Original Article

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Microbiology Section

Intestinal Cryptosporidiosis and the Profile of the CD_4 Counts in a Cohort of HIV Infected Patients

RASHMI K.S., RAVI KUMAR K.L.

ABSTRACT

Background: Cryptosporidium is an infectious enteric pathogen which is capable of causing life-threatening illnesses in immunocompromised patients.

Aims: This prospective study was planned to know the frequency of intestinal cryptosporidiosis in HIV infected patients and its correlation with their immune status. Also, the conventional diagnostic methods were compared with the copro-antigen detection test by using Enzyme Linked Immuno Sorbent Assay (ELISA).

Settings and Design: This was a prospective cohort study.

Methods and Material: Three consecutive stool samples which were collected from 90 HIV seropositive patients and 50 seronegative controls were screened for cryptosporidiosis by wet mount, direct modified ZN (Ziehl Neelsen) staining, modified ZN staining with formol ether concentration and copro-antigen detection by ELISA. Their immune statuses were measured by CD_4 + cell counting.

Statistical Analytical Tests which were Used: Odds ratio, Chi square test, Fisher extract test.

Results: Cryptosporidiosis was detected in 15 HIV seropositive cases. 13 cases had CD_4 cell counts of < 100 cells/ µL. The formol ether concentration technique resulted in an increased number of oocysts/oil immersion field in 8 cases. ELISA was positive in 2 cases which were shown to be negative by modified ZN staining. All the controls were negative for cryptosporidium.

Conclusions: Cryptosporidiosis is an opportunistic infection in HIV infected people who present with diarrhoea. The wet mount technique, though it is simple and inexpensive, is insensitive for the detection of cryptosporidium. The conventional modified ZN staining and the modified ZN staining with concentration have a sensitivity and a specificity of 85.71% and 98.84% respectively. The copro antigen detection by ELISA which has a greater sensitivity and specificity, is a useful tool in epidemiological studies.

Key Words: Cryptosporidiosis, HIV/AIDS, CD_4 counts, Modified ZN staining, ELISA

INTRODUCTION

Cryptosporidium is a coccidial protozoan parasite that infects humans and animals. *Cryptosporidium* was first recognized in the gastric glands of laboratory mice by EE Tyzzer in 1907. The infection occurs through the ingestion of environmentally resistant oocysts. It causes short term gastrointestinal illnesses in immunocompetent patients with debilitating diarrhoea, which are often accompanied by severe abdominal cramps, weight loss, anorexia, malaise and low grade fever which lasts for a longer duration in immune deficient patients with no curative protocols [1- 3]. The diagnosis of cryptosporidiosis is established by the visualization of oocysts by using concentration and staining procedures in stool, duodenal aspirates and in tissue samples [3]. These methods are of low sensitivity and arduous serological methods like *Cryptosporidium* Specific Antigen (CSA) detection by ELISA [4-6]. An early rapid diagnosis of cryptosporidiosis benefits the patients and the physicians as it limits further evaluation and decreases the use of an empirical therapy [7].

MATERIALS AND METHODS

This study was conducted over a period of 18 months at Kempegowda Institute of Medical Sciences Hospital and Research Centre, a tertiary care hospital which caters to the people in and around Bangalore, India. The criteria for inclusion in the study was HIV seropositive patients, irrespective of their ages and CD_4 cell counts. Fifty age and sex matched healthy individuals were included as the controls. HIV seronegative individuals with other illnesses were excluded from the study. The present study reported the commonness of cryptosporidiosis in the HIV seropositive patients who sought care at our institution. It has also correlated the infection with the patients' CD_4 cell counts. The diagnostic techniques have also been evaluated for their efficacy and epidemiological usefulness.

The institutional ethics committee approved the study. A total of 140 subjects were incorporated into this study, who consisted of 90 HIV seropositive patients and 50 seronegative controls. The HIV infection in these patients was evaluated according to the NACO guidelines [8]. The CD_4 +cell counts of the seropositive patients were measured with a flow cytometer (BD FACS Count).

Stool examination: Stool samples were collected from each subject, on three consecutive days, according to the NCCLS guidelines [9]. All the stool samples were fixed in 10 percent formol saline. The smears of direct and concentrated (formol ether concentration) specimens were subjected to wet mount (0.85% NaCl solution) and iodine mount (Lugol's iodine) preparations for the detection of ova, larvae, trophozoites and cysts of intestinal parasites.

The specimens were screened for *cryptosporidium* by the modified acid-fast staining (1% HCL in alcohol) before and after the concentration technique was done [4,10].

The *Cryptosporidium* copro-antigen was detected by using an FDA approved, commercially available ELISA kit (Ridascreen R *cryptosporidium* kit - BioPharm AG, Germany). This ELISA kit contains micro titre wells which are coated with *Cryptosporidium* specific antibodies to detect the *cryptosporidium* antigen which is present in the stool samples. The protocol which was recommended by the manufacturers, was followed [11].

A biostatistical analysis was done by applying the Odds ratio, the Chi square test, the Fisher extract test.

RESULTS

15 out of the 90 HIV patients were positive for *cryptosporidium*. Out of the 15 positives, 6 (40%) had acute diarrhoea and 7 (46.7%) had chronic diarrhoea, with 2 (13.33%) cases being asymptomatic. The associated symptoms were generalized weakness 12/15 (80%), weight loss 9/15 (60%), abdominal cramps 5/15 (33.33%), vomiting 4/15 (26.7%), nausea 3/15 (20%) and fever 2/15 (13.33%).

The *Cryptosporidium* oocysts were more frequently associated with mucus mixed stool samples eight out of 15 cases, with a p value of < 0.001 and an Odds ratio of 23.14, in comparison to watery (5/15) , semisolid (2/15) and formed stool samples.

The detection of the *cryptosporidium* oocysts by wet mount, modified ZN staining (with / without concentration) and ELISA has been analyzed in [Table/Fig-1].

In the modified ZN staining, the oocysts were characteristically found to be round or slightly ovoid, with a size of about 4.5-5 um (range-4-6um). The acid fastness of the oocysts within a smear and between the specimens was variable. The oocysts showed variation from an unstained to a partial red staining to a complete staining. Fully sporulated forms could be found in which, red staining crescentric bodies, the sporozoites, could be seen inside an unstained oocyst wall (Note: The *Cryptosporidium* in histological sections and in empty excysted oocysts are not acid fast.) Care needs to be used in the interpretations, as a variety of structures can be confused with oocysts (the so called *cryptosporidium* like bodies). These include fungal spores (6-10 um) that are slightly larger than the cryptosporium oocysts (4-6um), mould spores, fat globules and bacterial spores. But these can be distinguished on the basis of their sizes. Excessive ZN staining can result in false positive reactions in yeast cells [12].

	Wet mount		Modified ZN staining		ELISA
	Before Concentration	Before Concentration	Before Concentration	Before Concentration	
Positive	1	1	13	13	14
Negative	89	89	77	77	76

[Table/Fig-1]: Comparison of Wet mount, Modified ZN Staining, Formol ether concentration and ELISA in detection of Cryptosporidium oocysts

CD4 counts/ul	HIV (n=90)	Number of Cryptosporidium infection (n=15)
>100	28 (31.08%)	12 (80%)
101-250	48(53.28%)	3(20%)
>250	14(15.54%)	-

[Table/Fig-2]: Association of CD_4 counts with Cryptosporidium infection

Parasites detected	HIV (n=100)	Non-HIV (n=50)
1. *Ascarislumbricoides.*	4 (4.0%)	4 (8.0%)
2. *Ankylostomaduodenale.*	2 (2.0%)	3 (6.0%)
3. *Trichuristrichuiria.*	1 (1.0%)	1 (2.0%)
4. *Enterobiasvermicularis*	1 (1.0%)	1(2.0%)
5. *Entamoebahistolytica*	3 (3.0%)	3 (6.0%)
6. *Cryptosporidium*	15 (15.0%)	-
7. *Isospora belli*	4 (4.0%)	-
8. *Cyclosporacyatenensis*	1 (1.0%)	-

[Table/Fig-3]: Non opportunistic Parasites detected in stool samples of HIV seropositive &Seronegative individuals

Study done by	Region	Number of cases included in the study	Methodology	Percentage of *cryptosporidium* prevalence
Aruna Agarwal [15]	Northern India	100 HIV seropositives	Modified ZN Staining	13.63%
Kava Mohandas [16]	Northern India	120 HIV seropositives	Modified ZN Staining	10.8%
S.V. Kulkarni [17]	Western India	137 HIV seropositives with diarrhoea	Modified ZN Staining	12%
LekhaTuli [18]	Eastern India	366 HIV seropositives with diarrhoea	Modified ZN Staining & Modified Safranin staining	21%
Satheesh S.Kumar [19]	Southern India	150 HIV seropositives	Modified ZN Staining	14%
Singh A.Bairy [20]	Southern India	100 HIV seropositives	Modified ZN Staining	47%

[Table/Fig-4]: Prevalence of *cryptosporidium*

ELISA was positive in one sample, which was found to be negative by the modified ZN staining. The sensitivity and the specificity of ELISA, as compared to those of the modified ZN staining, were found to be 92.30 and 97.40 respectively, with a positive predictive value of 85.71 and a negative predictive value of 98.68.

Correlation of the *cryptosporidium* infection in HIV patients with their CD_4 cell counts, proved that the patients with CD_4 counts of <100/ul were 6.09 times more likely to have the *Cryptosporidium* infection, with a p value of 0.002. None of the patients with CD_4 counts of >250/ul had the infection. All the seronegative controls were also negative for *Cryptosporidium* [Table/Fig-2]. The other parasites detected in the study is shown in [Table/Fig-3].

DISCUSSION

Intestinal parasitic infestations in HIV infected patients with an impaired immunity will result in severe diarrhoeal symptoms. Among these, cryptosporidiosis is one of the major causes of opportunistic intestinal coccidiosis [2, 3].

The diagnosis of Cryptosporidiosis is made by the identification of the oocysts in stool, duodenal aspirates or in tissue samples. The fresh or preserved stool specimens can be subjected to concentration and staining procedures for the visualization of the *cryptosporidium* oocysts. The Formol ether concentration, the modified cold Kinyoun acid fast staining and immunofluorescent techniques are the popular methods which are used in diagnostic laboratories [3,4,13]. However these methods are laborious

and time consuming requiring skill and experience with low sensitivities as compared to specific antigen detection by ELISA [5-7]. The serological assays which measure the IgM and the IgG responses to the 17 kDA and the 27 kDA *cryptosporidium* antigens, provide an alternative to the parasitologic methods for monitoring the *cryptosporidium* infection [3,14]. Several real time PCR procedures are being evaluated for the genotyping and speciation of *cryptosporidium*. Cell culture and animal models are being used for evaluating chemotherapeutic and immunotherapeutic agents, [3,13].

An established curative therapy is not available for this parasitosis as yet[2]. The presently available agents include parmomycin, spiramycin, clarithromycin and nitroxanide. Ionophores such as Lasalocid and maduramycin are moderately effective [3,13].

A failure in diagnosing cryptosporidiosis in immunocompetent patients with diarrhoea, will rarely be of significance, as the disease is self-limiting. In contrast, the diagnosis of cryptosporidiosis is essential in immunocompromised patients, because of its severity and its interference with therapeutical procedures [5, 7].

There are few studies that have tried to define the prevalence of *cryptosporidium* in HIV-positive individuals in India. Studies which were done across the country have reported a prevalence of 10.8- 47% in this group [Table/Fig 4], [15-20].

In the present study, Cryptosporidiosis was present in 15% HIV seropositive patients. It was found to be 16.9 times more common in HIV positive patients as compared to the HIV negative group ($p<0.003$).

Among those who were positive for *cryptosporidium*, a significant number had diarrhoea (13/15-6 acute and 7 chronic diarrhoea), generalized weakness (13/15) and weight loss (12/15). This clinical profile parallels with the findings which have been reported by Kumar S S et al., Kava Mohandas et al., and Claudio Viera Silva et al., [19,16,5]. The *Cryptosporidium* oocysts were commonly associated with stool samples which were mixed with mucus (53.3%) than with watery (33.3%) and semisolid stools (13.3%) [21].

The detection of *Cryptosporidium* and other protozoal parasites is a challenge which involves the examination of small or large bowel biopsy materials with different staining techniques and their modifications. These include methods like the modified ZN staining, Safranin staining Dimethyl sulfoxide staining, Giemsa staining, Auramine-rhodamine staining, immunofluorescence staining and so on. Many of these techniques are cumbersome and time consuming, with variable sensitivities and specificities, which result in missing of the parasites if just one method is used. If the yield, cost, ease of handling and the ability to process large numbers of specimens are given equal weightage, the Ziehl Neelsen staining would rank first, followed by the Auramine-rhodamine staining and the Giemsa staining [22].

The wet mount examination (with iodine) is used mainly for screening the stool samples which are suspected to have intestinal parasites. In the present study, it could detect only one case. The poor detection of the *cryptosporidium* oocysts by wet mount may be because of the lesser number of oocysts in the stool samples of varied consistencies [18].

The Modified ZN staining is regularly used as it is moderately inexpensive, specimens can be stained in batches and the slides can be stored as a permanent record. This staining can also be used to show oocysts in the sputum, in bronchial washings and in duodenal and jejunal aspirations. The detection limit of this staining technique is $1x10^6$ oocysts /ml of faeces [22, 23]. The recovery of the *cryptosporidium* oocysts in the present study by the modified ZN staining (13/90) was comparable with the findings of Aruna Agarwal (18/100) and Kava Mohandas (13/120) [15,16].

Concentration of the stool samples by Sheather's sugar floatation (SSF) or Formol ether concentration is a prerequisite for getting a better yield of the cryptosporium oocyts for microscopy. Despite the disadvantage of the irritation which is caused by formalin and ether, the carcinogenic potential of formalin and the inflammability of ether, the Formol ether concentration technique is preferred to SSF because it has several advantages like -formalin inactivates the oocyst and the ether or ethyl acetate helps in extracting fats from the faecal sample, which sequentially help in dispersion of the oocysts into the aqueous phase [7,24]. With the use of the formol ether concentration technique, in our study, there was an increase in the number of *cryptosporidium* oocysts/oil immersion field, though there was no increase in the number of positives. These findings were similar to the observations of Akujobi from Nigeria [25].

Serological methods like ELISA are rapid and sensitive techniques which can provide an early diagnosis of these *cryptosporidium* infections and the results can influence the therapeutic interventions [18]. The *Cryptosporidium* specific antigens (40, 41, 47 kDA antigens) are detected by qualitative immunoenzymatic microplate assay/ELISA and Rapid immunochromatographic tests. Fresh/ formalin/sodium acetate- acetic acid-formalin preserved stool samples can be used. The samples which are preserved in polyvinyl alcohol are not suitable for ELISA. ELISA does not require concentration of the stool samples as a prerequisite. This test can detect 10^3-10^4 oocysts / ml. ELISA may be an alternative method for detecting the *cryptosporidium*-specific copro antigen in HIV/AIDS patients [23]. Since many false positives and negatives are being reported, it is not recommended as a sole screening test. The other disadvantage of ELISA is its failure in detecting very low number of ooysts in asymptomatic individuals [6]. A factor which complicates the comparison of the copro-antigen detection is the absence of a true reference standard. In general, the reference standard is based on the microscopical pathogen detection, a method that is impossible to standardize, as it is influenced significantly by the individual skills of the microscopists who are involved. Additionally, an uneven distribution of parasites and a difficulty in homogenizing the solid specimen contribute to the false negative results [26]. To overcome these limitations, the reference standard in the present study was not based entirely on the microscopical methods, but we also took into account, the results of the copro-antigen tests.

The sensitivity and the specificity of the modified ZN staining were 85.71% and 98.8% and that of ELISA were 92.31% and 97.70% respectively. These findings correlated with those of other studies,with the sensitivity and specificity of ELISA in detecting cryptosporidiosis as 87.9 % & 100% by Robert d. Newman et al ,Virginia[6] , 93 % & 99% by Mary T. Parisi , New York [7] , 93.25% and 97% as were reported by Lekha Tuli et al., Varanasi, India [18],93 % & 99 % by Jone E . Rosenblatt et al ., Minnesota [23] and 94 % & 99 % by Karen Sue C. Kehl, Atlanta , Georgia [27].

The main advantages of ELISA are, that it can be easily interpreted and that it requires lesser time in comparison with the modified ZN staining, when large numbers of samples have to be screened. Stool ELISA offers a simple and a cost effective alternative to the conventional microscopy for the routine diagnosis of the *cryptosporidium* infection in diarrhoeal stool samples. The patients who are negative by ELISA, but who still tend to pose a high clinical suspicion of the *cryptosporidium* infection, should undergo several different methods of staining procedures for the microscopic examination, which remains the standard for the diagnosis of this infection [2,7].

Experimental models have shown that the CD_4+ T cells and interferon-γ, together are required to prevent the *cryptosporidium* infection. The CD_4 cells limit the duration of the infection, whereas IFN-γ limits its intensity [13]. There is good evidence that the risk of the faecal carriage, the severity of the illness, and the occurrence of unusual complications of cryptosporidiosis are directly proportional to the CD_4 counts. The high prevalence of *Cryptosporidium* spp. in AIDS patients probably relates to the augmented risk of acquiring the infection from infected contacts and a prolonged excretion, which consecutively enhance the risk of subsequent transmissions. One therapeutic intervention that has a remarkable effect on the cryptosporidiosis in AIDS patients is antiretroviral therapy, which leads to recovery of the CD_4 counts [28].

Correlation of the *cryptosporidium* infection with the CD_4 counts of the patients in the present study, showed that the HIV patients with CD_4 counts of <100 cells/μL were 6.09 times more susceptible for the *cryptosporidium* infection, with a p value of 0.002. The results were consistent with those of the studies of Javid Sudrei and Viroj Wiwanitkit, which reported *cryptosporidium* as an opportunistic infection in HIV seropositive patients with CD_4 cell counts of < 200/ μL [29,30].

CONCLUSIONS

The *Cryptosporidium* infection was significantly found in HIV patients with CD_4 counts of <100 cells/ μL and in those who suffered from diarrhoea. Cryptosporidial Coproantigen ELISA offers a diagnostic alternative to the conventional direct microscopy, as it is simple, cost effective and do not require skill and proficiency for the morphological detection and recognition of the oocyst. It has been recommended that HIV patients with CD_4 counts of < 200 cells/ μL should be screened for intestinal coccidial parasites, as prophylactic measures and early interventions will significantly alter the course and the outcome of these infections.

REFERENCES

[1] Chen XM, LaRusso NF. Human intestinal and biliary Cryptosporidiosis. *World Journal of Gastroenterology*. 1999;5(5):424-9.

[2] D Dioniso. Cryptosporidiosis in HIV–infected patients. *J Postgrad Med*. 2002;48(3):215-6.

[3] Current WL. Cryptosporidiosis. In Topley and Wilson's Microbiology and Microbial Infections. Volume 5 .9th Edition. Edited by Collier Leslie, Balows Albert, Max Sussman .Volume editors: Cox EG Francis, Kreier J P, Wakelin Derek. *Arnold publishers.*1998; 329-47.

[4] Weber R, Bryan RT, Juranek DD. Improved Stool Concentration Procedure for Detection of *Cryptosporidium* Oocysts in Fecal Specimens. *J Clin Microbiol*. 1992; 30(11):2869-7.

[5] Silva CV, Ferreira MS, Goncalves-Pires MR, Costa-Cruz JM. Detection of *Cryptosporidium* – Specific Coproantigen in Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome Patients by Using a Commercially Available Immunoenzymatic Assay. *Mem Inst Oswalo Cruz, Rio de Janerio*. 2003; 98(8):1097-99.

[6] Newman RD, Jaeger KL, Wuhib T, Lima AA, Guerrant RL, Sears CL. Evaluation of an antigen capture Enzyme Linked Immuno Sorbent Assay. *J Clin Microbiol*. 1993; 31(8):2080-4.

[7] Parisi MT, Tierno PM Jr. Evaluation of New Rapid Commercial Enzyme Immunoassay for Detection of *Cryptosporidium* Oocysts in Untreated Stool Specimens. *J Clin Microbiol*. 1995;33(7):1963-5.

[8] Guidelines for HIV testing National AIDS Control Organisation, Ministry of health and family welfare. March 2007.

[9] Shimizu RY. Procedures for the recovery and identification of parasites from the intestinal tract, Section 9, Approved Guideline, M28-A, National Committee for Clinical Laboratory Standards, Villanova, PA NCCLS. 1997.

[10] WHO Regional office for South East Asia. Standard operating procedures for the laboratory diagnosis of common parasitic opportunistic infections in HIV/AIDS patients. Guidelines on standard operating procedures for the laboratory diagnosis of common parasitic opportunistic infections in HIV-Opportunistic infections. *Blood safety and Clinical technology*.

[11] Ridascreen ® *Cryptosporidium* enzyme immunoassay for the detection of *cryptosporidium* 03-04-01,R-Biopharm A G ,Landwehrstr.54,64293 Darmstadt, Germany C12020116.Available from: URL: http://www.r_biopharm.com.

[12] Casemore DP, Sands RL, Curry A. Laboratory diagnosis of Cryptosporidiosis; *J Clin Pathol*. 1985 :38;1321-36.

[13] White JR AC. *Cryptosporidium* species. In: Mandell, Douglas and Bennet's Principles and Practice of Infectious Diseases. Seventh Edition. Edited by Mandell GL, Bennett JE, Dolin R. Elsevier, Philadelphia PA: *New York Churchill Livingstone*. 2010; 3547-60.

[14] Eisenberg JN, Priest JW, Lammie PJ, Colford JM Jr. The Serologic Response to *Cryptosporidium* in HIV–Infected Persons: Implications for Epidemiologic Research. Centres for Disease Control and Prevention, *Atlanta Georgia.* 2001 Nov- Dec; 7(6).

[15] Aggarwal A, Arora U, Bajaj R, Kumari K. Clinico- microbiological study in HIV Seropositive patients. JIACM .2005; 6(2):142-5.

[16] 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; 55:83-4.

[17] 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 immune suppression. *Indian J Med Res*. 2009;130: 63-6.

[18] Tuli L, Singh DK, Gulati AK, Sundar S, Mohapatra TM. A multiattribute utility evaluation of different methods for the detection of enteric protozoa causing diarrhoea in AIDS patients. BMC Microbiology. 2010;10:11 http://www.biomedcentral.com/1471-2180/10/11).

[19] Kumar SS , Anandan S and Lakshmi P. Intestinal Parasitic Infection in HIV Infected Patients with Diarrhoea in Chennai. *Indian J Med Microbiol*. 2002; 20(2):88-91.

[20] Singh A, Bairy I, Shivananda PG. Spectrum of opportunistic infections in AIDS cases. *Indian J Med Sci*. 2003; 57(1):16-21.

[21] Y G Chen, F B Yao, H S Li, W S Shi, M X Dai and M Lu . *Cryptosporidium* infection and diarrhoea in rural and urban areas of Jiangsu, People's Republic of China. *J Clin Microbiol*. 1992 February;30(2):492-94.

[22] MacPherson DW, McQueen R. Cryptosporidiosis: Multiattribute Evaluation of six diagnostic methods. *J Clin Microbiol*. 1993 February; 31(2):198-202.

[23] J E Rosenblatt, L M Sloan. Evaluation of an Enzyme–Linked Immuno Sorbent Assay for detection of *Cryptosporidium* spp. in stool specimens. *J Clin Microbiol*. 1993 June; 31(6):1468-71.

[24] Bukhari Z, Smith HV. Effect of three concentration techniques on viability of *Cryptosporidium* parvum oocysts recovered from Bovine faeces. *J Clin Microbiol*. 1995 October; 33(10):2592-95.

[25] Akujobi CN, Ogunsola FT, Iregbu KC, Odugbemi TO. Comparative evaluation of direct stool smear and Formol-ether Concentration Methods in the identification of *Cryptosporidium* species. Abstract. *Nigerian Journal of Health and Biomedical Sciences.* 2005; 4(1): 5-7.

[26] Weitzel T, Dittrich S, Möhl I, Adusu E, Jelinek T. Evaluation of seven commercial antigen detection tests for Giardia and *Cryptosporidium* in stool samples. *Clin Microbiol Infect*. 2006 Jul; 12(7):656-9.

[27] K S Kehl, H Cicirello, and P L Havens. Comparison of four different methods for detection of *Cryptosporidium* species. *J Clin Microbiol*.1995 February; 33(2):416-18.

[28] Hunter PR, Nichols G. Epidemiology and Clinical Features of *Cryptosporidium* Infection in Immunocompromised Patients. *Clinical Microbiology Reviews*. 2002 January; 15 (1): 145-54.

[29] Sadraei Javid, Rizvi Moshahid A, Baveja U. K. Diarrhoea, CD_4+ cell counts and opportunistic protozoa in Indian HIV–infected patients. *Parasitology Research* 2005; 97 (4): 270-73.

[30] Wiwanitkit Viroj. Intestinal parasitic infections in Thai HIV – Infected patients with different immunity status. *BMC Gastroenterology.* 2002; 1(3).

AUTHOR(S):

1. Dr. Rashmi K.S.
2. Dr. Ravi kumar K.L.

PARTICULARS OF CONTRIBUTORS:

1. Assistant Professor, Department of Microbiology, KIMSH & RC, Bangalore, India.
2. Professor & HOD, Department of Microbiology, KIMSH & RC, Bangalore, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Dr. Rashmi K.S.,
Assistant Professor, Department of Microbiology,
No 53, Sree Nilayam, I Cross, D block ,
Krishna Garden, R V College Post,
Bangalore-59, India.
Phone: 9845269790
E-mail: rashminaga05@gmail.com

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