International Journal of Research in Medical Sciences
Varghese AN et al. Int J Res Med Sci. 2025 Nov;13(11):4823-4831
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012
DOI: https://dx.doi.org/10.18203/2320-6012.ijrms20253608

Original Research Article

Enteric opportunistic parasites in HIV positive patients and associated risk factors

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Received: 25 August 2025
Revised: 07 October 2025
Accepted: 09 October 2025

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ABSTRACT

Background: Depletion of CD4+ T-cells following HIV infection leads to immune system collapse, leaving patients susceptible to opportunistic infections. In developing countries like India, parasites cause diarrhoea in over 90% of HIV patients. This study was conducted to identify the enteric opportunistic parasitic diseases that affect HIV-positive patients and associated risk factors. This study also focuses on the identification of *Cryptosporidium* species by conventional methods and comparing them with ELISA.
Methods: A cross-sectional study was carried out in the Department of Microbiology, GMCH, Aurangabad, from January 2023 to June 2024. Total 104 stool samples of HIV-positive patients were collected. Proper history was obtained to identify the risk factors. The samples were subjected to direct wet mount, concentrated using formalin-ether method, followed by modified Ziehl-Neelsen staining, Auramine O fluorescent staining, and ELISA.
Results: The parasites identified include *Cryptosporidium* (34), *Cyclospora cayetanensis* (1), *Strongyloides stercoralis* (1), and *Entamoeba* (1). The risk factors associated with opportunistic infections were high HIV viral load, low CD4 count, shorter duration of ART, chronic diarrhea, and occupation. Auramine O staining was identified as superior to wet mount and modified Ziehl-Neelsen staining for identification of *Cryptosporidium* spp.
Conclusions: Clinicians can fight AIDS more successfully with proper knowledge about opportunistic infections. Since the spectrum of opportunistic infections varies with geography, their pattern in a particular area is of utmost importance. Understanding the risk factors and optimal diagnostic techniques for opportunistic infections helps in prompt treatment, contributing to delaying the progression to AIDS.

Keywords: *Cryptosporidium*, HIV, Opportunistic infections, Risk factors

INTRODUCTION

The Human immunodeficiency virus (HIV) pandemic is identified as one of the biggest health emergencies that humanity has ever faced.[1] UNAIDS (Joint United Nations Programme on HIV/AIDS) 2023 data shows that today, 39 million [33.1 million-45.7 million] people are living with HIV worldwide.[2] It is currently present in over 190 countries worldwide, and the overall number of people living with HIV in Asia and Africa is rising.[3] As per the National AIDS Control Organization (NACO), there were 24.01 (19.92-29.07) lakh people living with HIV/AIDS (PLHIV) in India in 2021.[4] Southern States of India have the greatest number of PLHIV, including Maharashtra, Andhra Pradesh, and Karnataka in the first three positions.[4] AIDS-Related Deaths (ARD) are estimated at 40,000 [26,000-62,000] in 2022 in India.[2]

During HIV infection, CD4+ T-cell depletion is a hallmark of AIDS (Acquired Immunodeficiency Syndrome). It manifests earlier, is extensive at mucosal sites, and antiretroviral therapy (ART) does not completely reverse

it, especially if it is started when T-cell activities are impaired. [5] As CD4+ T-cell counts decline, people living with chronic HIV infection become more vulnerable to a wide variety of illnesses known as "opportunistic infections (OIs)," which are infections that seldom affect immunocompetent hosts.[6] Because AIDS is characterized by progressive CD4+ T cell deterioration and eventual collapse of immune system functions, opportunistic bacterial, viral, and parasitic infections typically cause morbidity and eventually death.[7] Opportunistic infections via bacterial agents include *Mycobacterium tuberculosis*, *Mycobacterium avium complex*, *Salmonella species*, *Shigella species*, *Campylobacter*, *Escherichia coli*, *Clostridioides difficile–associated infection (CDI)*, *Streptococcus pneumoniae*, *Treponema pallidum*, etc.[7] Common opportunistic fungal infections are candidiasis, Coccidioidomycosis, *Cryptococcus neoformans*, *Pneumocystis jirovecii*, *Talaromyces marneffei*, *Histoplasma capsulatum*, *Aspergillus* species, etc.[7] *Examples of viral opportunistic infections include* Cytomegalovirus, Hepatitis B and C virus, Herpes simplex virus, Human papillomavirus, Varicella-zoster virus, etc.[7] Parasitic opportunistic infections in HIV are commonly *Cryptosporidium*, *Cystoisospora belli*, *Cyclospora*, *Leishmania*, Malaria, *Toxoplasma gondii*, Microsporidium, *Strongyloides*, etc.[7] Among these opportunistic infections, the majority of reports show that diarrhoea is a substantial cause of morbidity and mortality.[8] In developing countries like India, parasites cause diarrhoea in over 90% of HIV patients.[9] The quality of life is significantly reduced by diarrhoea, which also results in weight reduction, dehydration, and nutritional deficiencies.[8]

The cause of diarrhoea may be due to bacterial agents like Salmonella species, *Campylobacter jejuni*, and *Clostridium difficile*, and viral agents like Rotaviruses, Enteric Adenoviruses, Noroviruses, and Astroviruses.[10] Typically, parasitic infections causing diarrhoea linked to HIV-positive individuals are either well-established intestinal pathogens, e.g., *Entamoeba histolytica* and *Giardia lamblia*, or enteric opportunistic parasites like *Cryptosporidium*, *Cystoisospora belli*, *Cyclospora*, Microsporidia, and *Strongyloides stercoralis*.[11] Among these enteric parasitic infections in HIV patients, the most common etiological agents are found to be *Cryptosporidium parvum* and *Cystoisospora belli* with higher prevalence seen for *Cryptosporidium* infection, especially in those patients in whom HIV viral load is high and CD4 T cell count is low.[12]

Both the clinical advancement of HIV infection and the pattern of opportunistic infections differ across individuals and between geographical locations. Knowing the geographical areas along with the pattern or trends of infections in that particular area is helpful for early and accurate opportunistic infection diagnosis and treatment.[1,3] Due to the aforementioned, this study was conducted to identify the enteric opportunistic parasitic diseases that affect HIV-positive patients and associated risk factors.

METHODS

This was a cross-sectional study carried out in the Department of Microbiology, GMCH, Aurangabad, Maharashtra, India, from January 2023 to June 2024. Institutional ethical committee approval was obtained before the start of the study. A total of 104 stool samples of HIV-positive patients having diarrhoea from wards and OPD of the hospital were included in our study as per the inclusion criteria. HIV-negative patients having diarrhoea, other opportunistic infections in HIV-positive patients, and patients who received anti-parasitic treatment within 14 days were excluded from the study.

Stool samples were collected in a sterile, wide-mouthed, clean, leak-proof screw-capped container. Patients were asked to avoid contaminating the stool sample with urine and to transfer a portion (about a spoonful) of the specimen, especially that which contains mucus, pus, or blood. A detailed history of the patients according to the attached routine Performa was taken, including diarrhoea, associated symptoms, treatment history, occupation, etc., along with the estimation of CD4 cell count and HIV viral load to identify various risk factors associated with enteric opportunistic parasites. The samples were processed in the laboratory as soon as possible, preferably within 30 minutes. The collected samples were divided into two parts, one part for routine examination and the other part for *Cryptosporidium* antigen detection by ELISA. The samples for ELISA were stored at -20^{0}C without adding any preservatives.

From the first part of the stool sample, macroscopic examination was done, followed by direct wet mount and iodine mount. All the samples were concentrated using the formalin-ether concentration technique. The sediment obtained after stool concentration was used for the identification of opportunistic intestinal parasites using modified Ziehl-Neelsen staining and Auramine O fluorescent staining. The second part of the sample, which was stored at -20^{0}C was subjected to ELISA.

The risk factors associated with enteric opportunistic parasitic infections in HIV-positive patients were identified, and the results obtained for the identification of *Cryptosporidium* species using conventional methods and ELISA were compared. Data were collected and noted in Microsoft Excel, and evaluation and tabulation were done. Statistical analysis was done using the SPSS statistical program.

RESULTS

The study was conducted as a hospital-based cross-sectional study with 104 participants. The mean age among the total participants (104) was 36.8 years, with a standard deviation of 12.12. The most common age group among the participants was 31 to 40 years. The males and females were almost equally distributed in the study population, with a slight male predominance of 50.9%.

The major category among the participants was educated till high school (33.65%), followed by illiterates (25.96%) (Table 1).

Table 1: Socio-demographic profile of Study participants (n=104).

Parameters		Frequency	Percentage
Age in completed years	≤20	9	8.65
	21-30	28	26.92
	31-40	37	35.58
	41-50	18	17.31
	>50	12	11.54
Gender	Male	53	50.96
	Female	51	49.04
Education	Illiterate	27	25.96
	Primary	2	1.92
	High school	35	33.65
	Higher Secondary	20	19.23
	Graduate	20	19.23

The mean age was 35.67% in the participants with opportunistic infections (n=36) with a standard deviation of 12.87, while the mean age among the participants without infections (n=68) was 36.31 years with SD 11.79. The most common age group among both the groups was 41 to 50 years. The frequency of participants in different age groups with and without opportunistic infection was compared using the chi-square test. The difference was found to be not significant (p value 0.94).

The prevalence of opportunistic infection among males was 27.5%, and among females it was 41.5%, and the difference in prevalence was found to be not significant (p-value 0.13). The difference in educational status among the participants with and without opportunistic parasitic infections was also found to be not significant (p value 0.49).

Among the occupational status of study participants (n=104) The most common occupation among the participants was business (18.27%), followed by agriculture at 16.35%. 14.42% were homemakers and 33.65% were unemployed in this study. The occupation and opportunistic parasitic infection were significantly associated. The participants with agriculture-related jobs were at a higher risk of 8.1 times more to get parasitic infections compared to unemployed individuals (Table 2).

Table 2: Occupation among the participants with and without opportunistic parasitic infections.

Parameters		Participants with opportunistic parasitic infections n=36, N (%)	Participants without opportunistic parasitic infections n=68, N (%)	
Education	Unemployed	8 (22.9)	27 (77.1)	Reference
	Agriculture	12 (70.6)	5 (29.4)	8.1 (2.2,29.9)
	Business	3 (37.5)	5 (62.5)	2.0 (0.3,10.3)
	Homemaker	5 (33.3)	10 (66.7)	1.6 (0.4,6.3)
	Carpenter	0 (0.0)	5 (100.0)	-
	Taxi driver/truck driver	2 (40.0)	3 (60.0)	2.2 (0.3,15.9)
	Government job	0 (0.0)	3 (100.0)	-
	Manual labour	3 (100.0)	0 (0.0)	-
	Teacher	0 (0.0)	1 (100.0)	-
	Student	0 (0.0)	1 (100.0)	-
	Small business	3 (27.3)	8 (72.7)	1.2 (0.2,5.9)
	Total	36 (34.6)	68 (65.4)	

Chi-square with continuity correction, p value 0.01, Significant

Table 3: Duration of diarrhoea in study participants (n=104).

Duration	Frequency	Percentage
<2 weeks	96	92.31
≥2 weeks	8	7.69
Total	104	100.0

The history regarding diarrhoea episodes in study participants was inquired, and 92.31% of the participants had a duration of less than 2 weeks, while 7.69% had chronic diarrhoea that lasted more than or equal to 2 weeks (Table 3). 75% of participants with diarrhoea for more than 2 weeks had opportunistic parasitic infections (n=36), and this increased frequency was found to be significant with a p value of 0.01.

Substance abuse was the most common comorbidity among the study participants; alcohol intake was prevalent in 6.73%, and smoking in 4.81%. Hypertension was in 5.77%, and only 1.92% had diabetes mellitus. There was no significant association between comorbidity and opportunistic parasitic infections in the study **(**p value 0.09).

The majority of the study participants had an HIV viral load as Target Not Detected (TND), 73.08%. Followed by participants with a viral load of less than 1000 (11.54%). Similarly, in study participants with a viral load of more than 1000 copies/ml, 81.25% were infected with opportunistic parasitic infections (n=36), while only 26.14% of the participants with a count less than or equal to 1000 were infected. The association was found to be significant; the odds of having opportunistic parasitic infections in a viral load of more than 1000 copies/ml group was found to be 12.25 times more than participants with a count ≤1000 copies/ml viral load (Table 4).

Table 4: Viral load among the participants with and without opportunistic parasitic infections.

Parameters		Participants with opportunistic parasitic infections n=36, N (%)	Participants without opportunistic parasitic infections n=68, N (%)
Viral load in copies/ml	>1000	13 (81.25)	3 (18.75)
	≤1000	23 (26.14)	65 (73.86)
	Total	36 (34.6)	68 (65.4)

Chi-square=18.17, p value <0.0001, Significant, Crude odds ratio= 12.25 (3.19,46.88)

The CD4+ count in study participants (n=104) shows a total of 38.46% of the participants had less than 200 cells/mm³ CD4+ T cell count, and 36.54% had more than or equal to 500 cells/mm³. In study participants with a CD4+ T cell count less than 200 cells/microliter, 47.5% were infected with opportunistic parasitic infections (n=36), while only 26.6% of the participants with a count more than or equal to 200 cells/microliter were infected. The association was found to be significant (p value=0.03); the odds of having opportunistic parasitic infections in the CD4+ T cell count <200 cells/microliter group was found to be 2.5 times more than participants with a count ≥200 cells/microliter.

The history of exposure in study participants (n=104) shows the most common method of exposure to HIV among the study participants was heterosexual unsafe coitus (85.58%), followed by mother-to-child transmission in 3.85%, and only one participant had a history of unsafe injection. The study's 10 participants (9.62%) were unaware of the exposure status. There was no homosexual mode of exposure in this study. The type of exposure and opportunistic parasitic infections were not significantly associated with the study (p value 0.137).

In relation to the duration of anti-retroviral treatment in study participants, the major category of participants was undergoing treatment for HIV for 6 to 10 years (29.81%), followed by 26.92% for 1 to 5 years. Among this, 73.08% of the participants belonged to stage 1, followed by stage 2 in 18.27%. The lowest number of patients was in stage 4, which is 2.88%. Almost 75% of the participants who had been taking ART for less than a year had opportunistic parasitic infections. The increased frequency of opportunistic parasitic infections in the participants with ART less than 1 year was found to be significant after the chi-square test, with a p-value of 0.01. The association between opportunistic parasitic infections and the clinical stage of HIV infection was found to be not significant (p value=0.06).

The prevalence of opportunistic parasites in the study participants was 34.62%. One participant was having a mixed infection with *Cryptosporidium* and *Cyclospora cayetanensis*. The prevalence of *Cryptosporidium* infection among the participants was 32.69%, while the prevalence of *Strongyloides stercoralis*, *Cyclospora cayetanensis*, and *Entamoeba* was 0.96% (Table 5).

Table 5: Organisms associated with infections among the participants (n=104).

Organisms	No. of positive samples	Percentage
Cryptosporidium	34	32.69
Strongyloides stercoralis	1	0.96
Cyclospora cayetanensis	1	0.96
Entamoeba	1	0.96
Total	104*	100.00

The wet mount method revealed positive results in 18.27% of the participants. 17 samples were positive for *Cryptosporidium* by the wet mount method. One sample each was positive for *Strongyloides stercoralis*, *Cyclospora cayetanensis*, and *Entamoeba* (Figure 1), (Figure 2).

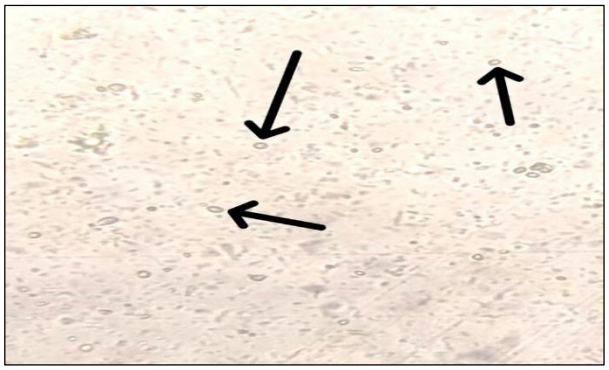

Figure 1: Oocyst of *Cryptosporidium* in stool wet mount.

The prevalence of *Cryptosporidium* by the modified Ziehl-Neelsen staining method was found to be 24.04%. The Auramine O fluorescent staining method revealed that 25.96% of participants had *Cryptosporidium* infection. The prevalence of *Cryptosporidium parvum* infection by the ELISA method was 32.69% (Figure 3) and (Figure 4).

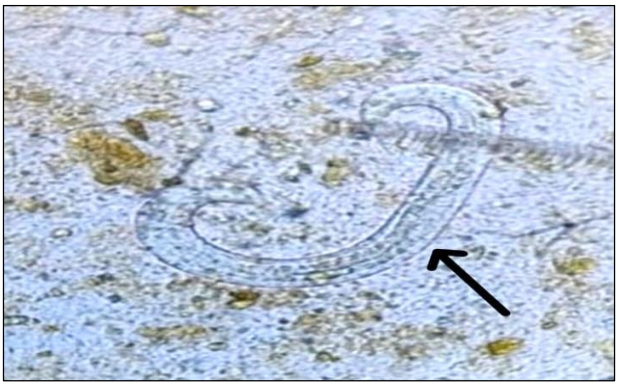

Figure 2: Larvae of *Strongyloides* in stool wet mount.

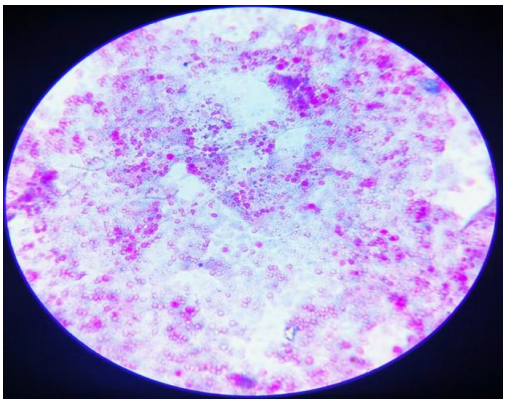

Figure 3: Oocyst of *Cryptosporidium* in modified ZN staining.

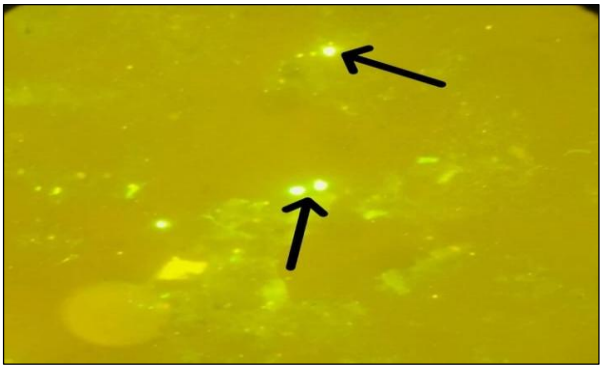

Figure 4: Oocyst of *Cryptosporidium* in Auramine O fluorescent staining.

The diagnostic accuracy parameters of wet mount in the identification of *Cryptosporidium* species in comparison with ELISA were identified as 83.65%, meaning that wet mount can identify the case correctly in 83.65 cases out of 100 (Table 6).

Diagnostic accuracy parameters of modified Ziehl-Neelsen staining in the identification of Cryptosporidium species in comparison with ELISA were 91.35%. Compared to the wet mount method, Modified Ziehl-Neelsen staining method had better sensitivity (73.53%), negative predictive value (88.61%), and diagnostic accuracy (91.35%). Compared to the wet mount method and modified Ziehl-Neelsen staining, the Auramine O fluorescent staining method had better sensitivity (79.41%), negative predictive value (90.91%), and diagnostic accuracy (93.27%).

Table 6: Diagnostic accuracy parameters of Wet mount in identification of *Cryptosporidium* species in comparison with ELISA.

Parameter	Estimate	Lower-Upper 95% CIs
Sensitivity	50	(34.07, 65.93)
Specificity	100	(94.8, 100)
Positive predictive value	100	(81.57, 100)
Negative predictive value	80.46	(70.92, 87.43)
Diagnostic accuracy	83.65	(75.37, 89.54)

DISCUSSION

The spectrum of intestinal parasites has significantly changed as a result of the HIV/AIDS pandemic. HIV significantly lowers a patient's immune system, rendering them more vulnerable to opportunistic infections. Among these opportunistic infections, intestinal parasites are a significant factor since they frequently cause diarrhea, which can have potentially fatal consequences. AIDS-related illnesses such as opportunistic intestinal parasitic infections cause the death of about 80% of people living with HIV/AIDS, not the virus itself.

This study was conducted to identify various enteric opportunistic parasites causing diarrhea in HIV-positive patients. The risk factors associated with these infections were considered and studied in detail. This study was also conducted to compare different methods for identifying *Cryptosporidium* species, including direct wet mount, modified Ziehl-Neelsen staining, and Auramine O fluorescent staining with ELISA. This study was carried out in our hospital from January 2023 to June 2024. A total number of 104 stool samples of HIV-positive patients having diarrhea were evaluated. The sex-wise distribution of intestinal parasitic infections showed a higher prevalence in females (41.5%) as compared to males (27.5%). The survey conducted by Dickson et al showed a higher prevalence of parasites in females (84.3%) than in males (76.5%), similar to this study.[13] Endalamaw et al also identified female predominance in their study (69.8% for females and 30.2% for males).[14] This gender disparity may be caused by biological factors such as suppressed immunity, socially restricted access to healthcare, social stigma, discrimination associated with HIV/AIDS, and poor nutritional status.

In this study, the age-wise distribution of intestinal parasitic infection showed a higher prevalence in the age group of 41 to 50 years. A similar observation was seen in the study by Barcelos et al.[15] Whereas studies carried out

by Endalamaw et al and Dereb E et al showed a higher prevalence of parasites in the age group 31-40 years.[14,16] Out of 104 participants in this study, 36 participants were identified to have opportunistic intestinal parasitic infections. The identified parasites include *Cryptosporidium, Cyclospora cayetanensis, Strongyloides stercoralis, and Entamoeba.* In this study, the prevalence of enteric opportunistic parasites among HIV-positive participants was found to be 34.62%. Similar findings were found in studies conducted by Kulkarni et al (35%), Saleem et al (37.5%), and Mohamud et al (37.1%).[17-19] However, some other studies showed a higher prevalence of opportunistic intestinal parasites, including Manish Mathur et al (50.36%), Gupta et al (59.3%), Kaushal Dwivedi et al (62.7%).[20,21,9]

Cryptosporidium was the most isolated parasite in this study. Prevalence of *Cryptosporidium* was 32.69%. Studies conducted by Nitya et al, Rakesh et al, and Dickson et al also came to the same conclusion, that *Cryptosporidium* was the most prevalent intestinal parasite in HIV-positive patients.[22,23,13] On the contrary, in a study conducted by Mehta et al, *Isospora belli* was the most prevalent intestinal parasite.[24] In another study conducted by Purbey et al, Hookworm was the most predominant intestinal parasite.[25] The prevalence of *Cyclospora* in the present study was identified as 0.96%, which is comparable to the study conducted by Kulkarni et al where the prevalence of *Cyclospora* was 0.7%.[17] The study conducted by Mohanty et al showed a higher prevalence of *Cyclospora*, that is 2%.[26] No *Cystoisospora* or Microsporidia species were identified in this study. This was in correlation to the survey conducted by Purbey et al in Varanasi in 2017.[25] In some other Indian studies, a higher prevalence of *Cystoisospora* and Microsporidia has been identified. The example includes a study conducted by Mohanty et al, prevalence was 4% and 2%, respectively for *Cystoisospora* and Microsporidia.[26] The lower positivity of Microsporidia in this study may be due to the use of modified Ziehl-Neelsen staining rather than Modified Trichrome stain. Trichrome stain has higher sensitivity than the Ziehl-Neelsen staining technique, and modified Ziehl-Neelsen staining may lead to false negative results due to the spore's tiny size and thick wall.[27] The positivity of *Entamoeba* was identified as 0.96%, which was lower compared to studies conducted by Kulkarni et al and Mathur et al which were 7% and 9%, respectively.[17,20]

The cause of diarrhea in participants negative for parasites (n=68) could be bacterial, viral, fungal, neoplastic or idiopathic. Only 8 participants out of 36 who were found positive for *Cryptosporidium* reported that their symptoms subsided after taking Nitazoxanide 500 mg twice daily for 14 days. The rest of the participants did not give consent for follow-up. The risk parameters monitored in this study include HIV viral load, CD4 T cell count, age, gender, education, occupation, comorbidities, duration of diarrhea, duration of ART, type of exposure, and clinical stage. The risk factors identified in this study associated with enteric opportunistic parasites include high HIV viral load (p=0.0001), low CD4 count (p=0.03), shorter duration of ART (p=0.01), longer duration of diarrhoea (p=0.01), and occupation (p=0.01). The HIV viral load of more than 1000 copies/ml and a CD4 cell count of less than 200 cells/microliter are identified as risk factors in this study, similar to studies conducted by Endalamaw et al and Dereb et al.[14,16] This is because a high viral load and low CD4 counts indicate a compromised immune system of the patient, making them more prone to parasitic infections. In this study, 75% of the participants who were taking ART for less than a year had opportunistic parasitic infections. The increased frequency of opportunistic parasitic infections in the participants with ART less than 1 year was found to be significant after the chi-square test, with a p value of 0.01. Similar conclusions were made by Abange et al and Zeynudin et al.[28,29] These two studies identified that patients who are on ART for a long duration have a lower prevalence of intestinal infections than those who are on ART for a short duration. This is possible because ART reduces the risk of HIV-related opportunistic infection by stimulating a strong immune response as indicated by CD4 cell count, presumably as a result of maximal viral suppression. A difference in opinion was made by Tumaini et al where they identified that there was no significant change in the prevalence of intestinal parasites between ART-treated and non-treated subjects.[30]

A total of 75% of participants with diarrhoea for more than 2 weeks (chronic diarrhoea) had opportunistic parasitic infections, and this increased frequency was found to be significant with a p-value of 0.01. This is in correlation to the study by Mohammed et al.[31] They concluded that the prevalence of intestinal parasites in HIV patients is more common in those with chronic diarrhoea than with acute diarrhoea (less than 2 weeks). The participants with agriculture-related jobs were at a higher risk of getting parasitic infections. Similar to this study, Akinbo et al concluded that agriculture workers pose a higher risk of getting intestinal infections.[32] This could be because of exposure to more soil-transmitted parasites, making them prone to infections. The following components were not identified as risk factors of parasitic infections in this study including age (p=0.94), gender (p=0.13), education (p=0.49), co-morbidity (p=.09), mode of transmission (p=0.137), and clinical stage of HIV (p=0.06). Studies done by Abebe et al and Alemayehu et al showed the same results: that age and gender are not risk factors associated with intestinal infections.[33,34] Similarly, level of education was not found as a risk factor associated with intestinal infections. However, a difference in findings was made by Dickson et al and Akinbo et al where poor education was identified as a risk factor.[13,32] The present study concluded that co-morbid conditions are not a risk factor for intestinal parasitic infections, but the presence of co-morbidities was identified as a risk factor in a study conducted by Mohamud et al.[19] Dwivedi et al in their study identified homosexual activity as a risk factor.[35] Still, in the present study, there were no homosexual modes of exposure, and the types of exposure and opportunistic parasitic infections

were not significantly associated. In study participants in clinical stage 1, 27.6% were infected with opportunistic parasitic infections, while 47.4% of the participants in stage 2, and 66.7% of stages 3 and 4 were infected. The association was found to be not significant. A similar conclusion was made from the study conducted by Alemayehu et al where no significant association between the clinical stage of the disease and intestinal parasitic infections was found.[34] Hence, even though factors like age, gender, education, comorbidity, mode of transmission, and clinical stage of HIV were not identified as associated with intestinal parasitic infections in this study, these are important since they can be potential risk factors according to various research.

In this study, 104 stool samples were subjected to parasitological examination. Out of the 34 samples ELISA positive for *Cryptosporidium parvum*, wet mount identified only 17 samples as C*ryptosporidium* positive. The sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic accuracy of wet mount were 50%, 100%, 100%, 80.46%, and 83.65%, respectively. The lower rate of parasite detection in direct wet mount may be because if parasites are scanty in stool, routine microscopy may fail to detect them. The wet mount of stool samples was done before the formol-ether concentration, which might have contributed to the same. Also, since oocysts can readily be mistaken for other materials in fecal samples, wet mount preparation techniques are not very useful for detecting *Cryptosporidium*. A similar finding was seen in a study conducted by Omoruyi et al.[36]

Out of the 34 samples ELISA positive for *Cryptosporidium parvum*, modified Ziehl-Neelsen staining identified 25 samples as *Cryptosporidium* positive. The sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic accuracy of Ziehl-Neelsen staining were 73.53%, 100%, 100%, 88.61%, and 91.35%, respectively. With 27 samples positive for *Cryptosporidium*, the Auramine O staining method showed sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic accuracy of 79.41%, 100%, 100%, 90.91%, and 93.27%, respectively. These results showed that the Auramine O staining method is superior to wet mount and Modified Ziehl-Neelsen staining. In the study conducted by Ninama et al for detecting *Cryptosporidium* using various staining methods, including Auramine O and Modified Ziehl-Neelsen, Auramine O fluorescent staining was identified as superior to the Modified Ziehl-Neelsen staining method.[37] Similar to findings in the present study, Jaiswal et al and Hanscheid et al stated that Auramine O is a simple, rapid, highly sensitive, and specific technique and does not require skill to observe the oocyst under the microscope.[38,39] ELISA is the gold standard test for detecting the presence of *Cryptosporidium* in a low-resource setting. There was an increasing trend in the percentage of detection of *Cryptosporidium* from wet mount to ELISA. Compared to ELISA, Wet mount, Modified Ziehl-Neelsen staining, and Auramine O fluorescent staining had a sensitivity of 50%, 73.53%, and 79.41%, respectively. Auramine O staining was identified as superior to wet mount and Modified Ziehl-Neelsen staining with higher sensitivity, negative predictive value, diagnostic accuracy, and rapidity.

The limitation of the present study was that it used modified Ziehl-Neelsen staining rather than modified trichrome stain for the identification of Microsporidia. Since Trichrome stain has higher sensitivity than the Ziehl-Neelsen staining, it might miss out on the parasite in patient samples and can alter the information regarding the incidence and prevalence of infections due to Microsporidia.

CONCLUSION

This study concludes that *Cryptosporidium parvum* is the commonest opportunistic intestinal parasite in the study population, with a significant correlation to a high HIV viral load of more than 1000 copies/ml, a CD4 count of less than 200 cells/microliter, a shorter duration of ART, chronic diarrhea, and agriculture workers. *Cryptosporidium parvum* antigen ELISA is a simple, rapid, reliable, sensitive, and specific test that can be used for a larger number of samples. Since ELISA is costlier, Auramine O can be used as an alternative for the diagnosis of *Cryptosporidium*. This study identifies the spectrum of opportunistic infections in the study area, as the pattern of these infections in a particular geography is of utmost importance for early diagnosis and treatment. Knowing the risk factors and best methods of diagnosis for opportunistic infections helps in prompt treatment, contributing to boosting immunity and increasing the life expectancy among infected patients by delaying the progression to AIDS.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Gautam H, Bhalla P, Saini S, Uppal B, Kaur R, Baveja CP et al Epidemiology of opportunistic infections and its correlation with CD4 T-lymphocyte counts and plasma viral load among HIV-positive patients at a tertiary care hospital in India. J Int Assoc Physicians AIDS Care (Chic). 2009;8(6):333-7.
2. The path that ends AIDS: UNAIDS Global AIDS Update 2023. Geneva: Joint United Nations Programme on HIV/AIDS; 2023. Licence: CC BY-NC-SA 3.0 IGO.
3. Bhuvana KB, Hema NG, Patil RT. Prevalence and risk factors for opportunistic infections in HIV patients who developed adverse drug reactions (ADRs) to antiretroviral therapy (ART) in a tertiary-

care teaching hospital. Nat J Physiol Pharm Pharmacol. 2015;5(3):200-.
4. National AIDS Control Organization- ICMR-National Institute of Medical Statistics. India HIV Estimates 2021 Fact Sheet, 2021. Available at: https://naco.gov.in/sites/default/files/India%20HIV%20Estimates%202021%20_Fact%20Sheets__Final_Shared_24_08_2022_0.pdf. Accessed 01 June 2025.
5. Suryawanshi M, Kalshetti V, Telele K, Wadile R, Haswani N, AHIRE K. The Intestinal Parasitic Infections and the CD4 Counts in HIV Seropositive Individuals in the Dhule District in Maharashra, India. J Clin Diagnos Res. 2012;6(7).
6. Meng S, Tang Q, Xie Z, Wu N, Qin Y, Chen R, et al. Spectrum and mortality of opportunistic infections among HIV/AIDS patients in southwestern China. Eur J Clin Microbiol Infect Dis. 2023;42(1):113-20.
7. Council A. Guidelines for the Prevention and Treatment of Opportunistic Infections in Adults and Adolescents With HIV, 2024. Available at: https://clinicalinfo.hiv.gov/sites/default/files/guidelines/documents/adult-adolescent-oi/guidelines-adult-adolescent-oi.pdf. Accessed 01 June 2025.
8. Sahu S, Mohanty I, Parida B. Parasitic enteropathogens in HIV seropositive patients in relation to CD4 count at a tertiary care hospital. Int J Clin Biomed Res. 2018;4(2):27-30.
9. Dwivedi KK, Prasad G, Saini S, Mahajan S, Lal S, Baveja UK. Enteric opportunistic parasites among HIV infected individuals: associated risk factors and immune status. Jap J Infect Dis. 2007;60(2-3):76-81.
10. Basile FW, Fedele MC, Lo Vecchio A. Gastrointestinal diseases in children living with HIV. Microorg. 2021;9(8):1572.
11. World Health Organization. Guidelines on standard operating procedures for laboratory diagnosis of HIV-oportunistic infections. WHO Regional Office for South-East Asia; 2001. Available at: https://iris.who.int/server/api/core/bitstreams/621d444c-d1a1-4b73-b553-9576d47d13a2/content. Accessed 01 June 2025.
12. Joseph AA, Ano-Edward GH. Correlation of viral load to intestinal parasitosis in HIV seropositive patients attending UITH Ilorin. Ann Trop Pathol. 2016;7(1):49-61.
13. Nsagha DS, Njunda LA, Assob NJ, Ayima CW, Tanue EA, Kibu OD, et al. Prevalence and predisposing factors to intestinal parasitic infections in HIV/AIDS patients in Fako division of Cameroon. Am J Epidemiol Infect Dis. 2017;5(3):42-9.
14. Endalamaw M, Alemneh A, Amare GA, Fenta A, Belew H. Magnitude of intestinal parasitic infections and its determinants among HIV/AIDS patients attending at antiretroviral treatment centers in East and West Gojam Zones, Northwest, Ethiopia: institution based cross-sectional study. AIDS Res Ther. 2024;21(1):32.
15. Barcelos NB, Silva LFE, Dias RFG, Menezes Filho HR, Rodrigues RM. Opportunistic and non-opportunistic intestinal parasites in HIV/ AIDS patients in relation to their clinical and epidemiological status in a specialized medical service in Goiás, Brazil. Rev Inst Med Trop Sao Paulo. 2018;60:e13.
16. Dereb E, Negash M, Teklu T, Damtie D, Abere A, Kebede F, et al. Intestinal Parasitosis and its Association with CD4+ T Cell Count and Viral Load among People Living with HIV in Parasite Endemic Settings of Northwest Ethiopia. HIV AIDS (Auckl). 2021;13:1055-65.
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 immunesuppression. Ind J Medi Res. 2009;130(1):63.
18. Saleem M, Sivasankaran MP, Sujitha K, Innocent JP. Identification of Opportunistic Intestinal parasites in HIV Seropositive patients attending a Tertiary care hospital in Madhurandagam, India. Int J Medi Pharma Virol. 2011;1(2):52-9.
19. Mohamud AK, Ahmed OA, Mohamud AA, Dirie NI. Magnitude of opportunistic infections and associated factors among adult people living with human immune deficient virus on art at selected public hospital, mogadishu somalia: cross-sectional study. Ann Med Surg (Lond). 2023;85(7):3364-71.
20. Mathur MK, Verma AK, Makwana GE, Sinha M. Study of opportunistic intestinal parasitic infections in human immunodeficiency virus/acquired immunodeficiency syndrome patients. J Global Infect Dis. 2013;5:164-7.
21. Gupta K, Bala M, Deb M, Muralidhar S, Sharma DK. Prevalence of intestinal parasitic infections in HIV-infected individuals and their relationship with immune status. Ind J Med Microbiol. 2013;31(2):161-5.
22. Vyas N, Sood S, Sharma B, Kumar M. The Prevalence of Intestinal Parasitic Infestation and the Related Profile of the CD4 (+) Counts in HIV/AIDS People with Diarrhoea in Jaipur City. J Clin Diagn Res. 2013;7(3):454-6.
23. Mohandas K, Sehgal R, Sud A, Malla N. Prevalence of intestinal parasitic pathogens in HIV-seropositive individuals in Northern India. Japan J Infect Dis. 2002;55(3):83-4.
24. Mehta KD, Vacchani A, Mistry MM, Kavathia GU, Goswami YS. To study the prevalence of various enteric parasitic infections among HIV infected individuals in the P.D.U. Medical College and Hospital, Rajkot, Gujarat, India. J Clin Diagn Res. 2013;7(1):58-60.
25. Purbey MK, Singh A, Kumari S, Banerjee T. High carriage rate of intestinal parasites among asymptomatic HIV-seropositive individuals on antiretroviral therapy attending the tertiary care hospital in Varanasi, India. Indian J Sex Transm Dis AIDS. 2021;42(2):101-5.
26. Mohanty I, Panda P, Sahu S, Dash M, Narasimham MV, Padhi S, et al. Prevalence of isosporiasis in relation to CD4 cell counts among HIV-infected

patients with diarrhea in Odisha, India. Adv Biomed Res. 2013;2:61.
27. Ghazali WA, Al-Talib H, Zaini AB. Microsporidiosis: Identification by a simple modified trichrome stain. Int J Infect Dis. 2020;101:430-6.
28. Abange WB, Nkenfou CN, Gonsu Kamga H, Nguedia CA, Kamgaing N, Lozupone C, et al. Intestinal parasites infections among HIV infected children under antiretrovirals treatment in Yaounde, Cameroon. J Trop Pediatr. 2020;66(2):178-86.
29. Zeynudin A, Hemalatha K, Kannan S. Prevalence of opportunistic intestinal parasitic infection among HIV infected patients who are taking antiretroviral treatment at Jimma Health Center, Jimma, Ethiopia. Eur Rev Med Pharmacol Sci. 2013;17(4):513-6.
30. Ringo TA, Ernest A, Kamala B, Mpondo BC. Intestinal parasitic infections and the level of immunosuppression in HIV seropositive individuals with diarrhoea in Kilimanjaro, Tanzania: A crosssectional study. South Sudan Med J. 2015;8(3):52-6.
31. Awole M, Gebre-Selassie S, Kassa T, Kibru G. Prevalence of intestinal parasites in HIV-infected adult patients in southwestern Ethiopia. Ethiop J Health Dev. 2003;17(1):71-8.
32. Akinbo FO, Okaka CE, Omoregie R. Prevalence of intestinal parasitic infections among HIV patients in Benin City, Nigeria. Libyan J Medi. 2010;5(1).
33. Alemseged M, Abebe R, Girma M. Prevalence and associated risk factors of opportunistic intestinal parasites among HIV positive and negative individuals in South Ethiopia: a case control study. BAOJ Hiv. 2015;1(001).
34. Alemayehu E, Gedefie A, Adamu A, Mohammed J, Kassanew B, Kebede B, et al. Intestinal parasitic infections among HIV-infected patients on antiretroviral therapy attending debretabor general hospital, Northern Ethiopia: a cross-sectional study. HIV AIDS (Auckl). 2020;12:647-55.
35. Dwivedi KK, Prasad G, Saini S, Mahajan S, Lal S, Baveja UK. Enteric opportunistic parasites among HIV infected individuals: associated risk factors and immune status. Jpn J Infect Dis. 2007;60(2-3):76-81.
36. Omoruyi BE, Nwodo UU, Udem CS, Okonkwo FO. Comparative diagnostic techniques for Cryptosporidium infection. Molecules. 2014;19(2):2674-83.
37. Ninama A. Detection of Cryptosporidium in fecal sample by three staining method from immunocompromised patients. Int J Med Microbiol Trop Dis. 2018;4(4):257-60.
38. Jaiswal V, Brar APS, Sandhu BS, Singla LD, Narang D, Leishangthem GD, et al. Comparative evaluation of various diagnostic techniques for detection of Cryptosporidium infection from the faecal samples of diarrhoeic bovine calves. Iran J Vet Res. 2022;23(3):247-54.
39. Hanscheid T, Cristino JM, Salgado MJ. Screening of auramine-stained smears of all fecal samples is a rapid and inexpensive way to increase the detection of coccidial infections. Int J Infect Dis. 2008;12(1):47-50.

Cite this article as: Varghese AN, Harbade MS, Iravane JA. Enteric opportunistic parasites in HIV positive patients and associated risk factors. Int J Res Med Sci 2025;13:4823-31.