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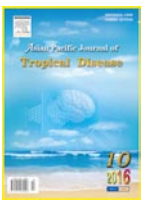

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# Modulation of cyptosporidiosis by CD4 levels in chronic diarrhoea HIV/AIDS individuals visiting Tarkwa Municipal hospital, Ghana

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ABSTRACT

**Objective:** To investigate the role $CD4^+$ levels play in controlling diarrhea conditions caused by intestinal coccidian infections among HIV/AIDS infected individuals visiting Tarkwa Municipal Hospital.

**Methods:** Fifty HIV/AIDS infected subjects with diarrhea conditions were enrolled into the study. Stool and blood samples were collected from each in two or three consecutive times to examine intestinal coccidian and microsporidian infections using microscopy and also estimate $CD4^+$ cells using BD FACSCount TM.

**Results:** Fourteen of the participants had intestinal coccidian or microsporidian representing 28% while 72% of the participants had diarrhea of unknown origin. *Cryptosporidium* recorded the highest prevalence of 42.86% whilst *Cyclospora* and Microsporidia equally recorded a prevalence of 28.57%. A significant protection against cryptosporidiosis was observed for $CD4^+$ count above 200 cells/μL ($\chi^2 = 6.522$, $P = 0.038$), but not cyclosporiasis ($P = 0.233$) or microsporidiosis ($P = 0.060$).

**Conclusions:** This study has shown that $CD4^+$ cells above 200 cell/μL of blood protect HIV-infected patients from cryptosporidiosis. Standardization of the association between $CD4^+$ cells and diarrhea condition caused by *Cryptosporidium* species is therefore suggested to serve as an indicator for prompt diagnosis and treatment of HIV-infected individuals with cryptosporidiosis.

## 1. Introduction

Chronic diarrhea in HIV/AIDS infection has many non-specific etiologies resulting in delayed diagnosis and treatment[1]. The etiological agent can either be infectious or noninfectious[2-6]. The infectious agents are the opportunistic parasites belonging to coccidia or Microsporidia[7-10]. These opportunistic parasites often go undetected in HIV/AIDS with chronic diarrhea. Untreated infections invigorate the clinical condition in HIV/AIDS patients.

*Cryptosporidium* hominis, *Cryptosporidium parvum*, and *Enterocytozoon bieneusi* infections are self-limiting in immunocompetent individuals but cause chronic diarrhea in HIV/AIDS patients[11,12]. Chronic diarrhea with persistent opportunistic parasitic infection has been linked to the depletion of $CD4^+$ cells in HIV/AIDS[12-15]. The $CD4^+$ cell depletion is attributed to HIV virus priming the $CD8^+$ T lymphocytes to kill the $CD4^+$ T lymphocytes cells[16-18].

The lower absolute $CD4^+$ cell count has shown to be the risk factor of disseminated pulmonary cryptosporidiosis among cancer patients on immunosuppressive agents with severe or atypical diarrhea in

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Saudi Arabia[19], however, withdrawal of immunosuppressive agents restored their immunity against enteric protozoa[20].

Although there are several effective treatment and diagnostic tools for opportunist pathogens in HIV/AIDS[21,22], routine diagnosis and treatment are often ignored at the antiretroviral therapy clinics. This study was conducted to investigate the role $CD4^+$ levels play in controlling diarrhea conditions caused by intestinal coccidian infections among HIV/AIDS infected individuals visiting Tarkwa Municipal Hospital.

## 2. Material and methods

### 2.1. Study site

Samples were collected from Tarkwa Municipal Hospital between July 2010 and April 2011. This hospital has an HIV clinic which offers voluntary counselling and testing for the populace. Tarkwa is the capital of Tarkwa-Nsuaem municipal district in the western region of Ghana (Figure 1). The municipality has a population of 90 477 inhabitants of which 34 941 have settled at Tarkwa, the capital city. The majority (about 56%) of the population are youth between the ages of 15 years and 50 years. The municipality is popular for its gold, manganese, iron ore and bauxite mining.

### 2.2. Ethical approval

The research protocols were reviewed and approved by the Department of Biomedical and Forensic Science Research Board, University of Cape Coast (DBFRB/09/0132) and Medical Administrative Committee of Tarkwa Municipal Hospital. Blood samples were obtained from subjects who had accepted to participate in the study to monitor their immune status in order to determine the appropriate time to put them on antiretroviral therapy.

### 2.3. Selection of study subjects

The aim of the study was explained to the potential subjects who were either HIV infected or having AIDS and experiencing diarrhea. The subjects who agreed to be part of the study were required to sign an acceptance document before being enrolled into the study. The subjects were required to give at least two to three stool and blood samples any time they experience diarrhea within a space of three months.

### 2.4. Sample collection

Participants of the study were given at least five 60 mL plastic leak

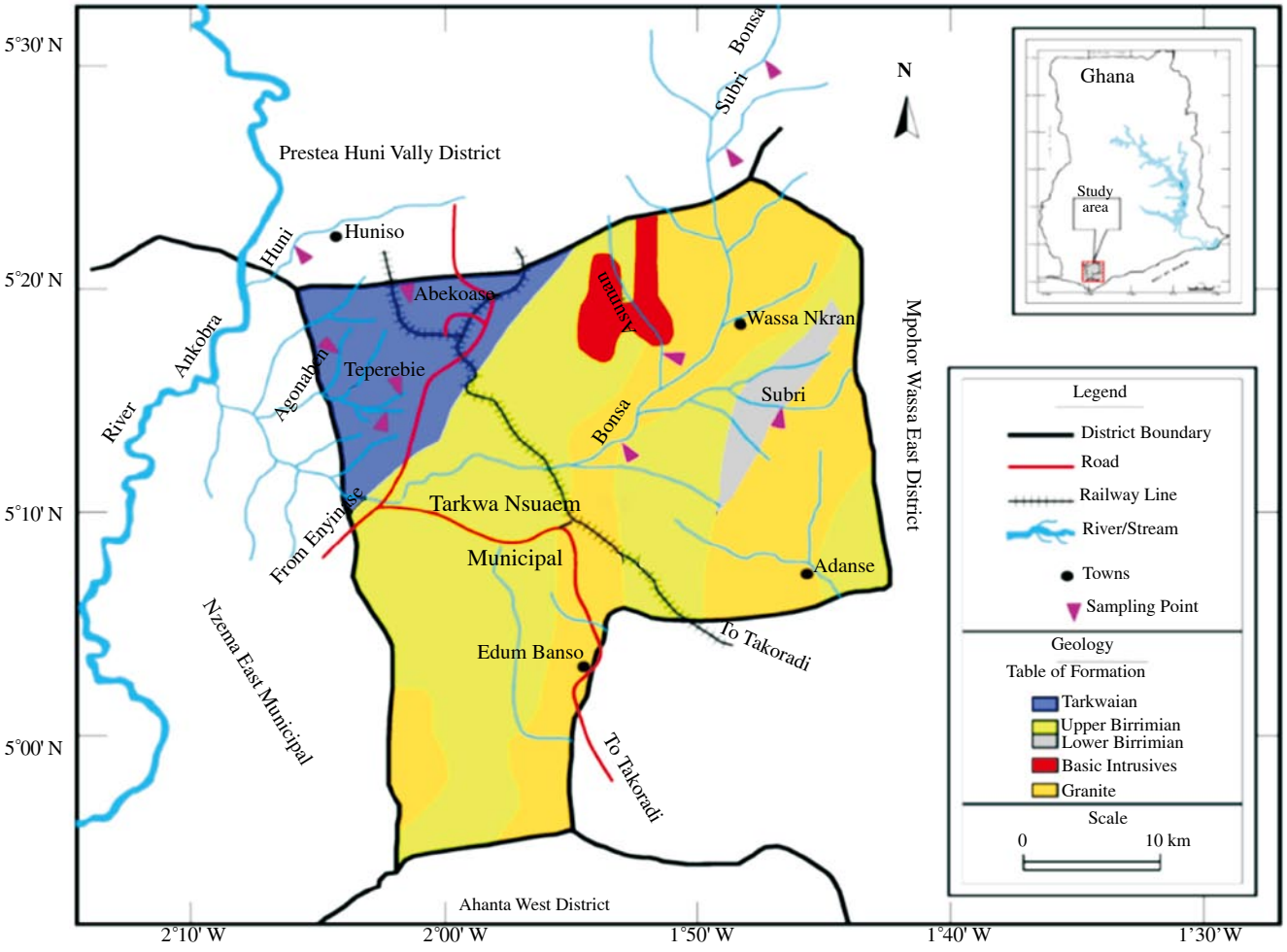


**Figure 1.** The map of Tarkwa Municipality and the sample collection sites in Ghana adapt from Google Earth Map.

proof stool containers with a spoon to collect stool samples prior to visiting the health facility any time they experienced diarrhea. The stool samples were either processed immediately upon collection or stored at –20 ℃ for a period of 48 h before processing. Five milliliters of capillary blood was taken per visit into ethylene diamine tetraacetic acid anticoagulant tubes for CD4 estimation.

### 2.5. CD4/CD8 estimation using FACSCount

BD FACSCount CD4/CD3 and CD8/CD8 two-tube format reagent kit (two-tube format): (340167, Becton Dickinson, USA) and FACSCount (software version 1.2) were used for the estimation of $CD4^+$ and $CD8^+$ lymphocytes levels in the sample[23].

### 2.6. Direct wet mount

One gram of each of the fresh stool samples was emulsified with 2–3 drops of physiological saline, and stained with a drop of Lugol's iodine. A total of 20 μL of the emulsified stool was transferred onto a microscopic slide and covered with a coverslip. The preparation was observed under the microscope with the 10× and 40× objective lenses.

### 2.7. Formalin-ethyl acetate concentration method

One gram of faeces with 8 mL of 10% formaldehyde was sieved using gauze into a centrifuge tube containing 4 mL of ethyl acetate. The suspension was mixed thoroughly for 1 min and centrifuged immediately at 1 000 g for 1 min. The supernatant was discarded and the residue resuspended. A few drops were transferred onto microscope slide, covered with coverslip and examined using 10× and 40× objective lenses.

### 2.8. Modified acid-fast and trichrome staining

Thin stool smears were made from formalin-ethyl acetate concentration and then fixed in methanol for 2–3 min and allowed to air dry. The smears were stained using modified acid-fast and trichrome staining methods as described[24,25].

### 2.9. Statistical analysis

The data generated were analysed using SPSS version 16. Statistical significance between $CD4^+$ level categories and identified coccidian or microsporidian parasites in stool samples were tested using Pearson *Chi*-square statistics.

## 3. Results

In all 50 subjects who were HIV/AIDS infected with recurrent diarrhea had their stool samples and CD4 cells examined at least twice within three months. Thirty six (72%) of the participants had their stool and blood samples examined thrice whiles the rest of the subjects had their samples examined twice. Each sample examination coincided with diarrhea episodes. All the 23 (46%) of the participants who had low $CD4^+$ cell count at the start of this study were on antiretroviral therapy treatment whereas 54% of the participants were not on antiretroviral therapy treatment but were being monitored at the HIV clinic. In all, 20 (40%) of the participants were males with a mean age of 35.80 years and 30 (60%) of them were females with the mean age of 39.03 years. The age range of the participants were from 21 to 57 years.

A total of 136 stool samples were examined for intestinal coccidia and Microsporidia from 50 participants. Fourteen of the participants had intestinal coccidian or microsporidian representing 28% whilst 72% of the participants had diarrhea of unknown origin. Among the participants with intestinal coccidian or microsporidian infection, 28.57% had *Cyclospora* and microsporidium each, and 42.86% had *Cryptosporidium* but no *Isospora* species was recovered in this study (Figure 2). There were equal occurrences of 50% *Cryptosporidium* and *Cyclospora* in both male and female participants. However, females had a higher prevalence (75%) of microsporidium infection

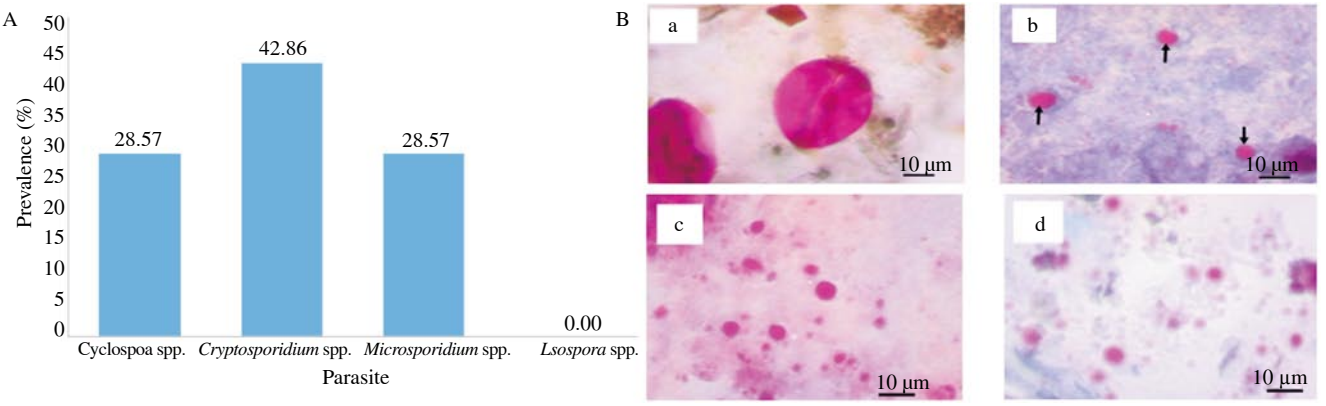


**Figure 2.** Prevalence of intestinal coccidian and microsporidian among study subjects.
A: Prevalence of *Cyclospora*, *Cryptosporidium*, microsporidian and *Isospora* among study subjects; B: Coccidian and microsporidian oocysts in stool; a: *Cyclospora* oocyst in trichrome staining; b: *Cryptosporidium* oocyst in trichrome staining; c: Microsporidian spores in modified acid-fast staining; d: Microsporidian spores in trichrome staining.

whilst male participants had 25% microsporidium infection (Figure 3).

Thirty-six (72%) of the participants had their $CD4^+$ level counted thrice whilst 28% of the participants had their $CD4^+$ level counted only twice. Fifteen (30%) of the participants had $CD4^+$ cell count above 500 cells/$mm^3$ of blood, 12 (24%) had $CD4^+$ cell count 200–499 cells/$mm^3$ and 23 (46%) had $CD4^+$ cell count lower than 200 cells/$mm^3$. The prevalence of *Cryptosporidium*, *Cyclospora*, and microsporidium infections were analyzed based on $CD4^+$ cells categories. In all 13 (56.52%) of the participants with $CD4^+$ cell levels lower than 200 cells/$mm^3$ had intestinal coccidian or Microsporidia infections; only 1 (6.67%) of the participants with a $CD4^+$ cell level above 500 cells/$mm^3$ had *Cyclospora* infection. Apart from *Cyclospora* infection, no other intestinal coccidian or Microsporidia infections were seen in $CD4^+$ category 200–499 cells/$mm^3$ or above 500 cells/$mm^3$ of blood. There was a significant difference in *Cryptosporidium* infection among the $CD4^+$ cells categories ($\chi^2 = 6.522$, $P = 0.038$) but not in *Cyclospora* ($P = 0.233$) or microsporidium ($P = 0.060$) (Table 1).

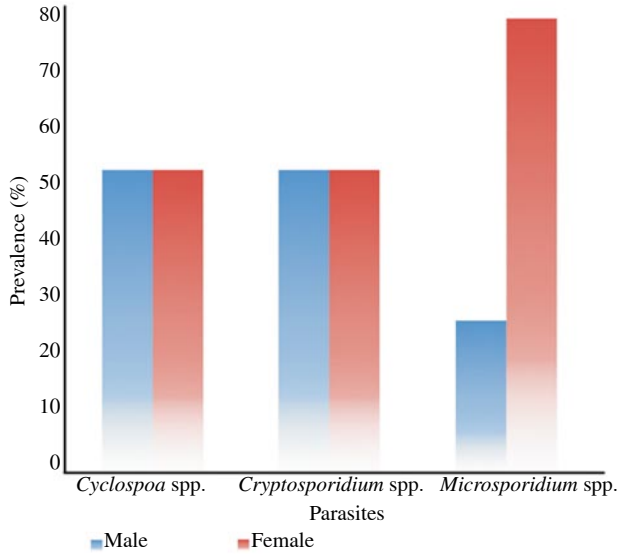


**Figure 3.** Gender and prevalence of intestinal coccidian and microsporidian among study subjects.

**Table 1**
Test of association between the prevalence of *Cyclospora*, *Cryptosporidium* and *Microsporidium* and $CD4^+$ T-cell counts among study subjects.

| $CD4^+$ T-cell count (cells/μL) | | Number of tested | Number of positive (%) | $\chi^2$ | *P* |
|---|---|---|---|---|---|
| *Cyclospora* spp. | > 500 | 15 | 1 (6.7) | 2.915 | 0.233 |
| | 200–499 | 12 | 0 (0.0) | | |
| | < 200 | 23 | 3 (13.0) | | |
| *Cryptosporidium* spp. | > 500 | 15 | 0 (0.0) | 6.522 | 0.038 |
| | 200–499 | 12 | 0 (0.0) | | |
| | < 200 | 23 | 6 (26.1) | | |
| *Microsporidium* spp. | > 500 | 15 | 0 (0.0) | 5.626 | 0.060 |
| | 200–499 | 12 | 0 (0.0) | | |
| | < 200 | 23 | 4 (17.4) | | |

## 4. Discussion

Diarrhea has become a cardinal feature of HIV/AIDS, affecting up to 90% infected individuals[26,27]. Chronic diarrhea has severe consequences on the quality of health, morbidity, and mortality of HIV-infected patients[1,27]. The etiology of diarrhea in HIV/AIDS is multi-factorial ranging from pathogenic infection to non-pathogenic factors[28,29]. Causes of diarrhea in HIV/AIDS are the profound intestinal coccidian protozoan infections such as *Cryptosporidium*, *Cyclospora*, *Isospora* and *Microsporidium* which naturally cause self-limiting infections in immunocompetent individuals[5,9,11,29]. However, it becomes chronic in HIV/AIDS leading to weight loss, wasting syndrome and finally resulting in acute morbidity and mortality[30]. These opportunistic parasites have an adverse health impact on HIV/AIDS patients, yet no routine diagnosis is performed for clients with diarrhea episodes. The severe complications caused by these opportunistic infections could be prevented with early diagnosis and treatment.

In this study, *Cryptosporidium* is the most frequent diarrhea-causing coccidian in HIV/AIDS followed by *Cyclospora* and *Microsporidium*. Cryptosporidiosis, cyclosporiasis, and microsporidiosis related diarrhea in HIV are worldwide, although their frequencies vary from one geographical area to another[31,32]. This finding is in agreement with several reports that had shown cryptosporidiosis as the most frequent diarrhea condition in HIV/AIDS infections[4,33,34]. Cryptosporidiosis responds to antiretroviral therapy or highly active antiretroviral therapy, however, its effects vary from case to case[35,36]. Cryptosporidiosis can resolve spontaneously or progress to severe and unremitting diarrhea[37]. Contrary, cyclosporiasis causes complication in both the immunocompetent and immunocompromised persons despite its low occurrence in HIV infection compared to cryptosporidiosis[38,39].

The study showed that more female subjects are infected with *Microsporidium* species compared to their male counterparts, whereas, *Cryptosporidium* and *Cyclospora* have similar occurrences in male and female subjects. Anuar *et al*. showed that microsporidiosis frequently affects females than males which is in agreement with our current data[40]. Other studies have shown that microsporidium infects more males than females contrarily to our findings[41]. The possible reason could be attributed to the frequency of contact with infectious sources. The previous study conducted by Wang *et al.* did not find any differences between *Cryptosporidium* infection and gender among HIV/AIDS patients with chronic diarrhea[42]. However, the study conducted in Kenya on the risk of *Cryptosporidium* exposure showed that *Cryptosporidium* infection depends on occupation which in turn affects gender[43]. The former which is similar to the current study was designed for internal predisposing factors (immunity) whereas the latter was designed to assess the external predisposing factors (exposure) and *Cryptosporidium* infection. Massouda *et al.* showed that there is no significant difference between gender and *Cyclospora* infections among immunocompetent individuals with diarrhea episodes which augments the findings of this study[44].

The study showed that there is a significant association between

$CD4^+$ cell count lower than 200 cells/$mm^3$ and cryptosporidiosis but not cyclosporiasis or microsporidiosis in HIV/AIDS. Cryptosporidiosis can occur at any point in time of HIV infections, but major morbidity and mortality exclusively occur at $CD4^+$ cell counts below 180 cells/$mm^3$ in patients whereas there is spontaneous recovery in patients with higher $CD4^+$ cell counts above this level[45]. HIV/AIDS mortality data from Milwaukee (USA) showed that patients with $CD4^+$ cell counts below 50 cells/$mm^3$ had 73% death rate caused by cryptosporidiosis and 36% death rate in patients with $CD4^+$ cell count ranging from 50 to 200 cells/$mm^3$[46]. The most profound effect of *Cryptosporidium* infection is the extraintestinal manifestations such as biliary cryptosporidiosis, cryptosporidial pneumonitis and cryptosporidial colitis which are associated with a $CD4^+$ cell count below 100 cells/$mm^3$ are unique to immunocompromised persons. Extraintestinal cryptosporidiosis is resistant to treatment by current anti-cryptosporidial regiments[47]. It is therefore important to promptly diagnose and treat *Cryptosporidium* infections and other opportunistic pathogens in HIV/AIDS infected individuals to reduce cryptosporidial associated morbidity and mortality.

The limitation of this study was the low sample size which could either underestimate or overestimate the results.

This study has shown that $CD4^+$ cells above 200 cell/μL of blood protect HIV-infected patients from cryptosporidiosis. Here we therefore suggested that standardization of association between $CD4^+$ cells and diarrhea condition caused by *Cryptosporidium* species could serve as an indicator for prompt diagnosis and treatment of HIV-infected individuals with cryptosporidiosis.

## Conflict of interest statement

We declare that we have no conflict of interest.

## Acknowledgments

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