Prevalence of intestinal protozoan infections among individuals living with HIV/AIDS at Felegehiwot Referral Hospital, Bahir Dar, Ethiopia

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**SUMMARY**

**Background:** HIV infection continues to pose a serious challenge to global health by predisposing patients to opportunistic infections. A cross-sectional study was conducted from December 2012 to February 2013 to assess the enteric protozoan infection status among individuals with HIV/AIDS in Felegehiwot Referral Hospital, Bahir Dar, northwest Ethiopia.

**Methods:** Stool specimens from 399 HIV-positive individuals were examined for the presence of trophozoites, cysts, and oocysts using direct wet mount, formol–ether sedimentation and modified Zielhl–Neelsen techniques. In addition, CD4+ T-cell counts were measured to evaluate the immune status of the study subjects.

**Results:** An overall prevalence of 30.6% enteric protozoan infections was recorded. Pre-ART (antiretroviral treatment) individuals were more infected than patients on ART, although this was not statistically significant (p > 0.05). The highest prevalence of enteric protozoan infection was due to *Entamoeba histolytica*/E. dispar (19.3%), followed by *Cryptosporidium spp* (5.8%), *Giardia lamblia* (4.3%), and *Isospora belli* (1.3%). A CD4+ T-cell count of <200 cells/µL and status of being diarrhoeic were significantly associated with the overall prevalence of enteric protozoan infection.

**Conclusions:** A relatively high prevalence of enteric protozoan infection was observed among individuals living with HIV/AIDS. Routine stool and CD4+ T-cell examinations should be conducted to monitor the status of HIV/AIDS patients.

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1. Introduction

Opportunistic and non-opportunistic intestinal parasitic infections are commonly known for the manifestation and presentation of various degrees of diarrhoea in immune-compromised individuals. Diarrhoea occurs in about 90% of individuals living with HIV/AIDS in developing countries. The presence of opportunistic protozoan parasites such as *Cryptosporidium spp*, *Cyclospora cayetanensis*, *Isospora belli*, and *Microsporidia spp* have been documented in individuals living with HIV/AIDS. In particular, *Cryptosporidium spp* and *I. belli* are implicated in severe, persistent, and life-threatening diarrhoea, especially in HIV-positive and AIDS patients. Non-opportunistic parasites such as *Entamoeba histolytica* and *Giardia lamblia* are also important parasites in diarrhoeal disease that occur in individuals living with HIV/AIDS. The incidence and prevalence of infection with a particular enteric protozoan parasite in individuals living with HIV/AIDS is likely to depend upon the endemcity of that particular parasite in the community. In general, *Cryptosporidium spp*, *I. belli*, and *E. histolytica* have been reported as the most frequently identified parasites, and these represent a significant cause of diarrhoea in the HIV/AIDS population.

Since the introduction of antiretroviral therapy (ART) in Ethiopia, the quality of life and survival of HIV-positive individuals have improved. A considerable decline in mortality of adult AIDS patients was shown to be related to the start of ART in Addis Ababa, Ethiopia. Although there has been an improvement in the survival of patients, parasitic infections pose a serious challenge with regard to reducing the morbidity and mortality of these individuals.

A few studies have been carried out on the prevalence of enteric protozoan infections among individuals living with HIV/AIDS in relation to ART in Ethiopia. The current study was undertaken to assess the prevalence of enteric protozoan infections in relation to socio-demographic and clinical factors in individuals living with HIV/AIDS at Felegehiwot Referral Hospital, Bahir Dar, in the northwest of Ethiopia.

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2. Materials and methods

2.1. Sampling procedure

A cross-sectional study was conducted to identify and determine enteric protozoan infections among individuals living with HIV/AIDS. Study subjects were HIV-positive individuals who were ART-naive and those on ART, aged between 18 and 60 years. Patients included in the on-ART group had been treated with ART for at least 3 months. Participants were excluded from the study if they were out of the age range, pregnant, or had been on ART for less than 3 months.

A systematic random sampling method was used. Every other subject called upon to attend a regular check-up was included in the study until the required sample size was reached. Almost equal numbers of samples were taken from each group: 199 study subjects were pre-ART and the other 200 were on ART.

2.2. Stool examination

Study subjects were given labelled, leak-proof, clean sterile plastic screw-cap containers to collect their stool samples. Samples were collected from the participant on the day that the study questionnaire was completed. Stool samples were evaluated by direct microscopic examination, formol–ether concentration technique, and modified Ziehl–Neelsen acid-fast method.13

2.3. Immunological assessment

Blood samples were collected in heparinised vacutainer tubes (PLK2) for CD4+ T-cell count measurement; these were analyzed using a fully automated fluorescence activated cell sorting (FACS) counter (Becton and Dickinson, Immunocytometry Systems, San Jose, CA, USA) in the ART clinic of Felegehiwot Referral Hospital, Bahir Dar, Ethiopia.

2.4. World Health Organization (WHO) clinical staging of HIV/AIDS

Study subjects were first examined by a medical doctor and, based on the clinical signs and symptoms stated in WHO guidelines, they were classified into WHO clinical stages I, II, III, and IV.14

2.5. Questionnaire

A pre-structured questionnaire was used to collect socio-demographic information such as age, sex, location of residence, level of education, marital status, occupation and monthly income, and knowledge, attitude, perception and practice related to the use of antiretroviral (ARV) drugs of the study participants. Health practices and behavioural habits with regard to health conditions with a history of symptoms were recorded. Environmental conditions such as the water supply, provision of a sanitation system, swimming practices, and method of waste disposal were also recorded.

2.6. Data analysis

Data were analyzed using SPSS software version 16 (SPSS Inc., Chicago, IL, USA). Values were considered significant when the p-value was less than 0.05 with the respective 95% confidence intervals.

2.7. Ethical considerations

The study was approved on November 8, 2012 by an ethics committee consisting of five members from Bahir Dar University (record number RCS/869/2005), and permission was also obtained from Felegehiwot Referral Hospital. Informed consent was obtained from all participants before testing and commencing the study. Subject confidentiality and privacy were protected by ensuring that no names appeared on any part of the report.

3. Results

3.1. Socio-demographic and general characteristics of the study participants

A total of 399 individuals living with HIV/AIDS were recruited into this study. Out of the 399 study subjects, 50.13% (n = 200) had been on ART over different periods of time and were assigned to the on-ART group and 49.87% (n = 199) were not on ART and were assigned to the pre-ART group.

Female subjects outnumbered males: females 60.40% (n = 241) and males 39.60% (n = 158). The mean age of the study participants was 35 ± 8.63 years, ranging from 18 to 60 years, with the majority of the study population belonging to the age group 29–39 years (46.87%, n = 187). In terms of location of residence, the majority were urban dwellers: 79.70% (n = 318) lived in urban areas and 20.30% (n = 81) in rural areas.

The mean monthly household income of the total study group was USS 54.00 ± 43.50, with a minimum income of USS 5.30 and maximum of USS 325.80. Out of the total study participants, the majority 39.1% (n = 156) of households earned between USS 26.90 and USS 53.80 monthly and 31.3% (n = 125) earned less than USS 26.90 per month. Seventy-nine (19.8%) participants had a monthly income between USS 53.80 and USS 107.50 and the remaining 9.8% (n = 39) of study subjects earned more than USS 107.50 per month.

3.2. Prevalence of enteric protozoan infections based on socio-demographic factors

Infection rates of 8.8% for E. histolytica/E. dispar, 3.5% for Cryptosporidium spp, 2.8% for G. lamblia, and 0.25% for I. belli were found (Table 1). Stool examinations revealed that 27.8% (44/158) of males and 27.8% (67/241) of females were infected with the enteric protozoan parasites. With regard to age, the infection rate appeared to be higher within the age group of 29–39 years (29.4%, 55/187) compared to the other age groups. In terms of location of residence, 26.7% (85/318) of the urban residents and 32.1% (26/81) of the rural dwellers were infected with enteric protozoan parasites.

Across occupational groups, the prevalence was 22.8% (13/57) among the unemployed, 22.2% (14/63) among casual labourers, 30.7% (27/88) among government employees, 30.3% (37/122) among the self-employed, and 40% (4/10) among students. Thirty-six percent (8/22) of peasant farmers and 21.6% (8/37) of privately employed study subjects had the infection.

3.3. Prevalence of enteric protozoan infections based on clinical status

Of the 399 persons examined for enteric protozoa, 122 were found to be infected with at least one of the enteric protozoa identified, with an overall prevalence of 30.6%. The results of the stool sample analysis showed that the main enteric protozoan parasites identified among the study participants in order of frequency were E. histolytica/E. dispar, Cryptosporidium spp, G. lamblia, and I. belli. The highest prevalence was due to E. histolytica/E. dispar (19.3%, 77/399), followed by Cryptosporidium spp (5.8%, 23/399) and G. lamblia (4.3%, 17/399); the lowest infection rate was due to I. belli (1.3%, 5/399) (Table 2).
Among 122 positive samples, a single infection was identified in 83.6% (102/122). Mixed infection with E. histolytica/E. dispers and Cryptosporidium spp was found in 4.1% (5/122). Infection with both E. histolytica/E. dispers and G. lamblia was found in 1.6% (2/122), and infection with E. histolytica/E. dispers and I. belli in 0.8% (1/122). Triple infection with E. histolytica/E. dispers, G. lamblia, and Cryptosporidium spp was found in 0.8%.

The overall prevalence of enteric protozoan infections in pre-ART subjects was 35.7% (71/199); the prevalence of E. histolytica/ E. dispers, G. lamblia, Cryptosporidium spp, and I. belli in these patients was 19.6% (39/199), 5.03% (10/199), 8.54% (17/199), and 2.5% (5/199), respectively. The most prevalent enteric protozoan infections in pre-ART patients were E. histolytica/E. dispers and Cryptosporidium spp.

With regard to the on-ART subjects, the overall prevalence of infection was 25.5% (51/200), with 19% (38/200), 3.5% (7/200), and 3% (6/200) due to E. histolytica/E. dispers, G. lamblia, and Cryptosporidium spp, respectively. I. belli was not identified in any on-ART patient in this study.

Relatively similar numbers of cases of infection with E. histolytica/E. dispers and G. lamblia were observed in the pre-ART and on-ART subjects. However, high rates of infection with cryptosporidiosis and isosporiasis were seen in the pre-ART subjects. A statistically significant difference was observed in the prevalence of cryptosporidiosis and isosporiasis between pre-ART and on-ART study participants (Table 2).

The overall infection rate with parasitic protozoa was higher in pre-ART participants than in on-ART participants. However, there was no significant association (p > 0.05) between the overall infection rate and ART status. Based on the diarrhoeal status, a higher prevalence of infection was identified among diarrhoeic patients than non-diarrhoeic ones. A significant association (p < 0.05) was observed between the prevalence of enteric protozoan infection and being diarrhoeic among study subjects. With regard to the WHO clinical stage of the patients, most were in stage III (49.1%, 196/399), followed by stage II (28.8%, 115/399), stage I (19.3%, 77/399), and stage IV (2.8%, 11/399) (Table 3). The presence of associated opportunistic infections was seen in only 28 (7.02%) HIV patients out of the total study subjects.

The effects of socio-demographic, clinical, and sanitation factors on the prevalence of cryptosporidiosis and giardiasis infection among individuals living with HIV/AIDS were assessed, and these were found to be determinants of the occurrence of these infections. There was a statistically significant association (p < 0.05) between the prevalence of cryptosporidiosis and ART status, CD4+ T-cell status, diarrhoea, work status, and access to a toilet. With regard to giardiasis, this was shown to be significantly associated with diarrhoea and residential location. Thus, the results further revealed location of residence to be independently associated (p < 0.05) with the prevalence of giardiasis (Table 5).

The enteric protozoan infection status with respect to the CD4+ T-cell count was variable among the study participants and there was an association between CD4+ T-cell count and the opportunistic coccidian parasite among patients on and not on ART. The maximum parasitic infection was 50.7% (34/67) in patients with a CD4+ T-cell count of <200 cells/µL, and was 25.4% (60/236) in patients whose CD4+ T-cell count was between 200 and 500 cells/µL and 17.7% (17/96) in patients whose CD4+ T-cell count was >500 cells/µL. Infection with cryptosporidiosis and isosporiasis was highly associated (p < 0.05) with a low CD4+ T-cell count (<200 cells/µL) (Figure 1).

The CD4+ T-cell counts of the study participants ranged from as low as 3 to 1274 cells/µL, with a median of 358 cells/µL and mean of 381.73 ± 202.62 cells/µL. HIV patients included in this study predominantly had a CD4+ T-cell count >200 cells/µL (83.2%, 332/399). The majority had a CD4+ T-cell count ranging between 200 and 400 cells/µL (71.4%, 284/399).

### Table 1
The prevalence of enteric protozoan infections according to sex, age, location of residence, and level of education; Felegehiwot Referral Hospital, Bahir Dar 2012–2013

| Variables          | Non-infected | Type of enteric protozoan infection (%) | Total positivity (N=399) |
|--------------------|--------------|----------------------------------------|--------------------------|
|                    | EHI          | GLA                                   | CRY                      | ISO                      |
| Sex                |              |                                        |                          |                          |
| Male               | 114          | 27 (6.8)                              | 6 (1.5)                  | 12 (3)                   | 3 (0.75)                 | 48 (12)                  |
| Female             | 174          | 50 (12.5)                             | 11 (2.75)                | 11 (2.75)                | 2 (0.5)                  | 74 (18.5)                |
| Age, years         |              |                                        |                          |                          |                          |                          |
| 18–28              | 72           | 21 (5.3)                              | 3 (0.75)                 | 1 (0.25)                 | 4 (1)                    | 29 (7.3)                 |
| 29–39              | 132          | 35 (8.8)                              | 1 (0.25)                 | 14 (3.5)                 | 1 (0.25)                 | 61 (15.3)                |
| 40–50              | 65           | 16 (4)                                | 4 (1)                    | 4 (1)                    | 1 (0.25)                 | 25 (6.3)                 |
| 51–60              | 19           | 3 (1.3)                               | 1 (0.25)                 | 1 (0.25)                 | 0 (0)                    | 7 (1.75)                 |
| Residence          |              |                                        |                          |                          |                          |                          |
| Rural              | 55           | 13 (3.3)                              | 8 (2)                    | 2 (0.5)                  | 31 (7.8)                 |                          |
| Urban              | 233          | 64 (16)                               | 9 (2.5)                  | 15 (3.8)                 | 3 (0.75)                 | 91 (22.8)                |
| Level of education |              |                                        |                          |                          |                          |                          |
| No education       | 94           | 21 (5.3)                              | 4 (1)                    | 5 (1.3)                  | 1 (0.25)                 | 31 (7.8)                 |
| Informal           | 17           | 3 (0.75)                              | 2 (0.5)                  | 2 (0.5)                  | 3 (0.75)                 | 10 (2.5)                 |
| Primary            | 66           | 19 (4.8)                              | 3 (0.75)                 | 9 (2.3)                  | 0 (0)                    | 31 (7.8)                 |
| Secondary          | 77           | 22 (5.5)                              | 6 (1.5)                  | 6 (1.5)                  | 0 (0)                    | 34 (8.5)                 |
| Tertiary           | 34           | 12 (3)                                | 2 (0.5)                  | 1 (0.25)                 | 1 (0.25)                 | 16 (4)                   |

EHI, Entamoeba histolytica/E. dispers; GLA, Giardia lamblia; CRY, Cryptosporidium spp; ISO, Isospora belli.

### Table 2
Distribution of enteric protozoan parasites in pre-ART and on-ART study subjects; Felegehiwot Referral Hospital

| Enteric parasite                  | ART status          | Chi-square | df | p-Value |
|-----------------------------------|---------------------|------------|----|---------|
| E. histolytica/E. dispers         | Pre-ART (n = 199)   | 39 (50.6%) | 0.02 | 1 | 0.880 |
|                                  | On-ART (n = 200)    | 38 (49.4%) | 0.07 | 1 | 0.451 |
| G. lamblia                        | Pre-ART (n = 17)    | 10 (58.8%) | 5.64 | 1 | 0.018* |
|                                  | On-ART (n = 17)     | 7 (41.2%)  | 0.09 | 1 | 0.024* |
| Cryptosporidium spp (n = 23)     | Pre-ART (n = 5)     | 5 (100%)   | 0.09 | 1 | 0.024* |
|                                  | On-ART (n = 200)    | 0 (0%)     | 0.09 | 1 | 0.024* |
| I. belli                          | Pre-ART (n = 17)    | 10 (58.8%) | 5.64 | 1 | 0.018* |
|                                  | On-ART (n = 17)     | 7 (41.2%)  | 0.09 | 1 | 0.024* |
| Total                             | Pre-ART (n = 199)   | 71 (35.7%) | 51 (25.3%) | 122/399 (30.6%) |
| Overall infection both in pre- and on-ART |

ART, antiretroviral therapy.

* Statistically significant, Chi-square test.
Table 3
Prevalence of enteric protozoan infection in relation to the clinical profile of the study subjects; Felegehiwot Referral Hospital

| Characteristics          | Status of infection, n (%) | Total examined, n (%) | Chi-square | df | p-Value |
|--------------------------|---------------------------|-----------------------|------------|----|---------|
|                         | Infected                  | Not infected          |             |    |         |
| ART status               |                           |                       |             |    |         |
| Pre-ART                  | 71 (35.7)                 | 128 (64.3)            | 199 (49.9) | 1  | 0.208   |
| On-ART                   | 51 (25.5)                 | 149 (74.5)            | 200 (50.1) | 1  |         |
| CD4+ T-cell count/µL     |                           |                       |             |    |         |
| <200                     | 34 (50.7)                 | 33 (49.3)             | 67 (16.8)  | 2  | 0.000*  |
| 200–500                  | 60 (25.4)                 | 176 (74.6)            | 236 (39.1) |    |         |
| >500                     | 17 (17.7)                 | 79 (82.3)             | 96 (24.1)  |    |         |
| Diarrhoeal status        |                           |                       |             |    |         |
| Yes                      | 31 (52.5)                 | 28 (47.5)             | 59 (14.8)  | 1  | 0.000*  |
| No                       | 80 (23.5)                 | 260 (76.5)            | 340 (85.2) |    |         |
| WHO stages of HIV infection |                          |                       |             |    |         |
| I                        | 26 (33.8)                 | 51 (66.2)             | 77 (19.3)  | 3  | 0.311   |
| II                       | 26 (22.6)                 | 89 (77.4)             | 115 (28.8) |    |         |
| III                      | 57 (29.1)                 | 139 (70.9)            | 196 (49.1) |    |         |
| IV                       | 2 (18.2)                  | 9 (81.8)              | 11 (2.8)   |    |         |

ART, antiretroviral therapy; WHO, World Health Organization.

* Statistically significant.

Figure 1. Status of enteric protozoan infection with reference to CD4+ T-cell count.

500 cells/µL (59.1%, 236/399), of whom more than half (52.1%, 123/236) were on ART and just under half (47.9%, 113/236) were pre-ART. Approximately 24% (96/399) of the study subjects had a CD4+ T-cell count >500 cells/µL, 49% (47/96) of whom were on ART and 51% (49/96) pre-ART (Table 4). Sixty-seven patients (16.8%) had a CD4+ T-cell count of <200 cells/µL, with 44.8% (30/67) on ART and 55.2% (37/67) pre-ART (Figure 2).

3.4. Univariate analysis for the association of the prevalence of enteric protozoan infection in relation to the clinical profile and selected socio-demographic factors

In the current study, based on univariate analysis, two factors were found to be associated with intestinal protozoan infection among HIV/AIDS patients: study subjects with a CD4+ T-cell count <200 cells/µL and those with a positive diarrhoeal status had a higher infection rate. The univariate analysis showed that patients with a CD4+ T-cell count of <200 cells/µL were at increased risk of enteric protozoan infection (odds ratio (OR) 4.79, 95% confidence interval (CI) 2.35–9.74, p = 0.000). Patients with a CD4+ T-cell count <200 cells/µL were approximately five times more likely to have an enteric protozoan infection than those with higher CD4+ T-cell counts (>500 cells/µL).

The prevalence of enteric protozoan infection among study subjects with diarrhoea was 52.5%, whereas it was 47.5% in non-diarrhoeic patients. There was an association between the presence of enteric protozoan infections and diarrhoea (OR 3.60, 95% CI 2.04–6.38, p = 0.000). The odds of having an enteric protozoan infection were 3.6 times higher in HIV-infected patients with diarrhoea than in those who were non-diarrhoeic.

In the univariate analysis, CD4+ T-cell count and diarrhoeal status were associated with the overall prevalence of enteric protozoan infections. However, other socio-clinical, environmental, and sanitation variables had no significant association with the prevalence of enteric protozoan infections among people living with HIV/AIDS, both pre-ART and on-ART subjects.

3.5. Univariate analysis for the association of the prevalence of cryptosporidiosis and giardiasis with socio-clinical and sanitation variables

The results showed a higher Cryptosporidium spp infection in those patients who had a CD4+ T-cell count of <200 cells/µL. The odds of a patient with a CD4+ T-cell count of <200 cells/µL being infected with Cryptosporidium spp were about 15 times those of a

Table 4
Univariate association between enteric protozoal infection and clinical profiles of study subjects

| Characteristics          | Infected, n (%) | Not infected, n (%) | Total examined, n (%) | OR (95% Cl) |
|--------------------------|----------------|--------------------|----------------------|-------------|
| ART-status               |                |                    |                      |             |
| Pre-ART                  | 71 (35.7)      | 128 (64.3)         | 199 (49.9)           | 0.75 (0.49–1.17) |
| On-ART                   | 51 (25.5)      | 149 (74.5)         | 200 (50.1)           | 1.00*       |
| CD4+ T-cell count/µL     |                |                    |                      |             |
| <200                     | 34 (50.7)      | 33 (49.3)          | 67 (16.8)            | 4.79 (2.35–9.74)* |
| 200–500                  | 60 (25.4)      | 176 (74.6)         | 236 (39.1)           | 1.58 (0.87–2.89) |
| >500                     | 17 (17.7)      | 79 (82.3)          | 96 (24.1)            | 1.00*       |
| Diarrhoeal status        |                |                    |                      |             |
| Yes                      | 31 (52.5)      | 28 (47.5)          | 59 (14.8)            | 3.60 (2.04–6.38)* |
| No                       | 80 (23.5)      | 260 (76.5)         | 340 (85.2)           | 1.00*       |

OR, odds ratio; CI, confidence interval; ART, antiretroviral therapy.

* Reference category.
* Statistically significant.
patient with a CD4+ T-cell count >500 cells/μl (OR 15.16, 95% CI 4.3–53.3, p = 0.000). The pattern of Cryptosporidium spp infection in this study was highly associated among the diarrhoea status. The odds of having cryptosporidiosis were 3.4 times higher in patients with diarrhoea than in non-diarrhoeic individuals (OR 3.40, 95% CI 1.40–8.42, p = 0.008).

The present study, however, could not rule out access to a toilet as a potential risk factor for infection with cryptosporidiosis in the study population. Respondents who had no access to a toilet were about four times more likely to harbour Cryptosporidium spp compared to those respondents who had access to a toilet (OR 4.40, 95% CI 1.70–11.44, p = 0.002).

Giardiasis was significantly associated with the location of residence of the study participants. The odds of being infected with G. lamblia were almost four times higher in patients who resided in rural areas than in those who lived in urban areas (OR 3.76, 95% CI 1.04–10.08, p = 0.008). In the current study, the prevalence of giardiasis was highly associated with the diarrhoea status. The odds of the occurrence of giardiasis were 3.4 times higher in patients with diarrhoea than in those without diarrhoea (OR 3.40, 95% CI 1.20–9.04, p = 0.021) (Table 5).

### 3.6. Multivariate analysis

Multivariate analysis using logistic regression confirmed CD4+ T-cell count <200 cells/μl (adjusted OR (aOR) 3.62, 95% CI 1.73–7.57, p = 0.001) and being diarrhoeic (aOR 2.74, 95% CI 1.5–5.02, p = 0.001) as significant risk factors for intestinal protozoan infection among HIV/AIDS patients. However, when the influence of other confounders was taken into account, the contribution of ART status was no longer evident as a predictor for the prevalence of enteric protozoan infection among study subjects.

With regard to Cryptosporidium spp infection, multiple logistic regression analysis indicated that the variable CD4+ T-cell count <200 cells/μl (aOR 7.8, 95% CI 2.73–22.33, p = 0.000) maintained the significant association with Cryptosporidium spp infection that was identified previously in the univariate analysis. However, the previously identified contributions of ART status, being diarrhoeic, being a rural dweller, and having access to a toilet were no longer apparent.

Furthermore, in the multiple logistic regression analysis, location of residence (aOR 3.86, 95% CI 1.42–10.07, p = 0.008) and diarrhoea (aOR 3.50, 95% CI 1.22–10.07, p = 0.020) retained their positive association as predictors of giardiasis (Table 5).

### 4. Discussion

In this study, the prevalence of intestinal protozoan infections among HIV-seropositive individuals with different immune status was investigated. The examination of stool samples collected from study subjects revealed an overall prevalence of 30.6% (122/399) intestinal protozoan parasites; 71 cases (35.7%) were pre-ART patients and 51 (25.5%) were on-ART patients (Table 2).

Due to down-regulation of the immune system, numerous opportunistic infections with Cryptosporidium spp and I. belli occur in individuals living with HIV/AIDS. Non-opportunistic parasites such as E. histolytica/E. dispar and G. lamblia are also encountered frequently in individuals living with HIV/AIDS, but they are not considered opportunistic parasites.

The findings of this study also showed an indiscriminate prevalence of enteric protozoan infection among patients by age category, sex, level of education, occupation, work status, and other factors measured. Co-infections of coccidian parasites with other non-opportunistic intestinal protozoa (E. histolytica/E. dispers and G. lamblia) were encountered in this study. This is in agreement with results from Nigeria.

This study revealed that the prevalence of enteric coccidian infection was higher among pre-ART patients than on-ART patients. HIV subjects who had received ART had lower rates of opportunistic infection compared to those who had not received this therapy. This could be due to the fact that on-ART patients are administered ARVs, in contrast to pre-ART patients, who are not given ARVs. This is in agreement with a previous study that showed the use of ART to be associated with parasite clearance even when the patients responded poorly to ART in terms of the CD4+ T-cell count. Furthermore, it has been suggested that HIV protease inhibitors can act as antiparasitic drugs. For example, in experimental studies, the drugs indinavir, saquinavir, and ritonavir have been reported to have anti-Cryptosporidium spp effects both in vitro and in vivo.

In the present study, the overall prevalence of Cryptosporidium spp infection among individuals living with HIV/AIDS was 5.8%, which is similar to the prevalence reported previously in

![Figure 2. Evaluation of CD4+ T-cell count across ART status of study subjects.](image-url)
Ethiopia.\(^{11}\) However, it is lower than that of a previous report.\(^{20}\) ART-naïve HIV patients (8.5%) appear to be more vulnerable to Cryptosporidium spp infection than patients on ART (3%). This finding is in concordance with a relatively recent study undertaken in Dessie Hospital among pre-and on-ART patients that reported none of the on-ART patients to have a Cryptosporidium spp infection.\(^{21}\)

In the current study, with the exception of one case, the prevalence of cryptosporidiosis was significantly higher among those with a CD4+ T-cell count of <200 cells/µL. This finding is consistent with a previous report from Cameroon, which showed a significantly higher prevalence of cryptosporidial infection among patients with a CD4+ T-cell count of < 50 cells/µL.\(^{22}\) An association of enteric coccidian parasites with lower CD4+ T-cell counts has also been reported from India.\(^{23}\)

*I. belli* is another enteric coccidian parasite that was detected among HIV-positive persons. The prevalence of infection with *I. belli* in the present study was 1.3%, which is lower than the 15.5% reported from northwest Ethiopia.\(^{23}\) *I. belli* (1.3%) was detected only in pre-ART study subjects; no such infection was detected in patients on ART. *I. belli* was more common in patients with a CD4+ T-cell count of <200 cells/µL and the association between the CD4+ T-cell count and *I. belli* infection was significant (p < 0.05). A similar previous study from India reported a higher prevalence of *I. belli* in patients with a CD4+ T-cell count of <200 cells/µL.\(^{24}\) The low prevalence and variations of infection with *I. belli* could be due to the wide use of anti-opportunistic infection medications, ART, and geographic differences. Furthermore, it has been suggested that co-trimoxazole prophylaxis given for other infections in AIDS cases and the low number of oocytes excreted are possible explanations for the observed low prevalence of *I. belli* infection.\(^{25}\)

In the present study, the most prevalent parasite detected in individuals living with HIV/AIDS was *E. histolytica/E. dispar*. The overall prevalence of *E. histolytica/E. dispar* in this study accounted for 19.3% (77/399). This is in agreement with previous reports (20%) among HIV/AIDS patients in Gambi Higher Clinic, Bahir Dar, Ethiopia.\(^{26}\) Furthermore, the present finding is comparable with the prevalences of 25.3%, 16.8%, and 15% reported from Mexico, Malaysia, and India, respectively.\(^{26–28}\) The present finding, however, was relatively higher (19.3%) compared to the prevalence rate of 10.3% observed among individuals living with HIV/AIDS in a teaching hospital in Jimma, southwest Ethiopia.\(^{29}\)

The prevalence of giardiasis in the present study was 4.3%. This finding is in concordance with data from Jimma Teaching Hospital, southwest Ethiopia (3.8%).\(^{29}\) Findings from the current study showed that infection with giardiasis was considerably lower when compared to other studies carried out among individuals living with HIV/AIDS. A higher infection rate, 16%, was reported in Ethiopia in selected ART centres in Addama, Afar, and Dire-Dawa.\(^{11}\) A higher rate of infection has also been reported from Hawassa Teaching and Referral Hospital, southern Ethiopia (11.2%).\(^{30}\) A considerably higher prevalence of giardiasis compared to the present finding has also been observed in previous works performed in other tropical regions, such as 14.9% from a study conducted at a mission hospital in tropical West Africa\(^{31}\) and 8% from India.\(^{31}\)

Mixed infection was the other observation found in the present study. The combined prevalence of both double and triple infection accounted for 2.3%. The multiple infection rate in the current study was, however, relatively lower than that reported previously in Ethiopia (27.2%).\(^{29}\)

In the current study, 14.8% (59/399) of study subjects were identified with diarrhoea. On multivariate analysis, diarrhoea was found to be more frequent among HIV/AIDS study subjects with cryptosporidiosis, isosporiasis, amoebiasis, and giardiasis compared to those patients without diarrhoea. This association is in agreement with other earlier reports that indicated an association between the occurrence of diarrhoea and enteric protozoa infections among individuals living with HIV/AIDS. This association has been reported in different parts of tropical countries.\(^{30,32}\)

This study revealed the CD4+ T-cell status to be one of the clinical determinants of enteric protozoan infection in patients with a CD4+ T-cell count of <200 cells/µL. The infection was considerably higher in patients with a CD4+ T-cell count of <200 cells/µL. After adjusting for confounders, HIV-infected patients who had a CD4+ T-cell count of <200 cell/µL were at about four times greater risk (aOR 3.62, 95% CI 1.73–7.57, p = 0.001) of having an enteric protozoan infection compared to those who had a CD4+ T-cell count > 500 cells/µL. The association between the CD4+ T-cell count and intestinal protozoa infection has also been reported in previous studies from Ethiopia, India, Malaysia, and Senegal.\(^{30,27,28,33}\)

Most of the socio-demographic features in the present study were not statistically significant as predictors of the manifestations of enteric protozoan infection among individuals living with HIV/AIDS. Demographic variables such as age, sex, location of residence, marital status, work status, monthly income, and level of education were not implicated as determinants of the occurrence of enteric protozoal infection among the study subjects.

In conclusion, the overall prevalence of enteric protozoan infection in individuals living with HIV/AIDS was 30.6%. *E. histolytica* infection was the most prevalent, followed by Cryptosporidium spp, G. lamblia, and *I. belli* infections. The rate of infection with *Cryptosporidium spp* and *Isospora belli* was found to be higher in pre-ART patients than in on-ART patients.

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