea by *Cryptosporidium* antigen detection ELISA while only 10 were detected by both staining techniques [Table/Fig-2]. While in 26 patients the oocyst of *Isospora* sp. could be demonstrated in wet mount examination, other enteric coccidian parasites were not appreciated due to their small size [Table/Fig-3].

Enteric protozoal parasites	Wet mount examination only	Kinyoun staining technique	Hot safranin staining technique	ELISA for Ag detection in stool only
Isospora spp.	26	32	32	0
Cryptosporidium spp.	0	10	10	18
Cyclospora spp.	0	8	8	0

[Table/Fig-2]: Evaluation of staining techniques and ELISA.



[Table/Fig-3]: Wet mount showing oocyst of *Isospora* spp. (40X).

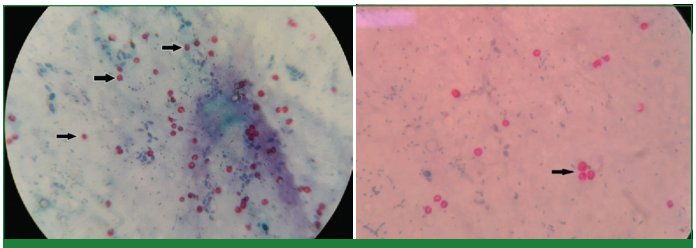
Out of 200 patients, 40 (20%) were treated for tuberculosis and 30 (15%) had oral ulcers while rest of the patients did not account for any opportunistic infections [Table/Fig-4]. Majority of patients were suffering from acute diarrhoea 98 (49%), while 24 (12%) patients were having chronic diarrhoea and the rest 78 (39%) were suffering from persistent diarrhoea [Table/Fig-4].

For accentuation and resolution of structural details of *Cyclospora* spp, *Cryptosporidium* spp. and *Isospora* spp, kinyoun staining technique was found to be better than hot safranin method [Table/Fig-5-10]. The rate of enteric infections was found to be higher in patients with CD4 cell counts below 250 cells/mm^3. These pathogens were found more in CD4<250 (44) as compared to CD4 250-500 (10) and CD4 >500 (4) respectively [Table/Fig-11].

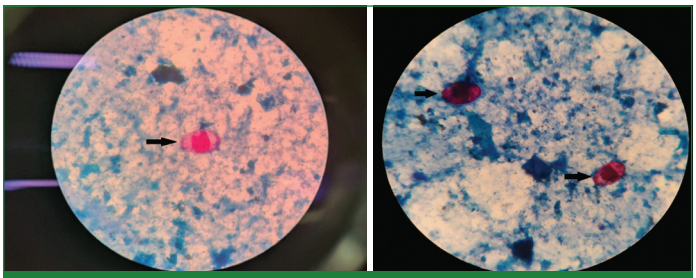
For detection of *Cryptosporidium* antigen, ELISA test had sensitivity of 100% (95% CI 69.2%-100%), specificity of 95.8% (95% CI 91.9%-98.2%), PPV of 55.6% (95% CI 30.8%-78.5%) and NPV of 100% (95% CI 98%-100%). This was in assumption with Kinyoun staining and hot Safranin staining as diagnostic test as they have sensitivity of 100% (95% CI 69.2%-100%), specificity of 100% (95% CI 98.1%-100%), PPV of 100% (95% CI 69.2%-100%), NPV of 100% (95% CI 98.1%-100%). With significance of p=0.008 both

Particulars		Sex		CD4 cell count (cells/mm³)						Opportunistic infections					
				Female			Male			Female			Male		
Age range (years)	Diarrhoea duration (days)	Female	Male	<250	250-500	>500	<250	250-500	>500	NA	Oral ulcer	TB	NA	Oral ulcer	TB
18-30	0-14	2	14	0	2	0	8	6	0	0	0	2	4	4	6
	15-28	0	4	0	0	0	2	2	0	0	0	0	4	0	0
	>28	6	16	2	2	2	6	8	2	4	0	2	14	0	2
31-50	0-14	12	56	6	0	6	18	18	20	6	2	4	40	10	6
	15-28	6	10	2	4	0	2	4	4	6	0	0	10	0	0
	>28	14	30	8	4	2	20	6	4	10	2	2	10	8	12
>50	0-14	4	10	0	4	0	2	6	2	4	0	0	6	0	4
	15-28	0	4	0	0	0	0	2	2	0	0	0	2	2	0
	>28	2	10	0	2	0	4	4	2	0	2	0	10	0	0
Grand total		46	154	18	18	10	62	56	36	30	6	10	100	24	30

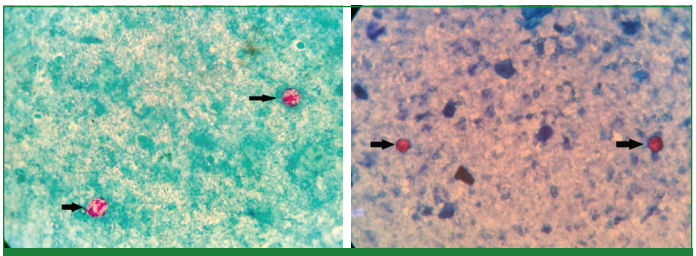
[Table/Fig-4]: Comparison of CD4 cell count, opportunistic infections and duration of diarrhoea in study population.
TB: Tuberculosis; NA: Not available



[Table/Fig-5]: Kinyoun staining showing oocysts of *Cryptosporidium* spp. (100X);
[Table/Fig-6]: Hot safranin staining showing oocysts of *Cryptosporidium* spp. (100X).
(Images from left to right)



[Table/Fig-7]: Kinyoun staining showing oocyst of *Isospora* spp. (100X);
[Table/Fig-8]: Hot safranin staining showing oocysts of *Isospora* spp. (100X).
(Images from left to right)



[Table/Fig-9]: Kinyoun staining showing oocysts of *Cyclospora* spp. (100X);
[Table/Fig-10]: Hot safranin staining showing oocysts of *Cyclospora* spp. (100X).
(Images from left to right)

Age group (years)	CD4 (cells/mm³)				
	Less than 250	250-500	More than 500	Total	*p-value
18-30	14	2	2	18	0.06
31-50	24	4	0	28	
>50	6	4	2	12	
Total	44	10	4	58	

[Table/Fig-11]: Distribution of CD4 counts.
*Fisher-exact test

Kinyon and hot safranin staining technique were correlated (100%, p<0.001). ELISA had 53% correlation with Kinyon staining and hot Safranin staining (p<0.001) [Table/Fig-12].

Variables	Wet mount	Kinyoun staining technique	Hot safranin staining technique	ELISA for Ag detection in stool
Wet mount	1.00			
Kinyoun staining technique	0.32* (p=-0.008)	1.00		
Hot safranin staining technique	0.32* (p=0.008)	1.00 (p<0.001)	1.00	
ELISA for Ag detection in stool only	-0.12 (p=1.0)	0.53* (p<0.001)	0.53* (p<0.001)	1.00

[Table/Fig-12]: Correlational matrix between various staining methods.
*: Bonferroni adjusted significance level

DISCUSSION

Enteric coccidian parasites are the most common cause of diarrhoea in HIV patients and have gained great significance as important causes of morbidity and mortality. Since, the line of treatment depends on the infecting parasite, it becomes absolutely essential that definite identity of the parasite causing diarrhoea is established to factor in the fatal outcomes in vulnerable groups.

In the present study, a total of 29% of enteric coccidian parasites were detected which is slightly lower when compared to other studies conducted in North India, which might be attributable to small sample size. Various studies from India and other countries have also reported a high prevalence of intestinal parasites ranging from 18.4-70% [5-7]. In the present study, the majority of the study population belonged to the age group of 18-50 years corresponding to the most active period of life and among all the age groups males predominated, reflecting a higher HIV prevalence in males in India as reported in other studies [10,11].

While earlier studies from North India had found *Cryptosporidium* to be the most common parasite and the prevalence of *Isospora belli* was found to be much lower but present study shows that *Isospora belli* was the most common parasite in HIV positive patients followed by *Cryptosporidium* spp [12-15]. In HIV-positive patients with diarrhoea, a study from south India found similar detection rates of *Isospora* spp., but it was significantly lower than present study research [16]. Similarly, another study has recorded a higher prevalence of isosporiasis, ranging from 18-26.1% [17,18]. However, studies in the northern part of India have shown a broad range of isosporiasis prevalence, ranging from 2.5-50% [1,5,10,13]. This difference may be attributed to the variation in geographical habitat of parasites, climate and detection methods. Because of asymptomatic oocyst shedding and treatment of other Opportunistic Infections (OI's) with trimethoprim sulfamethoxazole, which could confer some defense against this parasite, the level of identification of enteric coccidian parasites is sometimes underestimated.

The chance of detecting a pathogen in watery and semi-formed stools was found out to be 96.55% and 3.45%, respectively. Increased shedding, an increased inflammatory response, and increased pathogen virulence are all factors that contribute to watery diarrhoea. The current study could not be compared to other studies because most of them did not take stool characteristics into account. Cellular immunity is the major defence against intestinal parasitic infections and CD4 T cell count is a robust marker of status of Cell Mediated Immunity (CMI). CD4 cell counts below 500 cells/mm start predisposing the host to a variety of opportunistic infections. The normal CD4 count range is between 500 and 1400 cells/µL [19]. The maximum parasitic isolation was in the group of patients who had CD4 cell counts below 250 cells/µL and were most commonly associated with chronic diarrhoea. The detection rates decreased with the increase in the CD4 cell counts. This finding was in accordance with the study conducted by Basnet A et al., where 17 enteric coccidian parasites that were detected out of 22 isolates had CD4 count less than 200 [20].

In this study, the Kinyoun staining and hot safranin staining technique were compared with ELISA for *Cryptosporidium* spp. In present study, *Cryptosporidium* spp. was detected in 18 stool samples of HIV positive patients by *Cryptosporidium* antigen detection ELISA. Out of these 18, 10 specimens were positive with Kinyoun staining method and hot safranin technique. The reason for this could be non active excretion of oocyst at the time of specimen collection [21]. Jayalakshmi J et al., considered ELISA to be a quick, accurate, and less subjective test that could be very useful in routine diagnosis and screening a large number of specimens in a short period of time, especially in large scale epidemiological surveys, with a sensitivity and specificity of 90.9% and 98.7%, respectively [22]. The sensitivity of Barua P et al., assay, on the other hand, was just 36.4%, demonstrating that ELISA is not better than staining [23]. The assay may reduce the amount of ability required to conduct complex staining procedures and recognise the morphology of small *Cryptosporidium* oocysts. However, staining is important because it is inexpensive and has a similar efficacy to the assay.

The *Cryptosporidium* oocysts (4-6 µm) took up the Safranin stain and appeared bright pink against a green background. On Kinyoun staining the *Cryptosporidium* oocysts stained as discernable light pink to bright pink structures against a green background. In this study, Kinyoun staining technique gave better visualisation of *Cryptosporidium* oocysts as Safranin technique required heating and structural details of *Cryptosporidium* oocysts were poorly defined. This finding was in accordance with the study conducted by Kehl KS et al., [24].

For the diagnosis of other enteric coccidian parasites Kinyoun method and Hot Safranin method were comparable. Previous data have found Safranin staining superior for identification of *Cyclospora* spp as the *Cyclospora* oocysts appeared as uniformly stained bright pink structures and it was found to be 89.13% sensitive and 99.16% specific for *Cyclospora* spp. identification [12], while in this study, for identification and clear structural details of *Cyclospora* spp. and *Isospora* spp, Kinyoun staining technique was found to be better than hot Safranin method. Apart from an advantage of getting better structural details the techniques did not show any significant difference between the detection rates, hence any of these staining methods can be taken as reference method.

Limitation(s)

The present study did not find any *Microsporidium* spp. in the stool sample. This could have possibly happened due to incompetence of the staining techniques used in this study and because *Microsporidium* spp. are best picked up in techniques like hot chromotrope staining method.

CONCLUSION(S)

This study showed that Kinyoun staining performed better than hot Safranin method in bringing out morphology and structural details of the cyst of enteric coccidian parasites whereas ELISA performed better in detecting *Cryptosporidium* spp. than staining techniques. Hence, HIV positive cases presenting with diarrhoea and reported negative in staining methods should be investigated by ELISA to increase the likelihood of detecting enteric cryptosporidial parasite in stool samples. Therefore, routine screening of stool samples of HIV positive patients with diarrhoea should be undertaken to prevent delay in diagnosis and diarrhoea associated morbidity and mortality due to these parasites.

REFERENCES

[1] Prasad KN, Nag VL, Dhole TN, Ayyagari A. Identification of enteric pathogens in HIV positive patients with diarrhoea in Northern India. J Health Popul Nutr. 2000;18(1):23-26.

[2] Mitra AK, Hernandez CD, Hernandez CA, Siddiq Z. Management of diarrhoea in HIV-infected patients. Int J STD AIDS. 2001;12:630-39.

[3] Marcos LA, Gotuzzo E. Intestinal protozoan infections in the immunocompromised host. Current Opinion in Infectious Diseases. 2013;26(4):295-301.

[4] De A. Current laboratory diagnosis of opportunistic enteric parasites in human immunodeficiency virus-infected patients. Tropical Parasitology. 2013;3(1):7.

[5] Gupta S, Narang S, Nunavath V, Singh S. Chronic diarrhoea in HIV patients: Prevalence of coccidian parasites. Indian J Med Microbiol. 2008;26:172-75.

[6] Zali MR, Mehr AJ, Rezaian M, Meamar AR, Vaziri S, Mohraz M. Prevalence of intestinal parasitic pathogens among HIV-positive individuals in Iran. Jpn J Infect Dis. 2004;57:268-70.

[7] Inabo HI, Aminu M, Muktar H, Adeniran S. Profile of intestinal parasitic infections associated with diarrhoea in HIV/AIDS patients in a tertiary care hospital in Zaria, Nigeria. World J Lifr Sci Med Res. 2012;2:43-47.

[8] https://www.cdc.gov/dpdx/diagnosticprocedures/stool/specimenproc.html [Assessed on Mar 16, 2021].

[9] https://www.cdc.gov/dpdx/diagnosticprocedures/stool/staining.html [Assessed on Mar 16, 2021].

[10] Uppal B, Kashyap B, Bhalla P. Enteric pathogens in HIV/AIDS from a tertiary care hospital. Indian J Community Med. 2009;34(3):237-42.

[11] 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.

[12] Tuli L, Singh DK, Gulati AK, Sundar S, Mohapatra TM. A multiattribute utility evaluation of different methods for the detection of enteric protozoa causing diarrhea in AIDS patients. BMC Microbiology. 2010;10:11.

[13] Ballal M. Opportunistic intestinal protozoal infections in HIV infected patients in a rural cohort population in Manipal, Karnataka Southern India. Manuals on IndoUS Workshop on diarrhoea and enteric protozoan parasites. New Delhi: Indian Council of Medical Research; 2005.

[14] Mohandas K, Sehgal R, Sud A, Malla N. Prevalence of intestinal parasitic pathogens in HIV seropositive individuals in Northern India. Jpn J Infect Dis. 2002;55:834.

[15] Sadraei J, Rizvi MA, Baveja UK. Diarrhea, CD4+ cell counts and opportunistic protozoa in Indian HIV infected patients. Parasitol Res. 2005;97:2703.

[16] 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. The American Journal of Tropical Medicine and Hygiene. 2007;77(5):823-24.

[17] Mukhopadhya A, Ramakrishna BS, Kang G, Pulimood AB. Enteric pathogens in southern Indian HIV-infected patients with & without diarrhoea. Indian Journal of Medical Research. 1999;109:85.

[18] Ramakrishnan K, Shenbagarathai R, Uma A, Kavitha K, Rajendran R, Thirumalaikolundu subramanian 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.

[19] Li R, Duffee D, Gbadamosi-Akindele MF. CD4 Count. In: StatPearls [Internet]. Treasure Island (FL): Stat Pearls Publishing; 2021.

[20] Basnet A, Sherchan BB, Rijal B, Sharma S, Khadga P. Detection of coccidian parasites and their clinical manifestation, treatment and prophylaxis in HIV infected patients in Tribhuvan University teaching hospital. Scientific World. 2010;8(8):51-55.

[21] Ajjampur SS, Sankaran P, Kang G. *Cryptosporidium* species in HIV-infected individuals in India: An overview. Natl Med J India. 2008;21(4):178-84.

[22] Jayalakshmi J, Appalaraju B, Mahadevan K. Evaluation of an enzyme-linked immunoassay for the detection of *Cryptosporidium* antigen in fecal specimens of HIV/AIDS patients. Indian J Pathol Microbiol. 2008;51:137-38.

[23] Barua P, Hazarika N, Barua N, Rasul E, Laskar N. Microscopy for cryptosporidiosis screening in remote areas. Indian Journal of Medical Microbiology. 2008;26(2):203.

[24] Kehl KS, Cicirello H, Havens PL. Comparison of four different methods for detection of *Cryptosporidium* species. Journal of Clinical Microbiology. 1995;33(2):416-18.

PARTICULARS OF CONTRIBUTORS:
1. Senior Resident, Department of Microbiology, ABVIMS and Dr. RML Hospital, New Delhi, India.
2. Professor and Consultant, Department of Microbiology, ABVIMS and Dr. RML Hospital, New Delhi, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Rakesh Kumar Mahajan,
301, 3rd Floor, OPD Building, Dr. RML Hospital, New Delhi-110001, India.
E-mail: rks.mahajan@gmail.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Mar 19, 2021
- Manual Googling: Jul 26, 2021
- iThenticate Software: Aug 20, 2021 (14%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Mar 16, 2021**
Date of Peer Review: **May 03, 2021**
Date of Acceptance: **Jul 31, 2021**
Date of Publishing: **Jan 01, 2022**