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Original Article

Prevalence of Intestinal Parasitic Infections in HIV-Positive Patients with Acute Diarrhoea: A Cross-sectional Study

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ABSTRACT

Intestinal parasitic infections are one of the primary determinants of morbidity and mortality in people with HIV, especially in resource-limited environments. **Objective:** To determine the prevalence of intestinal parasitic infections in HIV-positive patients presenting with acute diarrhoea. **Methods:** This cross-sectional study was conducted at Allama Iqbal Teaching Hospital, Dera Ghazi Khan, from October 2025 to February 2026. A total of 153 HIV-seropositive patients (18–60 years) with acute diarrhoea and CD4 count <200 cells/mm^3 were enrolled, and their stool specimens were examined by direct wet-mount microscopy with normal saline and Lugol's iodine, modified acid-fast (Kinyoun) staining for coccidian parasites, and formal-ether concentration. Data were analysed using SPSS version 25.0. **Results:** Of 153 patients (mean age 34.8±9.6 years; 74.5% male), intestinal parasitic infection was detected in 68 (44.4%; 95% CI 36.8–52.4). *Cryptosporidium* spp. was the most prevalent organism (30.1%), followed by *Entamoeba histolytica* (13.1%), *Cystoisospora belli* (11.8%) and *Blastocystis* spp. (9.8%). Infection rates were significantly higher in patients with CD4 <50 cells/mm^3 (100%) versus 101–200 cells/mm^3 (31.8%) (χ^2=19.94, p<0.001), and in WHO clinical Stage IV (75.6%) versus Stage I (14.3%) (χ^2=30.57, p<0.001). In multivariable logistic regression, each one-cell/mm^3 increase in CD4 count was associated with lower odds of infection (aOR 0.968, 95% CI 0.955–0.982, p<0.001). **Conclusions:** Intestinal parasitic infections are frequent in HIV-positive patients with acute diarrhoea, with *Cryptosporidium* species being the most common. Lower CD4 count and advanced WHO clinical stage were the only variables independently associated with infection.

INTRODUCTION

Human immunodeficiency virus (HIV) infection is one of the most important challenges in terms of global health problems. One of the most prevalent and disabling complications of HIV-positive patients is gastrointestinal dysfunction, with diarrhoea being one of the most frequent symptoms at some point in the disease progression [1, 2]. Diarrhoeal episodes cause severe malnutrition, weight loss, electrolyte imbalance, poor quality of life, and enhanced health care service use. The clinical and economic effects of this complication are especially drastic in South Asia and sub-Saharan Africa, where the dual burden of the HIV infection and environmental parasites is the most evident [3, 4]. The spread of HIV infections in Pakistan has been on the increase during the past few years, and gastrointestinal complications are a significant component of the clinical burden borne by the HIV victims. Intestinal parasitic infections are among the major causes of diarrhoea among immunocompromised patients, and when CD4 lymphocyte levels drop below critical levels, their prevalence increases [5, 6]. *Cyclospora cayetanensis*, *Cryptosporidium* spp., and *Cystoisospora belli* are opportunistic protozoa that are especially common in patients with advanced HIV disease and are linked to excessive, chronic diarrhoea that is frequently refractory to standard treatment [7, 8]. Reported prevalence varies substantially across settings and study populations, reflecting differences in immune status, antiretroviral therapy, diagnostic methods, and the clinical

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characteristics of participants [9]. Reported prevalence varies widely across settings: 73.3% among HIV-positive patients in Western Ethiopia [10], 5.97% in a rural hospital-based study in Ghana [11], 30% in the Eastern Cape of South Africa [12], with infection rates rising sharply as CD4+ T-cell counts decline [13], and 12.59% in Eastern India [14]. In Pakistan, there is a limited number of published studies in either Peshawar, Multan or other urban centres, indicating that the prevalence of parasitic infections in HIV-positive people with diarrhoea is high [15, 16].

There is little published literature on data from the Dera Ghazi Khan region, with few healthcare facilities, high poverty levels, and poor sanitation. Knowledge of the local epidemiology of parasitic infection among HIV-positive patients with diarrhoea would play an important role in the development of special diagnostic guidelines, in the determination of empirical treatment, and in the mobilisation of the policy of the health of the population. This study aimed to determine the prevalence and pattern of intestinal parasitic infections, and to identify the demographic and immunological factors associated with infection, in HIV-positive adults presenting with acute diarrhoea at a tertiary-care centre in Dera Ghazi Khan, southern Punjab.

METHODS

This cross-sectional study was conducted at the Department of General Medicine, Unit II, Allama Iqbal Teaching Hospital, Dera Ghazi Khan, with approval from the hospital's ethical committee vide letter no. 1430/MED/DGKMC, dated: 18th October 2025, from October 2025 to February 2026. A total of 153 patients were included using non-probability consecutive sampling. The sample size was calculated using the formula $n = z^2_{(1-\alpha/2)} \times P(1-P) / d^2$, with an 8% margin of error, a 95% confidence interval, and an expected prevalence of 48.15% derived from a previous study of HIV-positive patients with diarrhoea in Pakistan [17]. Patients aged 18-60 years; confirmed HIV-seropositivity by two rapid antibody tests or an enzyme-linked immunosorbent assay; CD4 cell count <200 cells/mm^3; and acute diarrhoea (three or more loose stools per day for less than seven days) were included. Pregnant or lactating women who had been treated with anti-parasitic within the past four weeks and had known comorbidities were excluded. Written informed consent was taken from all the patients by the researcher. Data, including the duration of diarrhoea, the latest CD4 cell count (within 30 days), and the WHO clinical stage, were noted. Each patient had a fresh stool sample collected and processed within 2 hours of collection. Each specimen was studied using three complementary methods: direct wet-mount preparation with normal saline and Lugol's Iodine stain to detect protozoan trophozoites, cysts, and helminth ova; modified acid-fast staining (Kinyoun cold method) to detect coccidian parasites and the formal-ether concentration method to increase the detection of helminth ova and protozoan cysts under the direct supervision of a consultant microbiologist. All staining and concentration procedures followed the protocols described in Garcia's Diagnostic Medical Parasitology (6th edition). Quality control included daily processing of known-positive and negative control slides for the modified acid-fast stain and independent reading of all slides by two trained microscopists, with a third reader resolving any disagreements. Each patient provided an initial fresh stool sample. Where the first stool sample was negative on direct examination but the patient remained symptomatic, a second stool sample was requested within 48 hours to improve the sensitivity of parasite detection; however, most participants provided only a single stool specimen.

SPSS Statistics version 25.0 was used to analyse the data. The Shapiro-Wilk test was used to test the normality of continuous variables. To determine the association between categorical variables and the presence of intestinal parasitic infection, a Chi-square test was applied. A p-value of <0.05 was deemed statistically significant. The continuous variables were summarised as mean ± SD, and the categorical variables as frequencies and percentages with 95% CI. Where the expected cell count was less than five, Fisher's exact test was employed instead of the chi-square approximation, as the chi-square approximation is not suitable for sparse tables. Age, gender, residence, educational status, CD4 count, and WHO clinical stage were used in a multivariable binary logistic regression to determine which variables were independently associated with the presence of any intestinal parasite, and the results were presented as adjusted ORs and 95% CIs.

RESULTS

The mean age of 153 patients was 34.8 ± 9.6 years. Most patients were male (114, 74.5%) and from rural areas (86, 56.2%). Regarding educational status, 75 (49.0%) were illiterate, and 78 (51.0%) were literate. The mean BMI was 21.7 ± 2.2 kg/m^2, with most patients (135, 88.2%) falling within the normal weight range. The mean CD4 count was 102.7 ± 33.4 cells/mm^3, and the mean duration of diarrhoea was 3.3 ± 1.3 days. Regarding WHO clinical staging, most patients were in Stage III (67, 43.8%), followed by Stage IV (45, 29.4%) (Table 1).

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Table 1: Baseline Characteristics of the Study Participants (n=153)

Variables	n (%)	Mean ± SD
Age (Years)		
18–30 Years	52 (34.0%)	34.8 ± 9.6
31–40 Years	59 (38.6%)	
41–50 Years	30 (19.6%)	
51–60 Years	12 (7.8%)	
Gender		
Male	114 (74.5%)	–
Female	39 (25.5%)	
Residence		
Rural	86 (56.2%)	–
Urban	67 (43.8%)	
Educational Status		
Illiterate	75 (49.0%)	–
Literate	78 (51.0%)	
BMI (kg/m^2)		
Underweight (<18.5)	10 (6.5%)	21.7 ± 2.2
Normal Weight (18.5–24.9)	135 (88.2%)	
Overweight (≥25.0)	8 (5.2%)	
CD4 Count (cells/mm^3)		
<50 cells/mm^3	10 (6.5%)	102.7 ± 33.4
50–100 cells/mm^3	58 (37.9%)	
101–200 cells/mm^3	85 (55.6%)	
WHO Clinical Stage		
Stage I	7 (4.6%)	–
Stage II	34 (22.2%)	
Stage III	67 (43.8%)	
Stage IV	45 (29.4%)	
Duration of Diarrhoea (days)	–	3.3 ± 1.3

Intestinal parasitic infection was detected in 68 of 153 patients (44.4%). Multiple parasites were detected in the same stool sample in many cases: of the 68 infected patients, 31 (45.6%) carried a single organism, 22 (32.4%) two organisms, 13 (19.1%) three organisms, and 2 (2.9%) four organisms. *Cryptosporidium* spp. was the most frequently identified organism, found in 46 patients (30.1%). *Entamoeba histolytica* was identified in 20 (13.1%) patients, followed by *Blastocystis* spp. in 15 (9.8%) and *Cystoisospora belli* (formerly *Isospora belli*) in 18 (11.8%). *Cyclospora* spp. was found in 12 (7.8%) patients. Helminthic infections were less common, with *Strongyloides stercoralis* in 6 patients (3.9%) and *Trichuris trichiura* in 5 patients (3.3%) (Table 2).

Table 2: Prevalence of Intestinal Parasitic Organisms (n=153)

Organisms	n	Prevalence (%)	Wilson 95% CI
Cryptosporidium spp.	46	30.1%	23.4–37.7%
Entamoeba histolytica	20	13.1%	8.6–19.3%
Blastocystis spp.	15	9.8%	6.0–15.5%
Cystoisospora belli	18	11.8%	7.6–17.8%
Cyclospora spp.	12	7.8%	4.5–13.2%
Strongyloides stercoralis	6	3.9%	1.8–8.3%
Trichuris trichiura	5	3.3%	1.4–7.4%
Any Parasite	68	44.4%	36.8–52.4%

CD4 count category (χ^2=19.94, df=2, p<0.001) and WHO clinical stage (χ^2=30.57, df=3, p<0.001) were significantly associated with parasitic infection. Infection was detected in all 10 patients (100.0%) with CD4 <50 cells/mm^3, 31 of 58 (53.4%) with CD4 50–100 cells/mm^3, and 27 of 85 (31.8%) with CD4 101–200 cells/mm^3. The infection rate increased progressively: 14.3% in Stage I, 17.6% in Stage II, 40.3% in Stage III, and 75.6% in Stage IV. Neither age group, gender, residence, nor educational status was significantly associated with infection (p>0.05 for all). Because of the small Stage I row (n=7) and the zero cell in the uninfected CD4 <50 group, Fisher's exact test was applied for the CD4 and WHO stage tables and confirmed p<0.001 in each case. For the CD4 category, Cramér's V was 0.361, and for the WHO clinical stage, it was 0.447. Mean CD4 count was 88.43±32.86 cells/mm^3 in infected patients versus 114.18 ± 29.27 cells/mm^3 in uninfected patients (mean difference 25.75; 95% CI 15.81–35.69; t=5.12, df=151, p<0.001). We note that the p-value from the t-test (<0.001) is reported using the conventional threshold of 0.05. Given our stated threshold of <0.005, the result remains statistically significant under either threshold (Table 3).

Table 3: Association of Intestinal Parasitic Infection with Study Variables

Variables	Present, n (%)	Absent, n (%)	Test, Statistic	df	p-value	Cramér's V
Age Group (years)						
18–30 Years	23 (33.8%)	29 (34.1%)	χ^2 = 0.102	3	0.992	0.026
31–40 Years	26 (38.2%)	33 (38.8%)				
41–50 Years	14 (20.6%)	16 (18.8%)				
51–60 Years	5 (7.4%)	7 (8.2%)				
Gender						
Male	49 (72.1%)	65 (76.5%)	χ^2 = 0.387	1	0.534	0.005
Female	19 (27.9%)	20 (23.5%)				
Residence						
Rural	40 (58.8%)	46 (54.1%)	χ^2 = 0.340	1	0.56	0.047
Urban	28 (41.2%)	39 (45.9%)				
Educational Status						
Illiterate	34 (50.0%)	41 (48.2%)	χ^2 = 0.047	1	0.828	0.018
Literate	34 (50.0%)	44 (51.8%)				
CD4 Category						
<50 cells/mm^3	10 (14.7%)	0 (0.0%)	χ^2 = 19.94 (Fisher's exact p<0.001)	2	<0.001	0.361
50–100 cells/mm^3	31 (45.6%)	27 (31.8%)				
101–200 cells/mm^3	27 (39.7%)	58 (68.2%)				
WHO Clinical Stage						
Stage I	1 (1.5%)	6 (7.1%)	χ^2 = 30.57 (Fisher's exact p<0.001)	3	<0.001	0.447
Stage II	6 (8.8%)	28 (32.9%)				
Stage III	27 (39.7%)	40 (47.1%)				
Stage IV	34 (50.0%)	11 (12.9%)				

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In multivariable logistic regression, including age, gender, residence, educational status, CD4 count (continuous), and WHO clinical stage, CD4 count and WHO clinical stage were independently associated with intestinal parasitic infection after adjustment for age, gender, residence, and educational status. However, ART status, duration, and adherence were not assessed and therefore could not be included in the multivariable model. Each one cell/mm^3 increase in CD4 count was associated with a 3.2% reduction in the odds of infection (aOR 0.968, 95% CI 0.955–0.982, p<0.001), and each one-step advance in WHO clinical stage was associated with more than four-fold higher odds of infection (aOR 4.43, 95% CI 2.47–7.94, p<0.001). Age, gender, residence and educational status were not significant in the adjusted model (p>0.250 for all). Model fit (SPSS): Nagelkerke R^2=0.431; overall classification accuracy 76.5% (Table 4).

Table 4: Multivariable Binary Logistic Regression for the Presence of Any Intestinal Parasite (n=153)

Predictors	B (SE)	Wald	df	p-value	Exp(B)	95% CI for Exp(B)
Age (Per Year)	−0.002 (0.021)	0.008	1	0.930	0.998	0.957–1.041
Female Sex (Ref: Male)	+0.434 (0.466)	0.864	1	0.352	1.543	0.618–3.850
Rural Residence (Ref: Urban)	+0.465 (0.417)	1.247	1	0.264	1.593	0.704–3.605
Illiterate (Ref: Literate)	+0.097 (0.406)	0.057	1	0.811	1.102	0.497–2.444
CD4 Count (per cell/mm^3)	−0.032 (0.007)	19.912	1	<0.001	0.968	0.955–0.982
WHO Clinical Stage (Per Step)	+1.488 (0.298)	24.962	1	<0.001	4.427	2.470–7.936
Intercept	−1.826 (1.278)	2.042	1	0.153	0.161	—

DISCUSSION

In the present study, 68 of 153 (44.4%, 95% CI 36.8–52.4) HIV-positive individuals with acute diarrhoea had intestinal parasitic infections. These findings are supported by the pooled estimate of 40.24% found in a systematic review and meta-analysis of Ethiopian studies in people undergoing antiretroviral therapy and living with HIV, and fit the range of results reported in other resource-limited settings [8, 9]. A recent multi-centre study in Central Ethiopia reported a prevalence of 17.3% in patients attending antiretroviral therapy clinics, which is lower than our result, which can be due to the inclusion of symptomatic patients with active diarrhoea and severe immunosuppression in our study, unlike community-based ART cohorts, which include patients with different levels of immune recovery [3]. In studies conducted in Ghana and South Africa, comparable resource-limited settings have reported prevalence rates around 30%. The higher prevalence in our sample could be due to the advanced HIV disease condition of the individuals under study, with 43.8% in Stage III and 29.4% in Stage IV [11, 12]. The most common parasite found was *Cryptosporidium* spp. (30.1%) consistent with evidence from HIV-positive patients with diarrhoea in other resource-limited settings, where has been identified among the frequently detected intestinal pathogens [7, 15]. *Cryptosporidium* brings about a self-resolving disease in immunocompetent patients, yet in patients who have severe CD4 depletion, the disease causes chronic and life-threatening diarrhoea with intestinal malabsorption, wasting and lethality. The relatively high detection of *Cryptosporidium* in this study supports consideration of modified acid-fast staining as part of the diagnostic evaluation of HIV-positive patients with diarrhoea, particularly in resource-limited settings where molecular testing is unavailable. The second most common was *Entamoeba histolytica* (13.1%), a finding supported by Indian studies showing widespread intestinal amoebiasis. *Cystoisospora belli* (11.8%) and *Blastocystis* spp. (9.8%) were also detected at significant rates. *Strongyloides stercoralis* (3.9%) and *Trichuris trichiura* (3.3%) were less common but clinically relevant in the context of severe immunocompromise, where *Strongyloides* hyperinfection syndrome represents a potentially fatal complication [13, 14]. The variety of organisms observed in this case supports the need for comprehensive stool examination methods rather than relying on a single method. There was a statistically significant association between the CD4 cell count category and intestinal parasitic infection (χ^2 = 19.94, p<0.001). In patients with CD4 <50 cells/mm^3, all 10 (100%) had intestinal parasites, compared with 53.4% in the 50 to 100 cells/mm^3 group and 31.8% in the 101 to 200 cells/mm^3 group. A recent study from Peshawar involving adults with persistent or chronic diarrhoea also reported a higher parasitic burden among patients with lower CD4 counts; however, its population differed from the present study because it included persistent or chronic rather than acute diarrhoea [16]. According to Verma *et al.* HIV severity (number of CD4 cells) was a major predictor of the gastrointestinal pathogen burden of HIV-associated acute diarrhoea [18]. All these studies show the critical importance of cellular immunity in controlling intestinal parasitism and the relative vulnerability of patients with severe immunosuppression. WHO Clinical staging demonstrated a strong positive association with parasitic infection (χ^2=30.57, p<0.001). The infection rate increased from 14.3% in Stage I, 17.6% in Stage II, 40.3% in Stage III,

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and 75.6% in Stage IV. The observed stepwise increase in infection prevalence across WHO stages is consistent with greater vulnerability among patients with advanced HIV disease; however, the cross-sectional design precludes determining a causal relationship. These findings are supported by studies from Nigeria and South Africa, where patients with advanced clinical progression carried higher rates of parasitic co-infection [19, 20]. The strong relationship between clinical staging and infection also underscores its potential as an easily applicable surrogate measure of vulnerability to opportunistic intestinal parasites when CD4 counts are unavailable. On the contrary, demographic factors such as age group, gender, residential status, and educational level were not significantly associated with parasitic infection in this study. This outcome is supported by results from various sub-Saharan African environments in which immunological parameters, rather than sociodemographic factors, were identified as the main independent predictors of parasitic infection [8, 9]. The fact that our sample is predominantly male (74.5%) is not surprising, as this is the known epidemiological characteristic of HIV infection in Pakistan. The lack of association between education level and residence, although perhaps counter-intuitive given the influence that sanitation and understanding of hygiene are expected to have, may be an overriding result of extreme immunosuppression in defining infection vulnerability in this already susceptible group. WHO Clinical Stage (categorical, with Stage I as reference) was included in the model as a categorical variable using indicator coding. However, due to the small number of patients in Stage I (n=7), the model estimates for individual stages were unstable, with wide confidence intervals. As a result, the model was refit treating WHO stage as an ordinal variable (entered as a continuous linear term) after confirming the proportional odds assumption (test of parallel lines, p=0.71). This approach assumes a linear trend across stages, and the resulting odds ratio of 4.43 (95% CI 2.47–7.94) represents the increase in odds of infection for each one-stage advance. We acknowledge that treating ordinal data as continuous in logistic regression is statistically imperfect and may oversimplify the relationship; however, given the small sample size in early stages, this approach provides a parsimonious estimate of the stage-infection association. Sensitivity analyses using categorical coding (with Stages I-II combined as the reference group) yielded similar conclusions

This study has some limitations. The cross-sectional study design does not allow causal inferences about the relationships among CD4 count, WHO clinical stage, and parasitic infection. Non-probability consecutive sampling from a single tertiary care hospital can result in selection bias, and, where patients may differ in disease severity and healthcare access, where patients may be less immunosuppressed than in tertiary care. Molecular diagnostic techniques, including PCR-based assays, were not used and may have limited the detection of organisms that are difficult to identify reliably by conventional microscopy, particularly Microsporidia and some Cyclospora infections. Because most participants provided only a single stool specimen, intermittent or low-level parasite shedding may have resulted in under-detection of infection. The number of subjects in the CD4 <50 (n=10) and WHO Stage I (n=7) subgroups is small, and therefore, the estimates may be imprecise. Larger multicentre studies incorporating longitudinal follow-up and molecular diagnostic methods are warranted to confirm these associations and better characterise the burden of intestinal parasitic infections in this population.

CONCLUSIONS

Intestinal parasitic infections were frequently detected among HIV-positive adults with acute diarrhoea attending this tertiary-care hospital, with *Cryptosporidium* spp. being the most frequently detected organism. Lower CD4 count and more advanced WHO clinical stage were independently associated with intestinal parasitic infection after adjustment for selected demographic factors. However, these findings should be interpreted cautiously because of the cross-sectional, single-centre design, non-probability sampling, limited assessment of some potential confounders, including ART status, duration, and adherence, and the small number of patients with severe immunosuppression. The findings therefore should not be generalised to all HIV-positive patients with acute diarrhoea.

Authors' Contribution

Conceptualization: KAK
Methodology: KAK, MHB
Formal analysis: KAK
Writing and Drafting: KAK, MHB
Review and Editing: KAK, MHB

All authors approved the final manuscript and take responsibility for the integrity of the work

Conflict of Interest

All the authors declare no conflict of interest.

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