Cryptosporidiosis and Other Intestinal Parasitic Infections and Concomitant Threats Among HIV-Infected Children in Southern Ethiopia Receiving First-Line Antiretroviral Therapy

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Background: Children infected with human immunodeficiency virus (HIV) are at high risk of acquiring intestinal parasitic infections. This study aimed to determine the magnitude of Cryptosporidium and other intestinal parasitic infections and concomitant threats among HIV-infected children.

Methods: A hospital-based cross-sectional study was carried out at three antiretroviral therapy clinics in southern Ethiopia from February 2016 to June 2017 in 384 HIV positive children. Socio-demographic and clinical data were collected using structured questionnaires. Direct stool microscopic examination and modified Ziehl–Neelsen staining technique to identify parasites. Chi-square test was conducted to determine the real predictors of the infection. Significant association was considered when p-value <0.05 at 95% CI.

Results: The overall magnitude of intestinal parasitic infections among the study population was 16.9% (95% CI: 13.0–20.8%). The most predominant parasitic infections were Cryptosporidium spp. (9.6%) and the least was Taenia spp. (0.78%). Diarrheal status ($\chi^2=7.653$, df=2, p=0.022) was detected to be the only significant associated variable.

Conclusion: Cryptosporidium infection was found to be the most common intestinal parasitosis among HIV-infected children. Routine screening service for Cryptosporidium and other intestinal parasites is important in the clinical management of HIV-infected children.

Keywords: intestinal parasites, Cryptosporidium, HIV/AIDS, Southern Ethiopia

Background
Cryptosporidiosis is a diarrheal disease caused by the water-borne parasite, Cryptosporidium. The predominant symptom of cryptosporidiosis is watery diarrhea. In people with immunocompetent, the infection typically lasts approximately 2 weeks. However, those with decreased or compromised immune systems are at greater risk for developing serious, chronic diarrhea.1

Human immunodeficiency virus (HIV) was the most common community-acquired infection among approximately 2.1 million children under 15 years old globally in 2016.2 In people with HIV-infection, Cryptosporidium is an opportunistic infection and is considered an AIDS-defining illness.3 In addition to Cryptosporidium, other intestinal parasites are associated with both acute and chronic diarrhea which reduces the quality of life among HIV-infected patients.4
In developing countries, cryptosporidiosis is prevalent and has been demonstrated as high as 45% among children with associated impaired growth, malnutrition, and immune suppression. The lack of clean and potable water, poor hygiene, and unsanitary environmental conditions are generally responsible for the prevalence of intestinal parasites in most African nations. Parasites such as Cryptosporidium spp., Isospora belli, Giardia duodenalis, Entamoeba spp., Cyclospora cayetanensis, Blastocystis spp., Strongyloides stercoralis, and Ascaris lumbricoides are the most commonly reported among HIV-infected patients.

Children in emerging nations such as Africa, Asia, and Latin America are especially susceptible to chronic parasitic infections because they are immunologically naïve and often malnourished. Cryptosporidiosis has been shown to play a fundamental role in infantile malnourishment in unindustrialized nations, and has been associated with diminished growth in late childhood. Combination antiretroviral therapy (cART) has been found to improve physical, cognitive and nutritional outcomes among HIV-infected children and adolescents. Cryptosporidiosis among HIV-infected children while on cART may compromise the effectiveness of the regimen.

In a study in Gambian children, those consumed stored water, presence of large family size and had contact with animal are at risk of Cryptosporidium infection. Another study from Cameroon in under five children the presence of diarrhea was reported as concomitant treat for Cryptosporidium infection. In addition to this factors undernutrition, having previous hospitalization, older age and living in a day care institution was statically significant associated risk factors reported from Colombian children with HIV-infection. Therefore, this study evaluates the degree of opportunistic Cryptosporidium and other intestinal parasitic infections (IPIs) among children receiving cART and concomitant threat.

**Materials And Methods**

**Study Design, Area And Period**

A cross-sectional study of intestinal parasitic infection in HIV-infected children conducted in three public hospitals (Hawassa University Comprehensive Specialized Hospital (HUCSH), Adare General Hospital (AGH) and Wolyita Sodo Referral Hospital (WSRH)) in southern Ethiopia from February 2016 to June 2017. The hospitals are located in one of the administrative regions, the Southern Nations, Nationalities and Peoples’ Regional State of Ethiopia.

**Study Populations**

The study population was HIV-infected children who had regular follow-up visits at the HIV/ART clinic of each hospitals included for this study in one of their visit. Children who had received anti-parasitic treatment within a month prior to the time of data collection were excluded from the study. A total of 384 children (211 male and 173 female) met the inclusion criteria and were enrolled in the study. There were 217 children from HUCSH, 108 children from WSRH, and 59 children from AGH.

**Data Collection**

Socio-demographics, existing symptoms, and current or previous history of diarrhoea were collected using structured questionnaires. Diarrhoea was defined as a minimum of three watery or loose stools within 24 hrs. Diarrhoea lasting for 14 days was defined as chronic diarrhoea, and diarrhoea that lasted for fewer than 14 days was categorized as acute diarrhoea (Annex).

**Laboratory Diagnosis**

A single stool specimen approximately 2mg was collected from each participant using screw caped plastic container on the same day of the interview. Unconcentrated stool samples were examined using direct microscopy (normal saline and iodine preparation) techniques to identify the presence of trophozoite/cyst, larvae, or ova of intestinal parasites. The modified Zeihl–Neelsen staining technique was performed to visualize Oocysts of intestinal coccidian parasites as described elsewhere. Four milliliter (4mL) blood sample was collected using ethylene diamine tetra acetic acid (EDTA) to determine the CD4 T cell count using fluorescence-activated cell sorting (FACS) machine based on flow cytometry principle. All the samples were processed at the study sites with the close supervision of principal investigator.

**Data Analysis**

Data entry and analysis was conducted with SPSS Version 20. Mean, range, and proportions were calculated to summarize data as appropriate. Variances among magnitudes were assessed using Pearson’s Chi-square test ($\chi^2$), and a P-value <0.05 was considered significant.
Ethical Consideration
The Institutional Review Board of the College of Medicine and Health Sciences, Hawassa University approved the study. Involvement was voluntary and informed written consent was obtained from caregivers of the study participants. Physicians managed those found to be infected with any pathogenic intestinal parasites.

Results
Sociodemographic Characteristics Of The Study Subjects
A total of 384 children (211 male and 173 female) were included for the study. The children were grouped by age: 1–6 years old (33), 7–12 years old (161) and 13–18 (190). Of those tested, 54.9% (211) were male and 87.2% (335) were urban residents. Most of their mothers had completed primary school 66.9% (257). Variables considered were the age and sex of the child as well as the educational background of the child’s mother as the most likely caregiver. The living environment (urban/rural) as well as source of drinking water and regular contact with animals was considered. However, there were no statical significantly associated variables for Cryptosporidium as well as to other intestinal parasites (Table 1).

Magnitude Of Intestinal Parasites
The overall intestinal parasitic infections rate was 16.9% (95% CI: 13.0–20.8%). Cryptosporidium was the most frequently identified 37 (9.6%) parasite, followed by G. duodenalis 11 (2.8%), E. histolytica/dispar 19 (2.3%), A. lumbricoides 7 (1.8%) and Taenia spp. 3 (0.8%) (Figure 1). Mixed parasites were observed in two cases that are Cryptosporidium occurred with G. duodenalis (1%), E. histolytica/dispar (1%) and A. lumbricoides (1%) as co-infection.

Associated Risk Factors
The degree of diarrhea (absent/acute/chronic) and CD4 cells/µL levels were tracked from blood sample. There is a correlation between the source of drinking water and contact with animals with the degree of diarrhea, intestinal parasites and CD4 levels. Those children who reported drinking piped water (91.7%) and had no contact with animals (76%) were highly likely to report no diarrhea (81.8%). They had CD4 levels >500 cells/µL (64.8%).

Table 1 Socio-Demographic Characteristics Of Study Subjects For The Study Of Cryptosporidium And Other Intestinal Parasites Among HIV Positive Children In Southern Ethiopia

| Variables                  | Frequency | Presence Of Any Parasite | Presence Of Cryptosporidium | χ², df, (p-value) | χ², df, (p-value) |
|----------------------------|-----------|--------------------------|-----------------------------|------------------|------------------|
|                            | No. | %   | No. | (%) | χ² | df | p-value | χ² | df | p-value |
| Age groups years           |     |     |     |     |     |     |         |     |     |         |
| 1–6                       | 33  | 8.6 | 6   | 18.3| 0.3882 | (0.824)| 37 | 2 | 6.1 | 1.7592 | (0.415) |
| 7–12                      | 161 | 41.9| 25  | 15.5| 0.0381 | (0.845)| 18 | 8.5 | 11.0 | 0.6561 | (0.418) |
| 13–18                     | 190 | 49.5| 34  | 17.9| 10.9552 | (0.141)| 7  | 9.2 | 7.5  | 0.3732 | (0.830) |
| Sex                       |     |     |     |     |     |     |         |     |     |         |
| Male                      | 211 | 54.9| 35  | 16.6| 1.0572 | (0.590)| 2  | 12.5| 8.6  | 1.0422 | (0.594) |
| Female                    | 173 | 45.1| 30  | 17.3| 0.4055 | (0.528)| 26 | 10.2| 11.7 | 0.0211 | (0.885) |
| Mother’s occupation        |     |     |     |     |     |     |         |     |     |         |
| Government employee       | 76  | 19.8| 12  | 15.8| 0.1057 | (0.771)| 4  | 7.5  | 7.5  | 0.0211 | (0.885) |
| NGO                       | 53  | 13.8| 11  | 20.8| 0.9922 | (0.318)| 13 | 11.7 | 11.7 | 0.0211 | (0.885) |
| Others                    | 213 | 66.4| 42  | 16.5| 0.0022 | (0.969)| 5  | 10.2 | 9.6  | 0.0211 | (0.885) |
| Mother’s education         |     |     |     |     |     |     |         |     |     |         |
| Illiterate’s              | 16  | 4.2 | 2   | 12.5| 1.0422 | (0.594)| 2  | 12.5| 8.6  | 0.0211 | (0.885) |
| Primary school (1–6 grade)| 257 | 66.9| 41  | 16  | 0.1057 | (0.771)| 22 | 8.6  | 8.6  | 0.0211 | (0.885) |
| 2nd school (7–12 grade)   | 111 | 28.9| 22  | 19.8| 0.2791 | (0.598)| 5  | 10.2 | 9.6  | 0.0211 | (0.885) |
| Residence                 |     |     |     |     |     |     |         |     |     |         |
| Urban                     | 335 | 87.2| 58  | 17.3| 1.0422 | (0.594)| 32 | 9.6  | 9.6  | 0.0211 | (0.885) |
| Rural                     | 49  | 12.8| 7   | 14.3| 0.0022 | (0.969)| 5  | 10.2 | 9.6  | 0.0211 | (0.885) |

Abbreviations: χ², chi-square testing; df, degree of freedom; NGO, non-governmental organization.
However, children who drink spring water (16.7%), and had animal contact (22.8%) had higher rates of intestinal parasites than lacks animal contact. These children were more likely to have acute diarrhea (31.7%) and CD4 levels <200 cells/µL. Diarrheal status was the only significantly associated variable with intestinal parasites ($\chi^2=7.653$, df=2, p=0.022) as presented in Table 2.

There is also a correlation between low CD4 levels and parasitic infection rates. Children with CD4 count <200 cells/µL were more likely to have a parasite (22.6%)

![Distribution of IPIs](image)

**Figure 1** Distribution of intestinal parasites among HIV positive children in Southern Ethiopia, February 2016 to June 2017.

| Variables                  | Frequency | Presence Of Any Parasite | $\chi^2$, df, (p-value) | Presence Of Cryptosporidium | $\chi^2$, df, (p-value) |
|----------------------------|-----------|--------------------------|-------------------------|-----------------------------|-------------------------|
| Drinking water source      |           |                          |                         |                             |                         |
| Pipe                       | 352       | 91.7                     | 33                      | 9.4                         | 33                      | 9.4                     | 0.7122 (0.701)          |
| Hand pump                  | 20        | 5.2                      | 2                       | 10                          | 2                       | 16.7                    | 10.0                     |
| Spring                     | 12        | 3.1                      | 2                       | 16.7                        | 2                       | 10.0                    | 10.0                     |
| Animal contact             |           |                          |                         |                             |                         |                         |
| Yes                        | 92        | 24                       | 21                      | 22.8                        | 10                      | 10.9                    | 0.2121 (0.645)          |
| No                         | 292       | 76                       | 44                      | 15.1                        | 27                      | 9.2                     | 9.2                      |
| Diarrhea status            |           |                          |                         |                             |                         |                         |
| Absence                    | 314       | 81.8                     | 49                      | 15.6                        | 30                      | 9.6                     | 0.5612 (0.755)          |
| Acute                      | 41        | 10.7                     | 13                      | 31.7                        | 5                       | 12.2                    | 12.2                     |
| Chronic                    | 29        | 7.6                      | 3                       | 10.3                        | 2                       | 6.9                     | 6.9                      |
| CD4 level cells/µL         |           |                          |                         |                             |                         |                         |
| <200                       | 31        | 8.1                      | 7                       | 22.6                        | 5                       | 16.1                    | 16.1                     |
| 200–499                    | 104       | 27.1                     | 20                      | 19.2                        | 11                      | 10.6                    | 10.6                     |
| >500                       | 249       | 64.8                     | 38                      | 15.3                        | 21                      | 8.4                     | 8.4                      |

**Table 2** Assessment Of Risk Factors For Cryptosporidiosis And Other Intestinal Parasites Among HIV Positive Children In Southern Ethiopia
than those with CD4 levels of 200–499 cells/µL (19.2%) or CD4 >500 cells/µL (15.3%). In each group, the percentage of Cryptosporidium was consistent (16.1%, 10.6% and 8.4%, respectively). However, CD4 level is not statistically significant (Table 3).

In correlating CD4 levels to diarrheal level and parasitic infection directly in Table 3 children with low CD4 counts (<200 cells/µL) who had no diarrhea had a higher rate of parasitic infection than those whom children with acute or chronic diarrhea but not statistically associated to IPIs. In both the mid and high range CD4 levels, those children with acute diarrhea had higher degrees of parasitosis than those with no or chronic diarrhea (50% and 30%, respectively, as compared to 15.3/14.3% and 14.4/9.5%). However, statistically significant correlation was observed in the range of 200–499 cells/µL ($\chi^2=4.642$, df=1, p=0.031).

**Discussion**

Cryptosporidium spp. are recognized as intestinal parasites causing severe life-threatening diarrhoea in immunocompromised, immunosuppressed and malnourished patients worldwide. This study showed that not only Cryptosporidium but also other intestinal parasitic infections were common among HIV-positive children. Many studies in children and immunocompromised people suggest that the prevalence of cryptosporidiosis in developing countries is higher than in developed countries. These differences could be explained by the HIV treatment regimen of the study subjects and the study setting.

As compared to other study conducted somewhere else among HIV-infected children lower rates of parasitic infections were identified in our study for specific parasites, in Jamaica A. lumbricoides (2.4% vs 1.8%) and G. duodenalis (12.2% vs 2.6%), in Kenya G. duodenalis (11.1% vs 2.6%), in Addis Ababa G. duodenalis (7.6% vs 2.6%), E. histolytica/dispar (10% vs 2.0%), A. lumbricoides (6.3% vs1.8%), Taeniaspp. (3% vs 0.78%), in Nigeria A. lumbricoides (37.5% vs 1.8%). It is comparable with a study from Nigeria, A. lumbricoides (1.9% vs 1.8%). This lower incidence may be due to better laboratory testing, consistent patient follow-up, and better awareness of the patients themselves in implementing prevention and management actions against intestinal parasites in addition to anti-helminths and highly active antiretroviral therapy (HAART).

According to our findings, co-infection was observed with Cryptosporidium and G. duodenalis, E. histolytica/dispar, A. lumbricoides and Taenia spp. (1%) each which is different from previous studies. It is obvious that the availability of potable drinking water, reduced contact with animals, hygienic facilities and health education on sanitary practices contributed to the lower incidence of parasitic infestations.

**Table 3 Relation Of CD4 Count With Diarrheal Status For Cryptosporidiosis And Other Intestinal Parasites**

| CD4 Count Cells/µL | Patient Data | Any Par Schistosoma | | Cryptosporidium |
|---|---|---|---|---|
| | Diarrheal Status | No. | % | No. | % | $\chi^2$, df (p-value) | No. | % | $\chi^2$, (p-value) |
| <200 | None | 21 | 67.7 | 6 | 28.6 | 4 | 19 | 0.410 (0.522) |
| | Acute | 9 | 29 | 1 | 11.1 | 1 | 11.1 | 0 | 0 | 0.079 (0.778) |
| | Chronic | 1 | 3.2 | 0 | 0 | 0 | 0 | 0 | 0 | 0.079 (0.778) |
| | Total | 31 | 8.1 | 7 | 22.6 | 1.336 (0.248) | 5 | 16.1 | 0.410 (0.522) |
| 200–499 | None | 85 | 81.7 | 13 | 15.3 | 8 | 9.4 | 0.468 (0.414) |
| | Acute | 12 | 21.5 | 6 | 50 | 2 | 16.7 | 0 | 0 | 0 | 0.079 (0.778) |
| | Chronic | 7 | 6.5 | 1 | 14.3 | 1 | 14.3 | 1 | 10.6 | 0.079 (0.778) |
| | Total | 104 | 27.1 | 20 | 19.2 | 4.642 (0.031) | 11 | 10.6 | 0.079 (0.778) |
| >500 | None | 208 | 83.5 | 30 | 14.4 | 18 | 8.7 | 0.686 (0.408) |
| | Acute | 20 | 8 | 6 | 30 | 2 | 10 | 0 | 0 | 0 | 0.079 (0.778) |
| | Chronic | 21 | 8.4 | 2 | 9.5 | 1 | 4.8 | 0 | 0 | 0 | 0.079 (0.778) |
| | Total | 249 | 64.8 | 38 | 15.3 | 0.686 (0.408) | 21 | 8.4 | 0.079 (0.778) |
In this study, we assessed the possible associations of variables with Cryptosporidium and other parasitic infections. Diarrheal status was the only significant association observed for IPIs with (\(\chi^2=7.653, df=2, p=0.022\)), in agreement with a study from the same region conducted in 2009 on adult HIV patients and from Nigeria.\(^{40,41,42}\)

Conclusions
The overall magnitude of intestinal parasitic infestations was 16.9% with Cryptosporidium present in half of the infections. Therefore, based on our finding, health education should be provided to the children’s parents and caregivers of HIV-infected children receiving cART on how to prevent exposure to pathogens transmitted by exposure to non-potable water and animals. Direct stool examination and modified Ziehl–Neelsen staining should be routinely performed for HIV-infected children during follow up mainly based on diarrheal status.

Limitation Of The Study
We used a convenient sampling technique in which consecutive children were involved in the study, which may explain a bias toward children above 7 years, as compared to the younger children. A single stool sample was used to detect intestinal parasites, which may lead to false negative results. Two or more independently collected stool specimens are recommended to ensure diagnostic sensitivity to detect intestinal parasitic infections.\(^{43}\) Only HIV-infected children were tested; there was no control group of children not infected with HIV to compare the impact of HIV-infection on parasitic infection rates.

What Is Already Known About This Topic?
- HIV positive children are highly susceptible for opportunistic infections.
- Cryptosporidium is a common opportunistic parasite among these patients.
- Different risk factors were reported for intestinal parasites among HIV positive children other than immune status.

What This Study Adds
- Our study identified diarrheal status as the only statistical significant associated variable for IPIs.

Ethical Clearance
The Institutional Review Board of the College of Medicine and Health Sciences, Hawassa University approved the study. Involvement was voluntary and informed written consent was obtained from caregivers of the study participants. Physicians managed those found to be infected with any pathogenic intestinal parasites. This study was conducted in accordance with the declaration of Helsinki.

Availability Of Data And Material
All the raw data supporting the findings can be obtained from all authors.

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Author Contributions
All authors contributed to data analysis, drafting or revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

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Disclosure
The authors report no conflicts of interest in this work.

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