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Short Communication

Cryptosporidium Spp., a Frequent Cause of Diarrhea among Children at the Korle-Bu Teaching Hospital, Accra, Ghana

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SUMMARY: This report presents the results of a study conducted at the Child Health Department, Korle-Bu Teaching Hospital, Accra, Ghana, between the months of October 2001 and June 2002. Stool samples from 227 children with diarrhea and 77 children without diarrhea, aged less than 5 years, were tested for *Cryptosporidium* spp. Prevalence rates were 27.8 and 15.6% in children with and without diarrhea, respectively. *Cryptosporidium* infection was found to be high in children between the ages of 6 and 24 months. *Cryptosporidium* spp. was more common in malnourished children, but was not isolated in children under 6 months of age who were exclusively breastfed. Neither the presence of domestic animals, abdominal pain, blood in stool, nausea, vomiting, nor the consumption of untreated water was associated with *Cryptosporidium* spp. infection. *Shigella*, *Salmonella*, and yeast-like organisms were the most frequently identified enteropathogenic bacteria. In summary, this study demonstrates the prevalence of *Cryptosporidium* spp. among Ghanaian children.

In many developing countries including Ghana, gastroenteritis is the single biggest cause of early childhood mortality (1). In developed countries, gastroenteritis is hardly ever fatal; however, it is an important health and socioeconomic burden. Despite modern diagnostic techniques and treatment, diarrheal diseases continue to be the second most common cause of death of children younger than 5 years in less developed countries (2).

Cryptosporidium parvum is an enteric coccidian parasite that has recently emerged as an important cause of diarrhea worldwide, particularly in children and immunosuppressed individuals. The routes of transmission of this parasite include person-to-person contact, water contamination in community outbreaks with a common water source, and food contamination (3,4). The clinical spectrum of the disease is wide, ranging from asymptomatic carriers to a self-limited illness in immunocompetent individuals. However, in immunosuppressed individuals such as malnourished children and patients with AIDS, the disease frequently develops into a life-threatening, prolonged cholera-like illness (5).

Cross-sectional surveys of children with diarrhea suggest that cryptosporidosis is endemic in developing countries, with identification of this parasite in up to 26.9% of symptomatic children (6-8). Community-based longitudinal studies have also consistently found an association between *Cryptosporidium* infection and diarrheal illness (9,10). The role of cryptosporidosis among Ghanaian children with diarrhea has not been adequately documented. Out of 474 acute-phase stool samples collected during a previous survey of diarrheal disease in children aged 2 months to 5 years in Ghana, 61 (12.9%) were found to contain *Cryptosporidium* oocysts in moderate to large numbers (8). Cryptosporidiosis-related diarrhea is likely to be prevalent in Ghana for two reasons. First, various animals that are potential sources of transmission (dogs, goats, sheep, cats, rats, mice, pigs, and cattle) share a habitat with humans. Second, the common sources of drinking water, including tap water, may be contaminated because of the inadequacy of standard water treatment methods to remove the parasite. Here, we report the results of our 9-month study of cryptosporidiosis in Ghanaian children with and without diarrhea at the Korle-Bu Teaching Hospital (KBTH), Accra, Ghana. We also examined the association of cryptosporidiosis with various suggested risk factors for its transmission.

This study was conducted at the Child Health Department of the KBTH between October 2001 and June 2002. The KBTH is a leading tertiary referral hospital, which serves the city of Accra, its surrounding urban population, and the southern regions of Ghana. Accra, the capital city of Ghana, is a rapidly expanding city with a population of about 3 million.

Two hundred and twenty-seven children less than 5 years of age with acute diarrhea who visited the Child Health Department, the KBTH Emergency Room, and the Oral Rehydration Ward were prospectively enrolled in the study. The control group consisted of 77 children less than 5 years of age without diarrhea who were seen at the Emergency Room of the Child Health Department of the KBTH for other illnesses.

The rationale for conducting the present study was explained to the parents or guardians of all participants. Parents or guardians of these children gave written informed consent, permitting their children to be eligible for this cross-sectional study. The parents or guardians of these children were interviewed by a study nurse for the completion of a questionnaire that identified the children's age, sex, socioeconomic status (of the parent or guardian), weight, nutritional status (using the World Health Organization [WHO] and the National Center for Health Statistics age-height charts), breast-feeding habits, dietary and weaning information, water source and treatment, and presence of domestic animals. The presence of diarrhea was also determined by

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direct observation and by questioning the parents or guardians regarding the date of onset of diarrhea, number of stools per day, duration of diarrhea, clinical course, complications, and previous antibiotic treatment. The protocol for this study was approved by the Ethical and Protocol Review Committee of the University of Ghana Medical School, Accra, Ghana.

Parental consent or consent of the guardian was obtained in order to draw blood from the children (n = 90) and to determine the presence of serum HIV-1 and -2 antibodies. HIV testing was carried out by the particle agglutination test (Serodia HIV-1 and HIV-2; Fujirebio Inc., Tokyo, Japan) and was confirmed by Western Blot analysis (New Lav Blot I and II) or by synthetic peptide-based immunoassay (PeptiLav I and II), both obtained from Sanofi Diagnostic Pasteur, Marnes-la-Coquette, France.

The WHO criteria were used to determine the diarrheal episodes (11). A total of three or more unformed stools in one 24-h period was determined to be a diarrheal day. Diarrheal samples from each subject were collected directly into sterile disposable containers, and a portion of each sample was 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 h. Preserved stool specimens were concentrated using formalin-ethyl acetate at 800 x g in a fecal 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 fecal pellet was used to detect *C. parvum* oocysts by a modified Ziehl Neelsen technique. Briefly, slides were stained for 20 min with carbol fucshin, and were destained for 30-60 sec with a 10% solution of sulfuric acid. After being washed, the 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 specimens were plated onto various bacteriological agar media in order to isolate bacteria: MacConkey (*Shigella* spp., *Salmonella* spp.), xylose-lysine-deoxycholate (*Yersinia enterocolitica*), thiosulphate-citrate-bile-sucrose, trypticase soy with 5% defibrinated sheep blood, and ampicillin (*Campylobacter jejuni*). Culturing for enterotoxigenic *Escherichia coli* and demonstration of rota-virus could not be performed.

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

C. parvum was the most frequent pathogen isolated in the stools of children with or without diarrhea enrolled in our study. As shown in Table 1, of the 227 children with diarrhea, *C. parvum* oocysts were isolated from 63, giving an overall prevalence of 27.8%. Similarly, of the 77 children without diarrhea, *C. parvum* oocysts were identified in 12 of the stool samples, giving a prevalence of 15.6%. The majority of *C. parvum* cases occurred in children between the ages of 6 to 24 months; and among both groups of children (with or without diarrhea), significantly more oocysts (with diarrhea, 36.5% [23 out of 63]; without diarrhea, 50% [6 out of 12]) were isolated in the age group of 12 - 24 months (Table 1). There were no significant differences in the occurrence of cryptosporidiosis between male and female children of any of the age groups studied ($P > 0.05$).

Table 1. Clinical and socio-demographic data of children with or without diarrhea

	Cryptosporidium positive children	
	With diarrhea n = 63 (%)	Without diarrhea n = 12 (%)
Age		
< 1 months	0 (0)	0 (0)
1 - 5 months	10 (15.9)	2 (16.7)
6 - 11 months	16 (25.4)	3 (25.0)
12 - 24 months	23 (36.5)	6 (50.0)
2 - 5 years	14 (22.2)	1 (8.3)
Clinical manifestations		
Abdominal pain	25 (39.7)	5 (41.7)
Nausea	30 (47.6)	3 (25.0)
Vomiting	27 (42.9)	6 (50.0)
Fever	45 (71.4)	9 (75.0)
Blood in stool	25 (36.5)	2 (16.7)
Pathogens found		
Shigella spp.	4 (6.3)	2 (16.7)
Salmonella spp.	6 (9.5)	7 (58.3)
Yeast-like cells	11 (17.5)	7 (58.3)
Nutritional and socio-demographic factors		
Malnutrition (>25% deficit)	26 (41.3)	0
Breastfed (<6 months of age: n = 11)	0	0
Antibiotic therapy	37 (58.7)	5 (41.7)
Water source		
Tap water	46 (73.0)	10 (15.9)
Well	3 (4.8)	–
River	1 (1.6)	–
Domestic animals		
Dog	28 (44.4)	5 (41.7)
Cat	35 (55.6)	1 (8.3)
Goat	29 (46.0)	5 (41.7)
Sheep	5 (7.9)	2 (16.7)
Fowl	58 (92.1)	8 (66.7)

All the children (with or without diarrhea) reported watery stools that lasted from 3 - 90 days (diarrhea episodes, 3 - 10 stools per day). In addition to diarrhea, the most common symptoms in both groups of children included fever, nausea, vomiting, abdominal pain, and blood in the stool (Table 1). Interestingly, the symptoms observed in cases of cryptosporidiosis were similar to those observed in patients with enteritis caused by other pathogenic microorganisms. Other pathogens isolated from the pure cultures of diarrhea samples of both groups of children were *Shigella* spp., *Salmonella* spp., and yeast-like microorganisms. There was no concomitant association between *C. parvum* and any other intestinal parasites found in the stools of both groups of children. The presence of rotavirus and *E. coli* was not examined in the diarrhea samples.

A total of 26 malnourished children (41.3%) was identified; in these children a deficit of greater than 25% of the body weight expected for a particular age was observed among those with *Cryptosporidium*-associated diarrhea. Three children with only *Cryptosporidium* oocysts and none of the other intestinal pathogens studied in their stools died as a consequence of acute severe diarrhea. None of these three children who died of acute severe *Cryptosporidium*-associated diarrhea had HIV-associated immunodeficiency, malnutrition, or any other underlying clinical condition. In

the 11 children less than 6 months of age who were exclusively breastfed, no *Cryptosporidium* oocysts were isolated. Other factors such as a history of antibiotic treatment, water source, presence of domestic animals (dogs, cats, goats, sheep, and fowl) had no apparent influence on disease transmission.

Antibodies to HIV were examined in 90 serum samples from those authorized by the children's parents. Four (3 children with and 1 child without diarrhea) of the sera were positive for HIV antibodies, and all four children had *C. parvum* oocysts in their stools.

Cryptosporidiosis is a highly infectious illness with multiple modes of transmission through water, person-to-person contact, and zoonosis (3,4,12). This illness has a serologic prevalence as high as 30 - 60% in industrialized countries (13,14), and 95% in some tropical and developing countries (12). Cryptosporidia are etiological agents in up to 26.9% of cases of childhood-associated diarrhea (6-8), and cryptosporidiosis results in significant morbidity and mortality in children worldwide (5,15,16). However, there is little information available on the prevalence, significance, and prognosis of *Cryptosporidium* infection in Ghana, particularly in children with diarrhea.

Our findings demonstrate that *Cryptosporidium* infection is common among children <5 years of age who attended the Child Health Department of the KBTH Emergency Room. *Cryptosporidium* was isolated significantly more frequently from children with diarrhea than from those without diarrhea, suggesting that children with diarrhea are more likely to have *Cryptosporidium* oocysts identified in their stools; furthermore, it appears that *Cryptosporidium* may be an unrecognized pathogen responsible for diarrhea and other gastrointestinal disorders in the pediatric population.

In this study, the prevalence of cryptosporidiosis in both the children with and without diarrhea increased initially with age, peaking in the 12- to 24-month age group, and then decreased with age thereafter. A previous study in Ghana (8) reported that cryptosporidiosis was most common in the youngest age group (2 - 12 months), rather than in the 12- to 24-month age group observed in this study, and then prevalence decreased with age thereafter.

In the present study, a relatively high percentage of children in both groups with cryptosporidiosis exhibited abdominal pain, nausea, vomiting, fever, and blood in the stool. Nonetheless, we did not find any clinical symptoms specifically associated with cryptosporidiosis, when compared with other diarrhea-causing pathogens, thus rendering a clinical diagnosis of cryptosporidiosis difficult. Apart from *Cryptosporidium* oocysts, the other pathogens identified in the stools of both groups of children were *Shigella* spp., *Salmonella* spp., and yeast-like cells. However, there remains the possibility that viral agents and *E. coli*, which were not considered here, could have contributed to the cause of diarrhea among these children.

In the data reported herein, *Cryptosporidium* spp. was observed more frequently in malnourished children. In addition, we noted a protective effect of breast-feeding in children less than 6 months of age; however, we were unable to determine whether or not bottle-feeding was an actual risk. Furthermore, the lack of a clear association with possible risk factors for acquiring cryptosporidiosis (such as consumption of contaminated drinking water, and contact and presence of domestic animals) calls for more studies to identify the major routes of infection in this study area.

In summary, the data presented here demonstrate the frequent occurrence of *Cryptosporidium* among Ghanaian children with or without diarrhea. Hence, cryptosporidiosis should be considered as an important childhood diarrheal disease in Ghana. Since cryptosporidiosis appears to be more prevalent in developing countries than in the developed world, and its diagnosis is possible with simple staining techniques, we would like to re-emphasize a previous suggestion (8) that the inclusion of a search for *Cryptosporidium* oocysts be considered part of the routine clinical microbiological examination of cases of diarrhea in Ghana. Additionally, with the continuous rise in the number of HIV/AIDS-infected persons in Ghana (the current national prevalence rate is estimated at 4% by the National AIDS/STD Control Program, Ministry of Health, Accra, Ghana) and the designation of cryptosporidiosis as an AIDS-defining opportunistic infection, the routine examination for oocysts of *Cryptosporidium* in diarrheal stools may be used as a first-line screening measure for clinically suspected AIDS patients in Ghana.

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