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ABSTRACT

Background: **Although *Cryptosporidium spp.* infections in acquired immunodeficiency syndrome patients (AIDS) with chronic diarrhoea have been reported in several African countries, there is no information regarding cryptosporidial diarrhoea in Ghanaian AIDS patients.**
Objective: **To investigate the occurrence of *C. parvum* and other gastrointestinal parasitic agents in Ghanaian AIDS patients with chronic diarrhoea.**
Design: **Prospective study of HIV/AIDS patients with diarrhoea over a nine month period.**
Setting: **Korle-Bu Teaching Hospital and Korle-Bu Polyclinic Accra, Ghana.**
Results: **Analysis of stool specimens from clinically diagnosed HIV/AIDS (n=2I; mean CD4 count was 288 cells per microliter, 95% confidence interval of 237 to 340 cells per microliter) and HIV-seronegative (n=27) patients revealed *C. parvum* in six (28.6%) of HIV/AIDS and 10 (37.0%) of the HIV-seronegative patients, respectively. Three other HIV/AIDS cases had other infections involving *Strongyloides stercoralis* 4.8% (l/21) and *Salmonella spp*. 9.5% (2/21). There was no concomitant association between *C. parvum* and any other parasites found. Also, no enterobacteria was found in the HIV-seronegative patients.**
Conclusion: **This study demonstrates the prevalence of *Cryptosporidium* sp. in both HIV/AIDS and HIV-seronegative individuals in Ghana. However, there was no statistical association between cryptosporidiosis and HIV/AIDS (p>0.05).**

INTRODUCTION

Enteric cryptosporidiosis, caused by the coccidian parasite *Cryptosporidium* sp., has become of increasing interest since the first case of human cryptosporidiosis was reported in 1976(1). Much of this interest is due to its opportunistic behaviour in immunocompromised patients, particularly in those with acquired immune deficiency syndrome (AIDS) (2,3). AIDS predisposes individuals to several parasitic diseases, and among the intestinal parasites, the intracellular coccidia, *Isospora belli* and *Cryptosporidium parvum*, are important(4). As a result of increased cryptosporidial related diarrhoea in HIV/AIDS patients, the disease was recently listed as one of the AIDS-defining opportunistic infections(5).

Cryptosporidiosis develops in an estimated 10-15% of patients with AIDS in the United States of America and Europe(3,6,7) and 30-50% of patients with AIDS in the developing countries(8,9). Previous studies in Ethiopia, Zambia, Democratic Republic of Congo and other African countries have shown high prevalence rates of *C. parvum* infections in AIDS patients(10-12). The role of cryptosporidiosis in HIV/AIDS related diarrhoea in Ghana is virtually unknown. With a continuous rise in the number of HIV/AIDS infected persons, and with a prevalence rate currently estimated at 4% (National AIDS/STD Control Programme, Ministry of Health, Accra, Ghana), It is likely that *Cryptosporidium* sp. related diarrhoea may be prevalent in Ghana. This is even more likely because various animals that are potential sources of transmission (dogs, cats, cattle and pigs) and in some cases rats and mice share human habitations. Additionally, the common sources of drinking water including tap water, may be contaminated because of inadequacy of standard water treatment methods to remove the parasite. The aim of this study was to determine the prevalence of *Cryptosporidium* and other gastrointestinal parasitic agents in Ghanaian AIDS patients with chronic diarrhoea.

MATERIALS AND METHODS

Study area and subjects. This study was conducted at the Fevers Unit and the Polyclinic Unit of the Korle-Bu Teaching Hospital, Accra, Ghana, between February and September, 2001. Korle-Bu Teaching Hospital is a leading tertiary hospital which serves the city of Accra, surrounding urban population and the southern part of Ghana. Accra, the capital city of Ghana is a rapidly expanding city with a population of about 2 million. Twenty one HIV/AIDS patients (aged 8 months 54 years 11

males and 10 females) with chronic diarrhoea on admission at the Fevers Unit, and twenty seven HIV-seronegative individuals (control; aged 6–60 years 16 males and 11 females) who reported at the Polyclinic Unit with chronic diarrhoea were recruited in the study. The protocol for this study was approved by the Ethical and Protocol Review Committee of the University of Ghana Medical School, and written and informed consent was obtained from the patients. Following informed consent, a detailed demographic questionnaire including history and clinical status was completed with the help of a study nurse. The guidelines for the classification of HIV/AIDS in adults from the Centers for Disease Control (CDC), USA (13) were applied to both groups of patients. HIV testing was done by the particle agglutination test (Serodia HIV-1 and HIV-2: Fujirebio inc., Tokyo, Japan) and confirmed by Western Blot analysis (New Lav Blot I and II) or the synthetic peptide-based immunoassay (PeptiLav I and II), both obtained from Sanofi Diagnostic Pasteur, Marnes-la-Coquette, France.

Definition of diarrhoea episodes: The World Health Organisation (WHO) criteria was used to determine diarrhoeal episodes (14). A total of three or more unformed stools in one 24-hour period was determined to be a diarrhoeal day.

Specimen collection and processing: Diarrhoeal samples from each patient was collected directly into sterile disposable containers and a portion of each sample subsequently preserved in a tube containing 10% formalin for parasitological examination. Fresh stools were transported to the laboratory for parasitologic and microbiologic evaluation within 24 hours. Preserved stool specimens were concentrated using formalin-ethyl acetate at 800xg in a faecal parasite concentrator and two slides were made from the resulting pellet. One slide was stained with Lugol's iodine and examined microscopically at 400x magnification for the presence of endemic parasites: *Giardia lamblia* cysts, *Trichuris trichiura*, *Ascaris lumbricoides*, *Strongyloides stercoralis*, *Ancylostoma* spp., *Schistosoma mansoni* and *Entamoeba* spp. The second slide prepared with faecal pellet was used to detect *C. parvum* oocysts by modified Ziehl Neelsen technique. Briefly, slides were stained for 20 min with carbol fucshin and de-stained for 30-60 seconds with a 10% solution of sulphuric acid. After washing, slides were counterstained with methylene blue for 1 min. Red-stained *C. parvum* oocysts were observed microscopically using a 40x objective. Fresh wet stool preparations stained with Lugol's iodine were also examined for parasites. Unstained, fresh stool specimen were plated onto various bacteriological agar media to isolate bacteria: MacConkey (*Shigella* species, *Salmonella* species) xylose-lysine-deoxycholate (*Yersiniaenterocolitica*). thiosulphate-citrate-bile-sucrose, trypticase soy with 5% defibrinated sheep blood and ampicillin *(Campylobacter jejuni)*. Culturing for enterotoxigenic *Escherichia coli* and demonstration of rotavirus could not be performed.

Statistical analysis: Statistical analysis were performed by Fisher's exact or Wilcoxon rank test. Results were considered significant when the P-value was less than 0.05.

RESULTS

All the 21 HIV/AIDS (with a single infection from HIV-1) patients fell within the CDC clinical staging AI-C3 categories, representing asymptomatic AIDS to severe AIDS conditions. The patients had mean CD4 counts of 288 cells per microliter (95% confidence interval of 237-340 cells per microliter).

All patients reported watery stools lasting from 3-90 days (diarrhoea episodes, 3-10 stools/day). In addition to diarrhoea, the most common symptoms in both groups of patients (HIV/AIDS and HIV-negative patients) included abdominal pain, nausea, vomiting and fever. Presence of blood in stools was, less frequently reported (Table 1).

As shown in Table 1, significantly more HIV-negative patients 11 (40.7%) had abdominal pains compared to HIV/AIDS patients 5 (23.8%) ($p<0.05$). Nevertheless, none of the HIV/AIDS patients with abdominal pain and two out of 11 HIV-negative patients had *Cryptosporidium* oocysts in their stools. Of the 21 HIV/AIDS and 27 HIV-negative patients, six (28.6%) and 10 (37.0%), respectively had *C. parvum* only (Table 2).

There was no concomitant association between *C parvum* and any other intestinal parasites found in the stools of both categories of patients. Of the remaining HIV/AIDS cases, one patient had *S. stercoralis* and two had *Salmonella spp*, however, no enterobacteria was found in the HIV-negative patients (Table 2). Major risk factors such as drinking of untreated/ unboiled water and close association with domestic animals (chickens, pigs, cattle, sheep, goats, dogs, cats) had no significant effect on *Cryptosporidium* infections ($p>0.05$).

Table 1

Symptoms associated with chronic diarrhoea in HIV/AIDS and HIV-seronegative patients

Characteristic	HIV/AIDS (n=21)		HIV-seronegative (n=27)	
Symptom	Number (%)	*Cryptosporidium spp* positive	Number (%)	*Cryptosporidium spp* positive
Abdominal pain	5 (23.8)	0	11 (40.7)	2
Nausea	9(42.8)	2	12(44.4)	5
Vomiting	7(33.3)	3	9(33.3)	6
Fever	8(38.1)	2	13 (48.1)	3
Blood in stool	1 (4.8)	0	3 (11.1)	0

* Stool samples were loose and take the shape of the container

Table 2

Pathogens identified in HIV/AIDS and HIV-seronegative patients with chronic diarrhoea

Pathogen	HIV/AIDS n = 21 (%)	HIV-seronegative n = 27 (%)
Cryptosporidium spp.	6(28.6)	10(37.0)
Strongyloides stercoralis	1(4.8)	0
Salmonella spp.	2(9.5)	0

DISCUSSION

Cryptosporidiosis is a highly infectious illness with multiple modes of transmission through water, person-to-person and zoonosis(16). It has a serologic prevalence as high as 30-60% in industrialised countries(17,18) and 95% in some tropical and developing countries (16). Cryptosporidia are etiologic agents in 10-35% of' cases of AIDS-associated diarrhoea, and cryptosporidiosis results in a significant morbidity and mortality in patients with AIDS world-wide(5,7). However, there is little or no information available on the prevalence, significance, and prognosis of Cr*yptosporidium* infection in Ghana, particularly in HIV/AIDS patients.

In this study, *Cryptosporidium parvum* oocysts were identified by the modified Ziehl Neelsen method whose sensitivity and specificity are reported to be comparable to that of the immunofluorescence assay(19). C. *parvum* was isolated as the dominant pathogen from both HIV/AIDS and HIV seronegative groups of patients with diarrhoea than any of the other enteric pathogens investigated ($p<0.05$). This finding suggested that *Cryptosporidium* may be an unrecognized pathogen of diarrhoea and other gastrointestinal disorders in the Ghanaian population. However, there is the need to determine the importance of other agents of diarrhoea, such as enteropathogenic *E. coli*, viral and microsporidia infections which were not investigated in this study.

The results of this study are in agreement with that of Lucas (1990) who noted that the more 'common' intestinal parasites, *G. lamblia*, *T. trichiura*, *A lumbricoides* and *S. stercoralis* are not opportunistic in HIV/AIDS patients (20), Even though *Cryptosporidia* and other diarrhoea-causing pathogens produce similar symptoms, Tzipori (1988) reported that abdominal pains is associated with cryptosporidial diarrhoea(21). Nevertheless, the absence of abdominal pains in most *Cryptosporidium* infected diarrhoeal patients in this study may therefore suggest other causes of diarrhoea. Cryptosporidial diarrhoea is self-limiting in immunocompetent hosts and may be found in active disease. Furthermore, the lack of clear association with possible risk factors of acquiring cryptosporidiosis (such as consumption of contaminated drinking water and contact with domestic animals) calls for more work to identify the major routes of infection in the study area.

In summary, the data presented herein demonstrates the occurrence of *Cryptosporidium* among HIV/AIDS and HIV-seronegative diarrhoeal patients in Ghana. The disease was however not significantly associated with HIV/AIDS, and the high prevalence of infection in the HIV-seronegative population suggested that the transmission of *Cryptosporidium* in the study communities is high. In view of the increasing prevalence of HIV/AIDS in Ghana, findings from future studies will be useful in developing preventive measures for reducing infection among immunodeficient groups.

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