

# DEMOGRAPHIC STUDY OF ENTERIC PARASITIC INFESTATION IN CHILDREN ATTENDING TERTIARY LEVEL HOSPITAL AND PLHA CHILDREN RESIDING IN A COMMUNITY CARE CENTER OF WESTERN RAJASTHAN

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## ABSTRACT:

**Background:** Parasitic infestation is a major health problem in tropics, like in India leading to diarrhea and malnutrition. This study was conducted to determine the etiology of diarrhea in Western part of Rajasthan. This is a hospital based prospective study carried out in Umaid Hospital Dr. S. N. Medical College Jodhpur for a period of 17 months.

**Material and Methods :-** In study time 776 stool samples were received, after taking patient consent stool examination was done by Wet microscopy, modified ZN and Trichrome stain. Presence of ova and cysts were examined along with CD-4 count of all PLHA children.

**Results:** A total of 776 stool samples were examined for parasites out of them 718 samples from diarrhoea patients and 58 samples from PLHA patients. A total of 139 parasites were detected in diarrhoea patients. Cryptosporidium was the most common parasite identified with a prevalence of 5.29% followed by Giardia with prevalence of 5.01% in diarrhoea patients. In 58 sample 58 parasite are identified, out of them Cryptosporidium (27.59%) was the most common followed by E. histolytica (22.41%) and Microsporidium (15.52%).

**Conclusions:** Finding of our study demonstrated that Cryptosporidium was most frequent pathogen isolated from both immunocompetent and immunosuppressed patients.

**Key Words :-** Enteric parasite, PLHA, ART, CD-4 COUNT, Immunocompromised

## INTRODUCTION:

Diarrhoea remains the second leading cause of death among children under five globally. Infective diarrhea is one of the leading cause of morbidity and mortality among children under five years in the developing world<sup>1</sup> and can be caused by a wide range of viruses, bacteria, or parasites. The prevalence of the different enteric pathogens varies with the geographical area<sup>2</sup>.

Parasitic diarrhoea is also a major health problem in tropics, like in India. Cryptosporidium a protozoan, is now recognized

Parasitic disease<sup>3</sup>. India have reported the prevalence rates to be 4.3 to 13% (Nath et al, 1999)<sup>4</sup>.

Identifying the enteric pathogens that contribute significantly to the causes of diarrhoea in children will help to provide an accurate and region-specific estimate of disease burden. Traditional methods to detect the etiological agents of diarrhoea have ranged from microscopy and bacterial culture to immunoassays, and vary for each enteric pathogen. In a multicenter European study<sup>5</sup>, pathogens were identified in 65% of stool

samples from children with acute diarrhoea, a rate similar to that reported in developing countries<sup>6,7</sup>.

Diarrhoea is a common manifestation of HIV infection in both adults and children. In adults, it is usually the consequence of a deteriorating immune system late in the HIV disease cycle. In children with HIV, it is often the result of frequently aggressive common childhood infections caused by pathogens such as *Campylobacter*, *E. coli*, *Salmonella*, *Shigella* or *Rotavirus*<sup>8</sup>. When diarrhoea persists for more than two weeks in children, it may be an indicator of stage 3 HIV infection, according to WHO, and requires further evaluation and antiretroviral treatment<sup>9</sup>. Persistent diarrhoea occurs with increased frequency in HIV-infected children, and is associated with an 11-fold increase in mortality compared to uninfected children<sup>10</sup>. Causes of persistent diarrhoea in HIV-positive children include HIV-related malabsorption, gut manifestation of tuberculosis, gut infections and infestations of pathogens such as *Cryptosporidium parvum*, *Cyclospora cayentanensis*, *Isospora belli*, *Microsporidia* and *Cytomegalovirus*<sup>8</sup>.

Survey on the prevalence of various intestinal parasitic infestations in different geographic regions is a prerequisite to obtain an accurate understanding of the burden and cause of intestinal parasitic infestations in a particular area. The aim of the present study was to determine the intestinal parasitic infestation

among children in a tertiary level hospital and in PLHA children residing in CCC

**Material and Method:** This was a hospital based prospective study carried out for a period of 17 month from May 2016 to September 2017 on 718 children (< 18 years) attending tertiary level hospital with complains of diarrhoea and 58 HIV positive children living in a community care centre ( CCC) . Children having metabolic disorders and other non-infective causes of diarrhoea were not included in this study. Informed consent was taken from all patients and their attendants before enrolling the patients in study. Detailed demographic details (about age, sex, residence) were inquired along with clinical complaints of all patients .

**Ethical Committee :-** The study protocol was approved by the Ethical committee of Dr. S. N. Medical College Jodhpur, Rajasthan.

**Laboratory procedures:** All patients who gave consent were instructed to collect freshly passed stool sample in a clean sterile container. After proper labeling (e.g. Name, age, sex, reg. no., Time of collection) sample was transported to microbiology laboratory and processed immediately for Macroscopic and Microscopic examination (Wet Microscopy, Gram's staining, Modified Z.N. staining and Trichrome staining etc.) and Culture. Concentration method (Saturated salt floatation technique and Formalin ether sedimentation technique) was performed to concentrate parasitic agent.

## Statistical Analysis

Data was managed by Microsoft Excel spreadsheet and analysis was performed using Microsoft Excel and SPSS 22 using chi square test and strength association by P value .

**Results:** The present study is a prospective study of 776 patients whose stool samples were submitted for examination from May 2016 to September 2017. In present study a total of 718 patients had diarrhoea and were HIV negative out of which -417(58.08%) patients were male and 301(41.92%) patients were females . In 58 PLHA children who were HIV positive and were residing in Community Care Centre who complaint of diarrhoea , pain abdomen etc were included in study .

Maximum number of patients were in age group of up to 0-5years of age 602 ( 84.00%) , in age group 6-10 year 78(10.86%) patients and age group 11 & more 38(5.29%) with parasitic positivity 95,29 and 14 respectively in all age groups .In this study 387(53.90%) patients were from rural background and 331(46.10%) were from urban back ground. During study period a total of 718 stool sample were examined. Out of them 610(84.96%) stool samples were negative for parasite. In 108(15.04%) stool samples parasites were identified, out of them 80(74.07%) samples had one parasite, 26(24.07%) samples had two parasites and 2(1.85%) samples had three parasites.

Most common isolated parasite was cryptosporidium 38(27.54%) followed by Giardia 36(26.09%), Microsporidium 29(21.01%), E. histolytica 15(10.87%), E. coli

10(7.25%), Ascaris 5(3.62%) and others 4(2.90%).

Most common parasite in both male 21 (30%) and female 17(25%) was cryptosporidium followed by Giardia in male 20 (28.57%) and female 16 (23.53%). Parasitic prevalence in male was 16.78% (70/417) and in female was 22.59% (68/301). There was no significant difference between male and female (P value 0.077).

Over all 75(54.35%) parasites were identified from rural patient and 63(45.65%) parasites were identified from urban patient. Giardia 19(30.16%) was most common in urban patient and Cryptosporidium 20(26.67%) was most common in rural patient. During study period the prevalence of parasite in rural patients was 19.38% and in urban patients was 19.03%. There was no significant difference between these two groups (P value 0.86).

In this study most parasite were isolated in month of August 2017 18(13.04%) followed by in October 2016 16(11.59%). Most parasites were isolated post rainy season.

A total of 58 parasites were identified in 42 stool samples from PLHA patients with positivity rate 72.41%. No parasite was identified in 16(27.59%) stool sample. Single parasite was identified in 28(66.67%) stool sample, two parasites were identified in 12(28.57%) and three parasites were identified in 2(4.76%) of stool sample. Out of 58 identified parasite most common parasite in this group was Cryptosporidium 16(27.59%) followed by E. histolytica 13(22.41%), Microsporidium 9(15.52%) and Ascaris 6(10.34%).

Highest number of parasites were identified in CD4 count group 501-1000 followed by CD4 count group  $\geq 1001$  and 201-500 were

26(44.83%), 21(36.21%) and 11(18.97%) respectively.

In comparative evaluation of immunocompetent and PLHA patients opportunistic parasite Cryptosporidium (P value <0.001), Microsporidium (P value 0.0002) and E. histolytica (P value <0.001) were significantly associated with PLHA stool but Giardia was not show significant difference in prevalence (P value 0.95). In non-opportunistic pathogenic parasites Ascaris had significant difference of prevalence (P value 0.002).

**TABLE NO. 1 Demographic Distribution of samples :-**

	Diarrhoea cases	Parasite positive cases	Percentage(%)
male	417(58.08%)	70	16.79
female	301(41.92%)	68	22.59
Rural	387(53.90%)	75	19.38
Urban	331(46.10%)	63	19.03
AGE 0_5	602(83.84%)	95	(15.78%)
6_10	78 (10.86%)	29	(37.17%)
11 -18	38 (5.29%)	14	(36.84%)
Total	718	138	15.04

**Table No. 2 Age and Sex wise Distribution of parasite in diarrhoea patients :-**

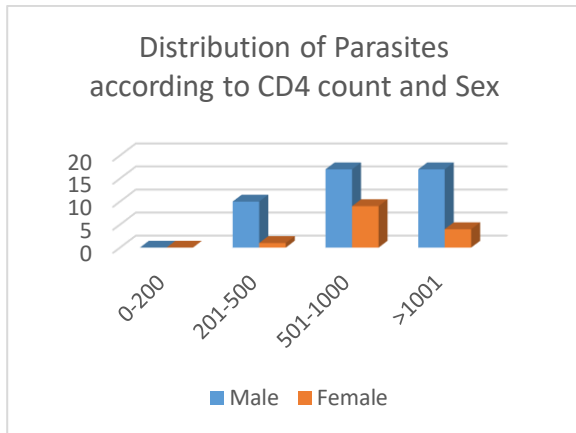
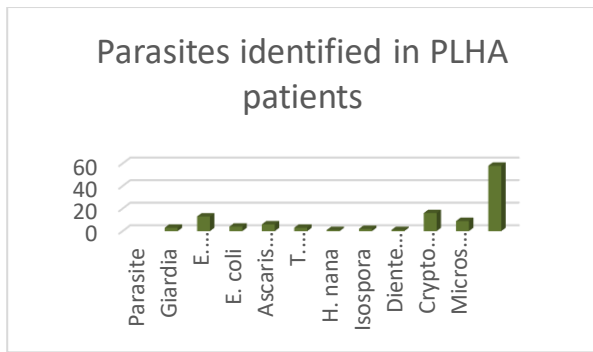
Age range	0_5 (602)		6_10 (78)		10_18 (38)		Total (718)	%
	M (103)	F (92)	M (40)	F (38)	M (22)	F (16)		
Parasite								
Giardia	15	12	3	3	2	1	36	26.09%
E. histolytica	4	7	2	1	0	1	15	10.87%
E. coli	3	5	0	1	0	1	10	7.25%
Ascaris Egg	4	0	1	0	0	0	5	3.62%
T. trichura	0	1	0	0	0	0	1	0.72%
H. nana	0	0	1	1	0	0	2	1.45%
Isospora	0	1	0	0	0	0	1	0.72%
Chilomastix	0	1	0	0	0	0	1	0.72%
Cryptosporidium	12	10	5	5	4	2	38	27.54%
Microsporidium	9	11	1	5	3	0	29	21.01%
Total	47	48	13	16	9	5	138	100%

**Table No. 3 Distribution of PLHA patient according to CD4 count and Sex**

	Male	parasite positive	Female	parasite positive	Total	parasite positive	Percentage
0-200	0	0	0	0	0	0	-
201-500	9	10	2	1	11	11	18.97%
501-1000	15	17	9	09	24	26	41.38%
≥ 1001	15	17	8	04	23	21	39.66%
Total	39	44	19	14	58	58	100%
Percent age	67.24%		32.76%		100%		

**Table No. 4 Distribution of Parasite in PLHA patient according to Sex and CD4 count**

Parasite	0-200		201-500		501-1000		≥ 1001		
	M (0)	F (0)	M (9)	F (2)	M (15)	F (9)	M (15)	F (8)	
Giardia	0	0	0	0	2	0	1	0	3
E. histolytica	0	0	4	1	4	2	2	0	13
E. coli	0	0	1	0	0	1	1	1	4
Ascaris Egg	0	0	1	0	0	0	2	3	6
T. trichura	0	0	0	0	3	0	0	0	3
H. nana	0	0	0	0	0	0	1	0	1
Isospora	0	0	0	0	2	0	0	0	2
Dientamoeba	0	0	0	0	0	0	1	0	1
Cryptosporidium	0	0	1	0	4	5	6	0	16
Microsporidium	0	0	3	0	2	1	3	0	9
	0	0	10	1	17	9	17	4	58



**Table No. 5:-**Comparison of parasite in Diarrhoea patient and PLHA

Parasites isolated	Patient with Diarrhoea (718 total Isolates 138)	Percentage	PLHA (58 Total Isolates 58)	Percentage	P value
Cryptosporidium	38	27.53%	16	27.59%	<0.001
Microsporidium	29	21.01%	9	15.52%	0.002
Isospora	1	0.72%	2	3.45%	-
Giardia	36	26.08%	3	5.16%	0.95
E. histolytica	15	10.86%	13	22.14%	<0.001
Ascaris	5	3.62%	6	10.34%	<0.001
T. trichiura	1	0.72%	3	5.17%	-
H. nana	2	1.45%	1	1.72%	-
E. coli	10	7.24%	4	6.90%	0.002
Chilomastix	1	0.72%	0	-	-
Dientamoeba	0	-	1	1.72%	-

## Discussion:

This was a hospital based prospective study. During the study period a total of 776 stool samples were examined for parasites. Out of them 718 samples were from seronegative

diarrhoea patients and 58 samples from PLHA patients. Out of 718 diarrhoea patient's stool samples 610 (84.96%) were negative for parasites and 108 (15.04%) samples showed presence of parasite. In 108 samples total of 138 parasites were identified by various method. Cryptosporidium was the most common parasite identified with a prevalence of 5.29% followed by Giardia with prevalence of 5.01%.

In various other study done in various part of world parasitic prevalence in diarrhoeal stool were 14.4% to 23.3%, which is higher than this study. According to study done by Ogunlesi Tinuade et. al.<sup>11</sup> in 2006 in Ilesa, Nigeria, parasitic agent was identified in 70 (23.3%) out of 300 samples. Most common parasite identified in this study was E. histolytica (65.7%) followed by Ascaris (18.6%) and Giardia (7.01%). Prevalence of Giardia is match with this study but prevalence of E. histolytica is much higher than our study.

Another study done by Moyo et. al.<sup>12</sup> in 2011, conducted at Dar es Salaam, Tanzania, according to this study parasitic agent were identified in 53 (19.2%) sample out of 270 diarrhoeal patient. In this study Cryptosporidium was identified in 18.9% sample and Giardia in 1.09% of sample. Cryptosporidium is most common identified parasite in present study, but prevalence is lower than this study. This may be due different geography and environmental condition.

Study done by Mandomando M. et. al.<sup>13</sup> (2007) in rural southern Mozambique, in this study 529 stool samples were examined for various pathogen. Out of them 76 (14.4%) were positive for parasites, Ascaris (9.4%) was most commonly identified parasite in this study followed by Giardia (2.5%). Parasitic prevalence of this study coincides with present study but prevalence of Ascaris (0.7%) in present study was lower than this study and prevalence of

Cryptosporidium is higher in present study than this study 5.29% versus 0.6% respectively.

A study done by Nair et. al.<sup>14</sup> (2010) in Kolkata India, out of 2519 stool samples collected, parasitic etiological agent was found in 532(21.12%) samples. Most common prevalent parasite in this study was Giardia (11.2%) followed by Cryptosporidium (6.3%) and E. histolytica (3.3%). The present study showed overall lower prevalence than this study but prevalence of Cryptosporidium and E. histolytica are nearly similar in both study.

In present study parasitic was least prevalent in age group <1 year. Parasitic prevalence in age group above 3 years nearly same, age group 3-5-year, 5-10 year and >10-year prevalence was 37.5%, 37.18% and 36.84% respectively. In present study parasitic prevalence is significantly lower in age group less than 3 year (P value <0.001).

According to study done by Mandomando M. et. al.<sup>13</sup> (2007) in rural southern Mozambique, distribution of parasitic was significantly higher in age group more than 2 year (P value for Ascaris <0.001 and for S. stercoralis 0.008). These findings are similar to present study.

Another study done by Nair et. al.<sup>14</sup> (2010) in Kolkata India, according to this study Cryptosporidium was more prevalent in lower age group than the higher age group. But in present study Cryptosporidium is more prevalent in higher age group (P value <0.001).

In present study parasitic prevalence in male and female are 16.78% and 22.59% respectively. There is no significant difference in prevalence. A community based study done by Ajjampur et. al. (2007)<sup>15</sup> also did not found any gender difference but study of O’Ryan et. al. (2005)<sup>16</sup> had male preponderance.

In present study there is no significant difference found according to demographic background of patients as rural and urban patient have prevalence 19.38% and 19.03% respectively.

Incidence of diarrhoea increases in rainy season, but in present study most of parasites were identified post rainy season (August to October). A study from Kolkata reported that the highest incidence of cryptosporidiosis occurred in the rainy season from June to October. According to study done by Nair et. al. (2010)<sup>12</sup> may be due to delayed Monsoon in western Rajasthan Giardia detected throughout the year but cryptosporidium and E. histolytica showed minor seasonal variability.

Prevalence of parasite in PLHA patient is higher in present study as compared to various other studies, prevalence as 32.5% by Khalil et. al. (2015)<sup>18</sup>, 38.66% by Nitya vyas et. al. (2013)<sup>19</sup>, 48.6% by Lehman et al. (2013)<sup>20</sup> and 55.8% Gupta et. al. (2008)<sup>18</sup>. This difference is may be due to maximum of sample in present study are from same community care center of orphan children and difference in age structure of sample population. Cryptosporidium is the most isolated parasite in HIV infected diarrhoea patient as in present study<sup>18</sup>.

In present study opportunistic parasites were significantly associated with PLHA patients (Cryptosporidium, Microsporidium and E. histolytica)<sup>17</sup>. Non-opportunistic parasites which were significantly associated with PLHA patient were Ascaris and E. coli. According to various studies opportunistic parasites associated with HIV patients are Cryptosporidium, Microsporidium and Isospora indicates same results<sup>18</sup>

## Conclusion:

Finding of our study demonstrated that *Cryptosporidium* was most frequent pathogen isolated from both immunocompetent and immunosuppressed patients. Routine screening for parasite in stool are strongly recommended in pediatric diarrhoea patients to avoid overuse of drugs in management in diarrhoea. Children are susceptible to parasitic infestation hence regular antihelminthic and antiprotozoal drugs should be given as treatment or prophylaxis. In children with HIV irrespective of their CD4 count regular stool examination is advocated for parasite screening . Prophylactic antihelminthic and antiprotozoal drugs should be given.

## References:

1. Bern C, Martines J, de Zoysa I, Glass RI: The magnitude of the global problem of diarrhoeal disease: a ten-year update. *Bull World Health Organ* 1992, 70:705-714.
2. Guerrant RL, Hughes JM, Lima NL, Crane J: Diarrhoea in developed and developing countries: magnitude, special settings, and etiologies. *Rev infect Dis* 1990, 12:S41-50.
3. Guerrant R L. Cryptosporidiosis: an emerging highly infectious threat. *Emerg infect Dis* 1997; 31: 51- 7.
4. Nath G, Singh TB, Singh SB. Prevalence of *Cryptosporidium* associated diarrhea in a community. *Indian Pediatr* 1999; 36: 180-3.
5. Guandalini S, Pensabene L, Zikri MA, et al. Lactobacillus GG administered in oral rehydration solution to children with acute diarrhoea: a multicenter European trial. *J Pediatr Gastroenterol Nutr* 2000; 30: 54–60.
6. Youssef M, Shurman A, Bougnoux M, Rawashdeh M, Bretagne S, Strockbine N. Bacterial, viral and parasitic enteric pathogens associated with acute diarrhea in hospitalized children from northern Jordan. *FEMS Immunol Med Microbiol* 2000; 28: 257–63.
7. Kang G, Ramakrishna BS, Daniel J, Mathan M, Mathan VI. Epidemiological and laboratory investigations of outbreaks of diarrhoea in rural South India: implications for control of disease. *Epidemiol Infect* 2001; 127: 107–12.
8. Tindyebwa, D., et al., ‘Common Clinical Conditions Associated with HIV’, in: *Handbook on Paediatric AIDS in Africa*, 2004.
9. Partners in Health, *The PIH Guide to the Community- Based Treatment of HIV in Resource-Poor Settings (Revised Second Edition)*, Partners in Health, Boston, 2008.
10. World Health Organization, *Antiretroviral Therapy of HIV Infection in Infants and Children: Towards universal access. Recommendations for a public health approach*, WHO, Geneva, 2006.
11. OgunlesiTinuade, Okeniyi John, OseniSaheed, OyelamiOyeku, Njokanna Fidelis I and DedেকেOlabisi. Parasitic Etiology of Childhood Diarrhea. *Indian Journal of Pediatrics*, 2006, 73:1081-1084.
12. Sabrina J Moyo, NjolstadGro, Mecky I Matee, Jesse Kitundu, Helge Myrmel, HaimaMylvaganam, Samuel Y Maselle, Nina Langeland: Age specific aetiological agents of diarrhoea in hospitalized children aged less than five years in Dar es Salaam, Tanzania. *BMC Pediatrics* 2011, 11:19.
13. Ina´ Cio M. Mandomando, Euse´ Bio V. Macete, Joaquim Ruiz, Sergi Sanz, Fatima Abacassamo, Xavier Vallès, Jahit Sacarlal, Margarita M. Navia, Jordi Vila, Pedro L. Alonso, And Joaquim Gascon: Etiology Of Diarrhea In Children Younger Than 5 Years Of Age Admitted In A Rural Hospital Of Southern Mozambique. *Am. J. Trop. Med. Hyg.* 2007, 76(3), , 522–527.

14. Gopinath Balakrish Nair, Thandavarayan Ramamurthy, Mihir Kumar Bhattacharya, Triveni Krishnan, SandipanGanguly, Dhira Rani Saha, Krishnan Rajendran, Byomkesh Manna, Mrinmoy Ghosh, Keinosuke Okamoto and Yoshifumi Takeda:Emerging trends in the etiology of enteric pathogens as evidenced from an active surveillance of hospitalized diarrhoeal patients in Kolkata, India.*Gut Pathogens* 2010, 2:4.
15. Ajjampur, S. S., B. P. Gladstone, D. Selvapandian, J. P. Mulyil, H. Ward, and G. Kang. Molecular and spatial epidemiology of cryptosporidiosis in children in a semiurban community in South India. *J. Clin. Microbiol.* 2007 45:915–920.
16. O’Ryan, M., Prado, V. & Pickering, L. K. (2005). A millennium update on pediatric diarrheal illness in the developing world. *Semin Pediatr Infect Dis* 16, 125–136.
17. Ganguly, S. K. Bhattacharya, A. A. Lal, and L. Xiao. Molecular characterization of *Cryptosporidium* spp. in children in Kolkata, India. *J. Clin. Microbiol.* 2006 .44:4246–4249.
18. Shehla Khalil, Bijay Ranjan Mirdha, Sanjeev Sinha, Ashutosh Panda, Yogita Singh, Anju Joseph, Manorama Deb;Intestinal Parasitosis in Relation to Anti-Retroviral Therapy, CD4+ T-cell Count and Diarrhea in HIV Patients.*Korean J Parasitol*; 2015; 53, 6,705-712.
19. Nitya Vyas, SmitaSood, Babita Sharma, Muneshkumar; Prevalence of Intestinal Parasitic Infestation and the Related Profile of the CD4<sup>+</sup> Counts in HIV/AIDS People with Diarrhoea in Jaipur City. *Journal of Clinical and Diagnostic Research.* 2013 March, Vol-7(3): 454-456.
20. Leopold G. Lehman, LafortuneKangam, Marthe-LilianneMbenoun, Eveline ZemoNguepi, Noël Essomba, Calvin Tonga, Charles F. Bilong;Intestinal parasitic and candida infection associated with HIV