Section: Microbiology

Original Research Article

COMPARATIVE STUDY OF PREVALENCE OF ENTERIC PARASITIC INFECTIONS AMONG HIV-POSITIVE SUBJECTS ATTENDING A TERTIARY CARE HOSPITAL, KOLKATA WITH CD4 ≥200 CELLS/µL AND <200 CELLS/µL

Sangeeta Pal[1], Maitreyi Bandyopadhyay[2], Bipasa Chakraborty[3], Meghna Majumder[4]

[1]Assistant Professor, Department of Microbiology, R.G. Kar Medical College & Hospital, Kolkata, West Bengal, India.
[2]Professor and Head, Department of Microbiology, R.G. Kar Medical College & Hospital, Kolkata, West Bengal, India.
[3]Associate Professor, Department of Microbiology, R.G. Kar Medical College & Hospital, Kolkata, West Bengal, India.
[4]Molecular Biologist, Department of Microbiology, R.G. Kar Medical College & Hospital, Kolkata, West Bengal, India.

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Corresponding Author:
Ms. Meghna Majumder,
Email: meghnamajumder430@gmail.com

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ABSTRACT

Background: Parasitic infections remain an important cause of morbidity and mortality in developing countries among HIV-infected persons. Our objective was to estimate their prevalence and compare between HIV infected subjects with CD4 >=200cells/µL and CD4 <200cells/µL. **Materials and Methods:**Freshly passed stool samples were collected into universal containers from HIV infected patients attending ART centre of our hospital for 9 months (01.11.24-30.07.25). 50 stool samples were collected (35 males and 15 females) with CD4 >=200cells/µL and 50 (39 males and 11 females) with CD4 <200 cells/µL from all age groups. Microscopic examination of sediment was performed by saline and iodine wet mounts after performing Formol-ether concentration technique to detect presence of ova, cysts or larva. Smears prepared from these samples were stained by modified acid-fast stain to detect oocysts of *Cryptosporidium, Cyclospora* and *Cystoisospora* species. **Results:** The prevalence of intestinal parasitic infections in HIV infected patients with CD4 count >=200cells/µL and <200cells/µL were 36% and 42% respectively. This study showed 37.14% parasitic prevalence in male and 33.33% in female, maximum in 21-40 years age group. Among 50 patients with CD4 count >=200cells/µL,18 were having 7 types of parasitic infections with *Cryptosporidium* spp having highest prevalence(24%), followed by Hookworm(6%), *Strongyloides stercoralis*(4%), *Cystoisospora belli*(4%), *Cyclospora cayetanensis*(2%), *Ascaris lumbricoides*(2%) and mixed parasitic infections(6%). Whereas in 50 patients with CD4 count <200cells/µL, 21 were having parasitic infections, *Cryptosporidium* spp showing highest prevalence(30%) followed by *Cystoisospora belli*(8%), *Cyclospora cayetanensis*(4%), Hookworm(2%), *Strongyloides stercoralis*(2%), and mixed infections(4%). **Conclusion:** Overall prevalence of parasitic infection was 39% among HIV infected patients. Though prevalence of parasitic infection was greater among CD4 count <200cells/µL, it was not statistically significant (p=0.538). *Cryptosporidium spp* had the highest prevalence, more among patients with CD4 count <200cells/µL. Hence routine microscopic screening of stool samples is recommended to estimate their burden and to guide clinicians for better management.

INTRODUCTION

Intestinal parasites are endemic in many regions of the world as well as in India where Human Immunodeficiency Virus/Acquired Immunodeficiency syndrome (HIV/AIDS) is also prevalent.[1] An estimated 33.0 million adults and children are living with HIV worldwide.[2]Opportunistic intestinal parasites, causing acute and chronic diarrhea are common

cause of morbidity and mortality.[1] Reports indicate that diarrhea occurs in 30%–60% of AIDS patients in developed countries and 90% of AIDS patients in developing countries.[1]The clinical spectrum caused by these parasitic intestinal protozoa among HIV positive patients ranges from asymptomatic infection to acute and chronic diarrhea, dehydration and mal-absorption.[3]Helminths and HIV co-infection have major effects on the host immune response.[4] Parasitic infections with helminths cause chronic immune activation, more toward T helper-2 immune responses.[5,6] According to research works, such immune modulation was shown to increase host susceptibility, promoting HIV infection and disease progression.[7] In India, parasitic diarrheal diseases in HIV/AIDS patients have been commonly reported in numerous studies but more evidences are required to prove this hypothesis. Although eastern part of India has many HIV/AIDS cases, but not many studies are done in West Bengal to study the prevalence of opportunistic intestinal parasitic infections in HIV infected subjects.[1]

Parasites frequently associated with acute and chronic diarrhea in HIV disease include various species of *Cryptosporidium* spp, *Cystoisospora belli, Microsporidia spp, Giardia intestinalis, Entamoeba spp, Cyclospora cayetanensis, Strongyloides,* Hookworm and *Ascaris lumbricoides.*[1]

As parasitic infection is under diagnosed and the data in eastern India is insufficient, routine examinations of stool samples for parasites would be significantly helpful for the HIV patients to reduce morbidity due to chronic diarrhea. Also there is progressive decline in immune system functions and decrease in CD4 cell counts in AIDS patients, and there is increase in opportunistic infections resulting in morbidity and mortality. So studies on correlation with CD4 cell count and parasitic intestinal infections are also essential.

So the objective of this study was to estimate the prevalence of intestinal parasites among HIV-positive/AIDS patients attending ART Centre at R.G. Kar Medical College and Hospital, to study their demographic profile and to do comparative study of occurrence of enteric parasitic infections among HIV-infected individuals with CD4+ T-cell count ≥200 cells/µL and <200 cells/µL.

MATERIALS AND METHODS

This study is a prospective and observational study, carried out in the department of Microbiology, R.G. Kar Medical College and Hospital, Kolkata after obtaining essential institutional ethical clearance and approval from NACO. All HIV/AIDS patients attending ART centre of our hospital with or without symptoms during 01.11.2024 to 30.07.2025 were randomly selected and included in this study. First to detect HIV 1 and 2 infections among the subjects, NACO testing strategy was followed using the following test kits. Three kits with different principle were used for detecting HIV1 and HIV2 infection. The different kits provided and used in our study period for HIV detection were as follows-

1.11.24 –11.02.25 Kit I CombAids.lot no 4000030332, expiry 4.5.26,, Kit II Maxline, R172417, 3.26, Kit III Aidscan , 37240204, 1.26

12.02.2025 – 28.02.25 Kit I Medsource , HIVc – 261124, 10.26, Kit II Comb Aids, 4000030332,, 4.5.2026, Kit III Aidscan , 37240204, 1.26

1.3.2025 – 18.03.25Kit I StandQ (Standard Q), C033151, 10.26, Kit II Comb Aids, 4000030332, 4.5.2026, Kit III Aidscan, 37240204, 1.26

19.03.25 – 23.04.25 Kit I Angcard, B HIV 2405, 1.27, Kit II Comb Aids .lot no 4000030332 ,expiry 4.5.26,Kit III Aidscan , 37240204, 1.26

24.04.25 – 07.05.25Kit I Med source, HIVc 040225, 1.27, Kit II Comb Aids 4000029677, 4.12.25, Kit III Aidscan, 37240204, 1.26

8.5.25 –09.05.25 Kit IMaxlineR172417, 3.26, Kit II Comb Aids 4000029677, 4.12.25, Kit III Aidscan , 37240204, 1.26

10.5.25 – 15.06.25 Kit I ANGSTROM, BHIV2405, 1.27, Kit II Comb Aids, 4000029677, 4.12.25, Kit III TREDRO, MI0224042, 15.2.26

16.06.25 – 30.07.25 Kit I Med source, HIVc 100525, 4.27, Kit II Comb Aids, 4000029677, 4.12.25, Kit III TREDRO, MI0224042, 15.2.26

After confirming HIV infections, informed consent was taken from the study subjects who participated in this study for providing their stool samples. Before sample collection, patient's sociodemographic data (name, age, sex) and CD4+ T-cell count were obtained. Treatment history was taken. A total of 100 stool samples from 100 HIV positive patients were tested for intestinal parasites.

Sample size in our study was calculated by the following formula, $n = z^2pq/d^2$[where n = Total number of sample, z = 1.96, p = Prevalence (38.7% from previous study[8]) and q= (100-p)= (100-38.7)= 61.3, d = 10 % (% of error)]. Here, n = (1.96 x 1.96 x 38.7 x 61.3)/100= 91.13.

50 stool samples was collected from the 50 HIV/AIDS patients with CD4 count ≥200 cells/µL and 50 stool samples from patients with CD4 count <200 cells/µL.

Freshly voided stool samples from these patients were collected into clean wide mouthed, screw capped, leak proof, sterile plastic containers (universal containers) and immediately transported to the parasitology laboratory for further proceeding. Fresh stool samples of these patients were examined both macroscopically and microscopically in the laboratory. Macroscopically we have observed for consistency, presence of mucus, blood, adult worms or their segments, and helminthic larvae if any. Microscopy was done on both direct and concentrated stool samples and examined under 10X and 40X magnification.[1] Microscopic examination of stool was performed by saline and iodine wet mounts before concentration technique to detect presence of any trophozoites, ova, cysts, larva and

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presence of pus cells. RBC, crystals and fat globules were also looked for. Formol-ether concentration technique of stool was performed. 7ml of 10% solution of formalin (Formol saline, i.e., 10 ml Formalin and 90 ml NS) was taken in a fresh sterile universal container and emulsified with 1 gm of stool sample taken out by spatula from patient's sample and left for 10 minutes for fixation. After straining this mixture through double layered woven gauge into a conical centrifuge tube, 3 ml of ether was added to the filtrate and screw capped. The filtrate was vigorously shaken for 1 minute and centrifuged at 2000 rpm for 2 minutes. The mixture was separated into 4 layers: the top layer is the ether containing the dissolved fat, the second layer is the fatty plug containing the faecal debris, the third layer is the formalin and the bottom layer is the sediment containing the parasite if any. With an applicator stick, after loosening the fatty plug, the tube was inverted and the fluid contents were poured carefully, leaving the sediment. Thus the supernatant was decanted and the sediment was used for detection of parasites.[9] Microscopic examination of the sediment was performed again by saline and iodine wet mounts to detect presence of any ova, cysts or larva. Smears prepared from these samples were stained by modified acid-fast stain method to detect oocysts of *Crytosporidium* spp, *Cyclospora cayetanensis* and *Cystoisospora belli*. Faecal smear was prepared from the deposits and heat fixed. The slide was flooded with cold carbol fuchsin (4%) for 5 min, then rinsed in tap water, further flooded with 1% H_2SO_4 as decolourizer about 30 seconds following Kinyoun's method. Counter staining was done using methylene blue (0.1%) for 3 minutes, rinsed in tap water and blotted dry with filter paper and smear examination was done under microscope using 100X oil emersion lens for detection of oocysts.[10]

In wet mount *Cryptosporidium* spp., oocyst appeared oval and colourless measuring 1.5-5 µm in diameter and highly refractile containing four elongated sporozoites. It does not stain with iodine but in modified acid-fast stain, it appears as bright red-stained acid-fast oocysts against blue background.[11]*Cystoisospora* oocysts were elliptical in shape, measuring 20-33 µm by 10-19 µm in direct wet mount. Each oocysts contained two sporoblasts, each of which contained four sporozoites. The sporozoites were long, slender and crescent shaped. The oocyst was surrounded by a two-layered cyst wall. Red coloured acid fast large oocysts were demonstrated in modified Z-N stain.[11] *Cyclospora* oocysts were round to ovoid, measuring 8-10 µm in diameter and acid fast. Each sporulating oocyst contained two sporocysts, each measuring 4 µm in diameter and containing two sporozoites.[11] The rhabditiform larvae of *Stongyloides stercoralis* found in faeces were actively motile, unsheathed, measuring 200-300 mm in length and 16 mm in breadth in wet mounts of stool by direct microscopy. The larvae was identified by short mouth, double bulb oesophagus and inconspicuous genital premordium.[12] Hookworm eggs were oval around 60 µm in length and 40 µm in breadth, thin shelled and non-bile stained, containing 4-8 blastomeres.[13] Fertilised egg of *Ascaris lumbricoides* were identified by their oval to subspherical shape, 45-70 µm in length and 35-50 µm in breadth, bile stained and golden brown in colour in saline mount. The egg was surrounded by thick shell with a light brown mammaliated albuminous outer coat containing a large conspicuous unsegmented ovum with a clear crescentic area at each pole.[13]

Each stool sample was tested in triplicate to increase the field in our study and findings were properly noted. After performing the laboratory procedures, the samples and the testing materials were discarded all following Biomedical Waste Management Rule, India, 2016 (including the amendment added in 2018 and 2019). Universal containers containing stool samples and plastic centrifuge tubes were discarded/disposed in red colored non chlorinated plastic bag or bin. Microbiological slides and cover slips were disposed in puncture, leak proof blue container. Gauge pieces were disposed in yellow colored non chlorinated plastic bag or bin according to Biomedical Waste Management Rule, India, 2016 (including the amendment added in 2018 and 2019).

Results were tabulated in Excel sheet and analyzed using Microsoft excel software and using simple biomedical statistical methods. Statistical significance was calculated using GraphPad Prism software version 5.0, and of p value ≤ 0.05 were considered as statistically significant.

RESULTS

Among the 100 study participants, 35 were male and 15 were female with CD4+ T-cell count ≥200 cells/µL, and 39 were male and 11 were female with CD4+ T-cell count<200 cells/µL.

Sociodemographic factor like gender and age of the study subjects were studied and prevalence of enteric parasite infections were analysed between the two groups CD4+ T-cell count ≥200 cells/µL and CD4+ T-cell count <200 cells/µL. It was found that overall prevalence was greater in CD4 <200 cells/µL group but was not statistically significant in terms of age group and gender. It was found that maximum parasitic infections were prevalent in the middle age group (21-40 years). Age and gender wise distribution of these parasitic infections is shown in [Table 1].

In our study, out of 50 HIV positive patients with CD4+ T-cell count ≥200 cells/µL, parasites were present in stool samples of 18 patients(36%) and out of 50 HIV positive patients with CD4+ T-cell count <200 cells/µL, parasites were present in stool samples of 21 patients (42%). So, overall prevalence of intestinal parasitic infections among HIV cases was 39%. Distribution of different parasitic infections in our study with CD4+ T-cell count ≥200

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cells/µL and <200 cells/µL are shown in Figure-1(a) and 1(b) respectively and few parasites detected from stool examinations from our study are as shown in [Figure-2(a) to 2(f)].Comparative analysis of prevalence of different parasitic infections in our study with CD4+ T-cell count ≥200 cells/µL and <200 cells/µL respectively are depicted in [Table 2].The findings of [Table 2] indicate that the prevalence of coccidian parasitic infection was more among the HIV subjects with CD4+ T-cell count <200 cells/µL but not statistically significant. However on the contrary, the prevalence of helminthic infection was more among CD4+ T-cell count ≥200 cells/µL but statistically not significant.

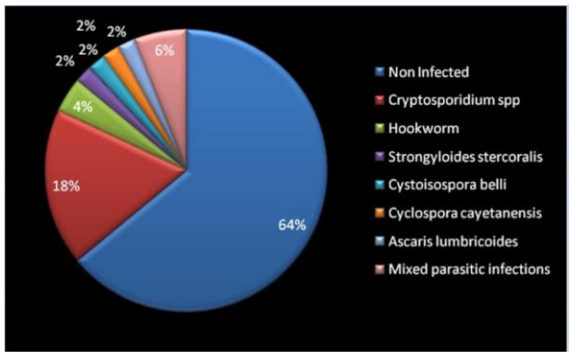

Figure 1(a): Distribution of different parasitic infections with CD4 T Cell count ≥200 cells/µL

Note: Mixed infections comprised co-infections of *Cryptosporidium* spp. with Hookworm, *Cryptosporidium* spp. with *Strongyloides stercoralis* and *Cryptosporidium* spp. with *Cystoisospora belli*.

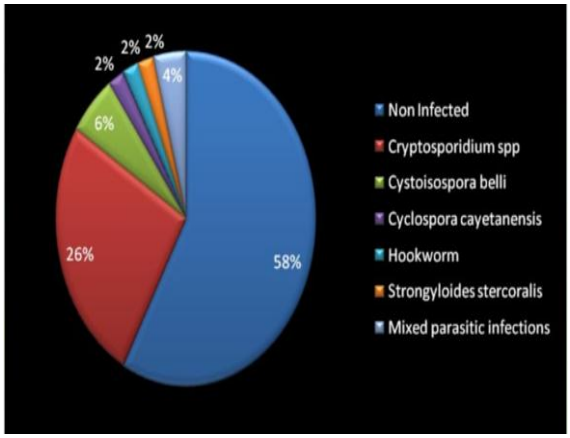

Figure 1(b): Distribution of different parasitic infections with CD4 T Cell count <200 cells/µL

Note: Mixed infections comprised co-infections of *Cryptosporidium* spp. with *Cystoisospora belli* and *Cryptosporidium* spp. with *Cyclospora cayetanensis.*

Table1:Age and gender wise distribution of parasitic infections with CD4≥200 and CD4<200

Socio-demographic factors (Gender)		CD4≥200 (N=50)			CD4<200 (N=50)			Chi square test (χ^2)/Fisher's exact test for (A vs B) compared with (C vs D)	P value
		Parasite detected A	Parasite not detected B	Prevalence %	Parasite detected C	Parasite not detected D	Prevalence %		
Gender	Male	13	22	37.14%	17	22	43.59%	χ^2=0.3180	0.5728
	Female	5	10	33.33%	4	7	36.36%	χ^2=0.0257	0.8726
Age in years	0 to ≤20	0	2	0%	0	1	0%	Odds ratio (OR) for Fisher's test = 0.6000	1.0000
	21 to ≤40	13	18	41.94%	12	15	44.44%	χ^2=0.0370	0.8475
	41 to ≤70	5	12	29.41%	9	13	40.91%	χ^2=0.5509	0.4580
	>70	0	0	0%	0	0	0%	N.A.	N.A.

Table 2: Comparative analysis of prevalence of different parasitic infections with CD4 T Cell count ≥200 cells/µL and <200 cells/µL respectively

Name of parasite	CD4≥200 (N=50)			CD4<200 (N=50)			Chi square test (χ^2)/Fisher's exact test for (A vs B) compared with (C vs D)	P value
	Parasite detected A	Parasite not detected B	Prevalence %	Parasite detected C	Parasite not detected D	Prevalence %		
Cryptosporidium spp.	12	38	24%	15	35	30%	χ^2=0.4566	0.4992
Cystoisospora belli	2	48	4%	4	46	8%	χ^2=0.7092	0.3997
Cyclospora cayetanensis	1	49	2%	2	48	4%	χ^2=0.3436	0.5578
Hookworm	3	47	6%	1	49	2%	χ^2=1.0417	0.3074
Strongyloides stercoralis	2	48	4%	1	49	2%	χ^2=0.3436	0.5578
Ascaris sp.	1	49	2%	0	50	0%	χ^2=1.0101	0.3149

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Note: A 2×2 contingency table was constructed comparing parasite detection (detected vs not detected) between CD4 ≥200 and CD4 <200 groups, and the association was assessed using the Chi-square test or Fisher's exact test where appropriate.

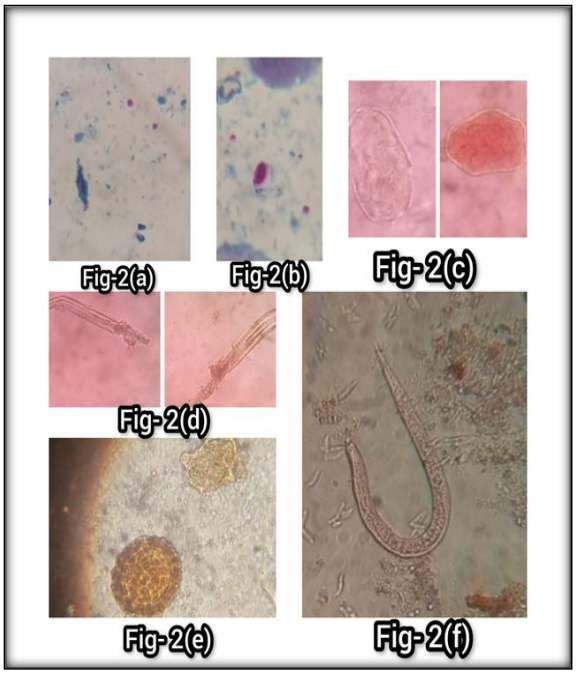

Figure 2(a): Oocysts of *Cryptosporidium* spp. in modified acid-fast stain
Figure 2(b): Oocysts of *Cystoisospora belli* and *Cryptosporidium* spp. in modified acid-fast stain
Figure 2(c): Egg of Hookworm in Saline and Iodine mount
Figure 2(d): Larva of *Strongyloides stercoralis* in Saline and Iodine mount
Figure 2(e): Fertilized egg of *Ascaris lumbricoides* in Saline mount
Figure 2(f): Larva of *Strongyloides stercoralis* in Saline mount

DISCUSSION

Routine microscopic examination of stool samples from HIV patients is usually not done. Hence to understand it's importance and necessity we did this study and found out that intestinal parasitic infections among HIV subjects was 39%. According to previous study, the incidence of parasitic infections was 50% in developed countries but 95% in developing countries.[14] In India, parasitic diarrheal diseases in HIV/AIDS patients is increasing in the last three decades and the Northeast India is the highest contributor of HIV/AIDS cases. Studies are very less to explore the prevalence of opportunistic intestinal infections in such patients.[1] Studies have shown that the prevalence of intestinal parasitic infection was 38.7% in HIV/AIDS patients in Madurai City, South India,[8] whereas 40.99% in North India within which coccidian parasitic infection was 85.13% of total intestinal parasitic infections.[1] In our study, the total prevalence of intestinal parasitic infection is 39% in HIV/AIDS within which coccidian parasitic infection is 81.82% of total intestinal parasitic infections which is similar to the above study. Frederick Olusegun Akinboet also carried out a study among 2000 HIV-positive patients and 500 HIV-negative individuals in Benin City, Nigeria to determine intestinal parasites from stool specimens and their correlation with CD4+ T-cell counts and demographics.[15] They examined for ova, cysts, or parasites in stool samples and also analyzed patient's blood samples for CD4 counts by flow cytometry. An overall prevalence was observed 15.3% among HIV-positive patients which is lower than the percentage reported in previous studies,[16,17] and in our study (39%). Better laboratory equipment, better detection rate and stage of HIV infection could probably explain the difference in the prevalence of parasites. HIV status was a significant ($p<0.0001$) risk factor for intestinal parasitic infections and male gender, CD4 count <200cell/µl and diarrhea were significantly associated with an increased prevalence of intestinal parasitic infections among HIV-positive patients in their study.[15] Similarly, in our study, prevalence of intestinal parasitic infection was more (42%) in patients with CD4+ T-cell count <200cell/µL in comparison with CD4+ T-cell count >=200cell/µL where it was 36%, but the finding was not statistically significant (p=0.538%). Also, male HIV patients had higher prevalence of parasitic infections but not statistically significant (p=0.594%). In the study by O.O Oguntibeju et.al in Malayasia, *Cryptosporidium parvum* had the highest prevalence (10%), followed by *Giardia intestinalis* (8.3%), *Entamoeba histolytica* (6.7%), *Isospora belli* (3.3%) and *Blastocystis hominis* (3.3%) partially similar to our study where we got *Cryptosporidium* spp. (27%) followed by *Cystoisospora belli* (6%), Hookworm (4%), *Cyclospora cayetanensis* (3%), *Strongyloides stercoralis* (3%) and *Ascaris lumbricoides* (1%). This study showed a significant prevalence ($p<0.05$) of intestinal parasites in HIV-positive/AIDS patients than in HIV-negative subjects.[18] Some authors observed a low prevalence of 28.4% in Abeokuta, Nigeria,[19] while others reported a high prevalence of 79.3% in Osun state, Nigeria,[20] indicating geographical variation of prevalence of intestinal parasitic infection among HIV subjects worldwide. So, prevalence studies should be done region wise to estimate the actual burden in that area.

Kuppamuthu et al. reported *Cryptosporidium parvum* the predominant pathogen (28.7%) in HIV/AIDS cases with chronic diarrhea in Madurai City, South India in 2007,[8] whereas it was found 2.3% at Kolkata in 2005,[21] 11.8% of cases at Chennai in 2002 by Kumar et al,[22] 8.5% at Mumbai,[23] and 10% at Vellore by Mukhopadhya et al. in 1999.[24]

These variations of results explained in these studies is it may differ due to the advanced stage of HIV

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and chronic diarrhea which recorded greater prevalence.[8]

By Mohammed Ashraf Ali S Namaji et al. *Cryptosporidium parvum* was the most common cause of diarrhea (70.64%), followed by *Cystoisospora belli* (23.81%) and *Cyclospora* spp. (5.55%).[1] Trend analysis of coccidian etiology during this study revealed a significant rise in the positivity of *Cryptosporidium* spp. and a decrease in the *Cystoisospora belli* infection in most of the studies. In our study, we took stool samples of all HIV subjects irrespective of diarrheal symptoms. We found 27% of stool samples positive for *Cryptosporidium* spp, which was also a predominant pathogen in our study like most of the above studies and also, we found it more prevalent among HIV patients with CD4+ T-cell count <200cells/µL but not statistically significant (p= 0.499). The common helminthic parasite was Hookworm (8.1%) followed by *Ascaris lumbricoides* (4.7%).[1]

In our study, the common helminthic parasites was also Hookworm (4%) but the prevalence of Hookworm infection was more in HIV subjects with CD4+ T-cell count >=200cells/µL but again, not statistically significant. Similarly, *Strongyloides* and *Ascaris* infection were more prevalent in HIV patients with CD4+ T-cell count >=200cell/µl indicating that immunosuppression do not play any significant role in causing infections with non-coccidian parasites. Mohondas et al. carried out a study with fecal samples from 120 HIV-sero positive subjects in North India and analyzed that 30% patients were found to harbour an intestinal parasite within which *Cryptosporidium parvum* was the most common (10.8%), followed by *Giardia lamblia* (8.3%), *Cyclospora cayetanensis* (3.3%) and *Blastocystis hominis* (3.3%), while *Isospora belli* and *Enterocytozoon bieneusi* were each detected in 2.5% of the patients[25].

Unlike our study, in a study by Mohanty I et al. in Odisha, they found prevalence of *Cystoisospora belli* greater than *Cryptosporidium parvum*. They found *I. belli* in 22% of the patients with diarrhea and in 4% without diarrhea (p=0.0019). *I. belli* was the most common parasite, followed by *Entamoeba histolytica/dispar* (8%) and *Cryptosporidium parvum* was only 5% in HIV-positive patients with diarrhea. In HIV-positive patients without diarrhea, the most common parasite detected was *E. histolytica/dispar* (12%) followed by *C. parvum* (6%) and *I. belli* (4%) indicating that prevalence of a particular parasitic infection varies with geographical location and clinical symptom of diarrhea.[26]

Similarly, Chinnambedu R Swathirajanet et al. also found *Cystoisospora belli* more than *Cryptosporidium parvum* in south india in 2017.[27]

But in our study, *Cryptosporidium* spp. infection was most common, followed by *Cystoisospora belli*. *Cystoisospora belli* was more prevalent among the HIV patients with CD4+ T-cell count<200 cells/µL(8%) than CD4+ T-cell counts >=200 cells/µL (4%) but not statistically significant.

CONCLUSION

In our study, overall prevalence of parasitic infection was 39% among HIV infected patients. *Cryptosporidium parvum*, *Cystoisospora belli*, *Cyclospora*, Hookworm, *Strongyloides stercoralis* were the opportunistic parasites observed in this study from stool samples of HIV infected subjects. *Cryptosporidium parvum* had the highest prevalence but more in patients with less CD4+ T-cell count. Different methods for identification of parasitic infections like serological and molecular methods are costlier and their testing facilities are unavailable in most of the laboratories. So proper identification of parasites by microscopy is a cost effective method though technically challenging and require expertise for identification. So significance of our study is that it generated highly valuable data regarding the burden of parasitic infections in this region among HIV patients in a very cost effective way. Hence routine microscopic screening of stool samples is highly recommended to guide clinicians for better management and better outcome of these patients.

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Ethical Approval

The study was conducted after obtaining approval from the Institutional Ethics Committee, R.G. Kar Medical College, Kolkata, vide Registration Number **EC/NEW/INST/2023/3404**; Memo No. **RKC/1085**; dated **02 May 2024.**

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