# Cryptosporidiosis among HIV-infected persons in the Niger Delta of Nigeria

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**ABSTRACT**

**BACKGROUND:** Since the discovery of acquired immunodeficiency syndrome (AIDS), many studies demonstrated that intestinal parasites were frequently associated with pictures of severe diarrhoea in patients with HIV.

**OBJECTIVES:** To determine the prevalence of Cryptosporidiosis among HIV-infected persons in the Niger Delta of Nigeria.

**METHOD:** Faecal samples from One hundred and five HIV/AIDS infected subjects made up of 48 males and 57 females aged 18-54 years, mean age and CD4 count of 36.14 $\pm$ 8.46 years and 320 $\pm$ 140 cells/µl respectively were evaluated for Cryptosporidiosis using the Modified Ziehl-Neelsen staining method.

**RESULTS:** Of the one hundred and five faecal samples examined, 3(2.9%) were positive for cryptosporidium oocyst. Prevalence was significantly higher among females 2/57 (3.5%) compared to males 1/48(2.1%), among subjects with diarrhoea 3(11.5%) and among subjects with CD4 lymphocyte count <200 cells/µl ($p<0.01$).CD4 count of subjects positive for cryptosporidium oocyst was significantly lower (150 $\pm$ 50 cells/µl) .The mean CD4 count of subjects with diarrhoea was significantly lower (mean 180 cells/µl) compared to those without diarrhoea (360 cells/µl).

**CONCLUSION:** Our study indicates that the prevalence of intestinal colonization due to Cryptosporidium is significantly higher among HIV-infected persons presenting with diarrhoea and low CD4 lymphocyte count of <200cells/ µl and re-emphasizes the need to incorporate routine faecal parasitological examination (FPE) in the follow up management of patients with HIV/AIDS. This is likely to optimise treatments in these patients by eradicating opportunistic pathogens and improve the quality of life of these patients.

**KEY WORDS:** Cryptosporidiosis, HIV, AIDS, Niger Delta, Nigeria.

## INTRODUCTION

*Cryptosporidium* is a protozoan that produces environmental stage (oocysts) that are eliminated in the faeces of the host. This parasite is a common cause of acute gastroenteritis in humans particularly those that are immunocompromised [1]. Human infection is usually acquired by direct contact between persons and by ingestion of contaminated food or water [2]. *Cryptosporidium* has a wide host range (reptiles, birds and mammals) and a wide geographical distribution in developing and developed countries [3].Chronic diarrhoea is a common presentation among HIV-infected Africans [4, 5]. *Cryptosporidium* is the commonest microbial cause of diarrhoea in the HIV-infected population worldwide [6, 7, 8]. Laboratory diagnosis is usually made by microscopic detection of microsporidial spores in faecal samples stained by a modified Ziehl-Neelsen method [9]. *Cryptosporidiosis* causes significant morbidity and mortality in human immunodeficiency virus (HIV)-infected population. In June 2002 antiretroviral therapy of two nucleoside reverse transcriptase inhibitors (stavudine and lamivudine) and one non-nucleoside (nevirapine) became available for HIV-infected patients in Nigeria. In this study in the Niger Delta Geopolitical zone of Nigeria, we sought to assess the prevalence of C*ryptosporidiosis* in our cohort of human immunodeficiency virus (HIV)-infected adults.

## MATERIALS AND METHOD

Between February 2005 and March 2006, we selected 105, consecutively recruited previously antiretroviral naive HIV/AIDS patients. They were enrolled into phase 3 antiretroviral therapy project in the Department of Haematology of the University of Port Harcourt Teaching Hospital (UPTH).This is a tertiary health facility with 500 beds, located in the Niger Delta geopolitical zone of Nigeria .Inclusion criteria included age $\geq$ 18 years, confirmed HIV positivity and written informed consent to enrol in the study. Subjects responded to standard questionnaire. Socio-demographic information (age, sex, educational status, previous antiretroviral and other anti-infective medications use 30 days prior to enrolment). Written informed consent was obtained from all study subjects. Subjects submitted faecal specimen for faecal parasitological examination at the Microbiology and Parasitology Department of University of Port Harcourt Teaching Hospital. Smears of faecal samples were prepared and stained using the Modified Ziehl-Neelsen staining .Previous study found method cost effective particularly in resource-limited setting [10]. They were read with a light microscope; including all of the fields of eight to ten slides per faecal specimen. Slides were

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considered positive when showing *Cryptosporidium* oocysts. Complementary CD4+ T lymphocyte counts of subjects were obtained using the Dynal beads method (Dynal Asa, Oslo, Norway) -an alternative to flow cytometry for the enumeration of peripheral CD4 T-lymphocyte particularly in resource poor settings. Previous study showed a positive correlation with results from flow cytometry [11].

## STATISTICALANALYSIS

Parametric and non-parametric methods were utilized in uni- and multivariate analyses. The maximum level of significance utilized for the statistical analyses was 5%. The analyses were carried out using the program "Statistical Package for Social Sciences" (SPSS Inc., Chicago, IL), version 9.

## RESULTS

The highest prevalence was observed in subjects in the 30-39 years age group (6.6%) followed by the 20-29 years age group (3.6%) as shown in table 1.The study population was made up of 48 males and 57 females aged 18-54 years, mean age and CD4 count of 36.14 ± 8.46 years and 320 ± 140 cells/µl respectively. Figure 1 shows the gender-related distribution of cryptosporidiosis among HIV-infected subjects. Of the faecal samples examined, 26(24.8%) was diarrhoeic while 79(75.2%) were non-diarrhoeic. The mean CD4 count of subjects with diarrhoea was significantly lower (mean 180 cells/µl) compared to those without diarrhoea (360 cells/ µl).Of the 105 faecal samples examined 3(2.9%) was positive for *Cryptosporidium* oocysts. Prevalence was significantly higher among females 2/57 (3.5%) compared to males 1/48(2.1%), among subjects with diarrhoea 3(11.5%) and among subjects with CD4 lymphocyte count <200 cells/µl 3/18(16.6%) ($p<0.01$).The mean CD4 count of subjects positive for *Cryptosporidium* oocyst was 150 ± 50. Table 2 shows the mean vales of subjects with diarrhoea and those without diarrhoea.

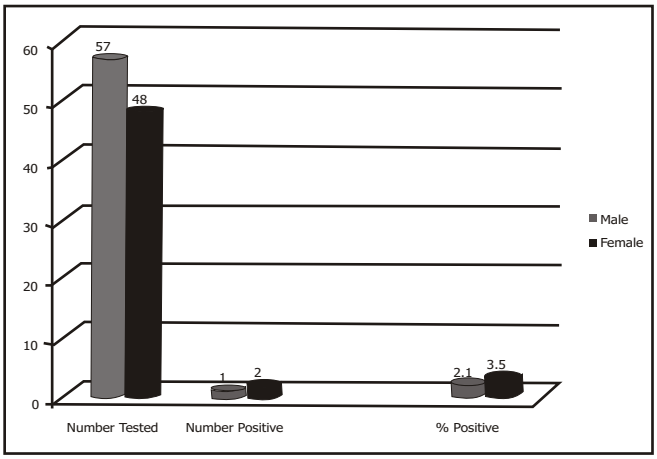
**Table 1: Age Distribution of study population**

| Age group (years) | Total screened | Number positive for cryptosporidiosis | % Positive |
|---|---|---|---|
| >20 | 15 | - | - |
| 20-29 | 28 | 1 | 3.6 |
| 30-39 | 30 | 2 | 6.6 |
| 40-49 | 18 | - | - |
| 50-59 | 14 | - | - |

**Table 2: Mean values of diarrhoeic and non-diarrhoeic HIV/AIDS-infected subjects.**

| Parameter | Diarrhoeic subjects | Non-diarrhoeic subjects | P-value |
|---|---|---|---|
| Age | 32.54 ± 8.40 | 31.33 ± 5.32 | > 0.05 |
| CD4+ Count | 180 ± 90 | 360 ± 100 | <0.01 |

**Figure 1: Gender-related distribution of Cryptosporidium infection among HIV-infected subjects.**




## DISCUSSION

In this study of HIV-infected adults in Port Harcourt Nigeria, the overall prevalence of *Cryptosporidium* oocysts was 2.9%. *Cryptosporidium* infection was found clustered among HIV/AIDS patients presenting with diarrhoea. This prevalence is consistent with findings from previous studies in different parts of the world among patients with HIV [12-15] but however at variance with previous reports by Kamisky *et al* in Honduras [16] and Nwokediuko *at al* in Nigeria [17] who did not find *Cryptosporidium* species among 133 and 161 HIV infected Hondurans and Nigerians respectively. It is possible that a significant number of subjects included in these studies may have been on anti-infective medication prior to the study coupled with the fact that subjects may not have presented with low AIDS defining CD4 Lymphocyte count as observed in our study. There seems to be a correlation between HIV and *Cryptosporidial* infection. Studies from other parts of the globe on HIV/AIDS-related diarrhoea have implicated *Cryptosporidium*; Taiwan (0.5%) [6], India (33%) [14] and Brazil (8.1%) [18]. The first reports of human cases of *Cryptosporidiosis* were in 1976 [19] followed over the next four years by a handful of reports largely of disease in immunosuppressed host. However, the Centres for disease control (CDC) in the USA recognized the importance of *Cryptosporidium* as a major human pathogen in 1982 when outbreak of cryptosporidial diarrhoea in 21 patients with advanced HIV disease [20].Chronic *Cryptosporidiosis* is a qualifying diagnosis for CDC-defined AIDS. In the United States 3-4% of persons at the time of CDC defined AIDS diagnosis have *Cryptosporidiosis* and an estimated 10-15% develop *Cryptosporidiosis* during the course of HIV disease [21].In Sydney Australia, 5% of patients with advanced HIV disease undergoing gastrointestinal evaluation have *Cryptosporidium* enteritis [22].

The prevalence of cryptosporidiosis was significantly higher among HIV/AIDS-infected patients with AIDS defining CD4 lymphocyte count of <200cells/µl. This finding is consistent with previous reports in Cameroon [7] by Sarfati *et al* who observed a significantly higher prevalence of opportunistic protozoa (32%) among patients with CD4 cell counts less than 50cells/µl and Dwivedi *et al* [14] in India who found enteric coccidian parasites significant associated with diarrhoea, especially among those a lower CD4+ cell count. Infection with HIV causes a gradual decline in the peripheral CD4 helper lymphocyte. These lymphocytes are part of the body's immune system and play a key role in cell mediated immunity. But as HIV destroys these lymphocytes,HIV-infected patients becomes predisposed to opportunistic infections.HIV/AIDS related cryptosporidial infection has been found to resolve following HAART related immune reconstitution of the immune system even without specific treatment of the parasite [23-24].Moreover there is evidence invitro and invivo to suggest that the control of *Cryptosporidiosis* in patients on HAART are also helped by the anti-HIV protease inhibitors which could be acting on the aspartyl-protease of the parasite [25]. Highly active antiretroviral therapy has changed the landscape of HIV/AIDS related care in the developed world in patients fortunate enough to have access but its long-term effect has not been well elucidated by many HIV/AIDS infected in Nigeria. There is need for the universal access to HAART in resource limited setting particularly in the Niger Delta geopolitical zone of Nigeria. This will facilitate the initiation of therapy in these patients before they develop full blown AIDS, their CD4 lymphocyte count fall below 200cells/µl and they become prone to a wide range of opportunistic infection including cryptosporidiosis. There is need to incorporate routine faecal parasitological examination (FPE) in the follow up management of patients with HIV/AIDS. This is likely to optimise treatments in these patients by eradicating opportunistic pathogens and improve the quality of life of these patients. This study had several limitations; the non availability of PCR based technology in our centre to characterize the three samples found positive for Cryptosporidiosis as well as compare techniques employed in this study with molecular techniques.

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