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Diarrhea Associated with *Cryptosporidium parvum* among Young Children of the Nile River Delta in Egypt

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Summary

Over a 2-year period, the prevalence and clinical characteristics of *Cryptosporidium*-associated diarrhea in the Nile River Delta of Egypt was studied. A stool sample was obtained from children with diarrhea attending one of two study hospitals and of the 1275 children evaluated, 214 (17%) were found to be infected with *Cryptosporidium*.

Younger age was a risk factor for developing *Cryptosporidium*-associated diarrhea. Children <12 months of age were 2.4 times more likely to be infected with Cryptosporidium ($p<0.01$) and children 12 to 23 months were 1.9 ($p<0.05$) times more likely to be infected with the organism as compared to older children. Breastfeeding had a trend towards protection against *Cryptosporidium*-associated diarrhea ($p=0.07$). Clinical findings associated with *Cryptosporidium* diarrhea included vomiting, persistent diarrhea and the need for hospitalization. Our data suggest that *Cryptosporidium* is common in Egyptian children and may be associated with severe diarrhea.

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The opinions and assertions contained herein are the private ones of the authors and are not to be construed as official or as reflecting the views of the U.S. Department of the Navy, U.S. Department of Defense, the U.S. Government, the World Health Organization, or the Egyptian Ministry of Health and Population.

The study protocol was approved by the U.S. Naval Medical Research Unit No.3 (NAMRU-3) Institutional Review Board (Protocol No. DoD#30988) in compliance with all Federal regulations governing the protection of human subjects. Written informed consent was obtained from each parent prior to the enrollment of their child into the study.

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Introduction

Cryptosporidium are intracellular protozoan parasites first linked to human gastroenteritis in 1976.[1] The infection gained in medical importance with the recognition that the organism was a common cause of severe, chronic diarrhea in immunocompromised patients, particularly those infected with HIV.[2] The 1993 outbreak of *Cryptosporidium* in Milwaukee demonstrated the organism also had the potential to cause significant illness in immunologically normal hosts.[3] Studies have also indicated that the parasite may be an important enteropathogen in developing countries.[4]

Little data is available regarding *Cryptosporidium*-associated diarrhea in Egypt. A study among children evaluated for diarrhea at two hospitals in the Nile River Delta was thus established to better understand the prevalence and clinical characteristics of *Cryptosporidium parvum*-associated diarrhea in Egypt. We now report the findings of the study.

Materials and Methods

Study Sites and Population

The study was conducted at the Benha Fever Hospital, a periurban community of 455,000 persons at the beginning of the Nile Delta and at the Abu Homos District Hospital, an agrarian community with a

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population of 348,000 located southeast of Alexandria, Egypt.

Children under five years of age presenting to one of the two study sites for evaluation of diarrhea comprised the study population. Due to the high volume of children evaluated at both sites, the study was designed to enroll every fifth child with diarrhea. If a parent or guardian did not wish their child to participate in the study, then the next child coming for evaluation for diarrhea was substituted for the refusal.

Data Collection

After written informed consent, a detailed clinical history and physical examination was obtained using a structured and pre-tested questionnaire. The clinical history, including the length of time the child had been ill, the frequency of loose or liquid stools and any associated symptoms, was recorded. Additionally, demographic data, dietary history (including breast feeding) and history of any treatment received for the current illness (including antibiotics) was recorded. While a complete physical examination was performed, particular attention was given to assessing the degree of dehydration. Also for every child, a rectal temperature was measured and recorded.

Specimen Collection

At the time of enrollment, a stool sample and two rectal swabs were collected from each study child. The stool sample was immediately delivered to the hospital laboratory where the specimen was aliquoted and stored frozen at −20°C. One rectal swab was placed in Cary-Blair transport media and the other in buffered-glycerol-saline (BGS) medium and both swabs were stored refrigerated at 2°C to 8°C. Within three days of collection, the specimens were transported to the US Naval Medical Research Unit #3 (NAMRU-3) in Cairo, a two to three hour drive from the study sites. Rectal swabs were transported to NAMRU-3 in a cooler containing wet ice while the stool aliquots were shipped in a container of dry ice. Upon arrival at NAMRU-3, the rectal swabs in transport media were again stored in a refrigerator at 2°C to 8°C and the stool samples were placed in a −70°C freezer.

Specimen Processing

The *Cryptosporidium* TEST (TechLab Inc., Blacksburg, Virginia, USA), a commercially available enzyme linked immunosorbent assay (ELISA), was used according to manufacturer's instructions to detect *Cryptosporidium parvum*. The test has been compared to microscopy and fluorescent antibody, other modalities routinely used to detect *Cryptosporidium*, and demonstrated both sensitivity and specificity above 90%.[5]

Standard microbiological methods were used to isolate *Salmonella*, *Shigella*, *Campylobacter* and *Vibrionaceae*.[6,7] The API 20E system (Analytab Products, New York, NY) was used to confirm the identity of presumptive enteric pathogens. In addition, five *E. coli*- like colonies were picked from the MacConkey plate and evaluated for being enterotoxigenic *E. coli* (ETEC) by detecting the expression of either labile toxin (LT) or stable toxin (ST) using GM1-ganglioside enzyme-linked immunosorbent assays.[8]

Stools were also tested for the presence of rotavirus and/or astrovirus using commercially available ELISA kits according to manufacturer's instructions. (Rotaclone, Meridian Diagnostics Inc., Cincinnati, Ohio; IDEIA Astrovirus, DAKO Diagnostics Ltd., United Kingdom).

Definitions

"Some dehydration" and "severe dehydration" were defined according to definitions of the World Health Organization.[9] For the current study, these 2 categories were collapsed into a single classification of "any dehydration". "Persistent diarrhea" was an episode lasting longer than 14 days. "Fever" was defined as a rectal temperature >38.5 and "severe fever" was defined as rectal temperature >40.0. "Breast-feeding" was defined as receiving any breast milk regardless of whether other fluids or solids were included in the diet. "*Cryptosporidium* infection" was defined as the identification of *Cryptosporidium* with or without copathogens while episodes where *Cryptosporidium* was the only organism identified, the case was defined as "sole pathogen".

Analysis

Crude odds ratios were obtained by adding a single independent variable into a logistic regression model. Adjusted or multivariate odds ratios were obtained by adding an independent variable and confounders to a logistic regression model. Odds ratios, p-values, and confidence intervals were calculated from model parameters for both the crude and adjusted odds ratios. Statistical significance was set at $p<0.05$. All data was double entered into Epi-info 6.12. SAS 6.12 (SAS, Cary, North Carolina) was used for all analyses.

Results

Between May 2000 and May 2002, 1275 children (714 from Abu Homos and 561 from Benha) were enrolled in the study. A rectal swab was collected from every study child and a stool sample was available for *Cryptosporidium* testing from 71% ($n=510$) of the children in Abu Homos and 91% ($n=508$) of the children in Benha. There were no observable differences in the study population enrolled at the two

TABLE 1

Crude and adjusted odds ratio for the association between selected demographic factors and presence of Cryptosporidium parvum *among Egyptian children aged less than six years old, Benha Fever and Abu Homos District Hospitals, 20 May 2000 to 19 May 2002*

Variables	*Cryptosporidium*			
	Positive[a] ($n=214$)	Negative ($n=1061$)	Crude Odds Ratio	Adjusted OR (95%CI)[b]
Age				
0–11 months	138 (19)[c]	575 (81)	2.5[d]	2.4 (1.4, 4.2)[d]
12–23 months	60 (16)	320 (84)	1.9[e]	1.9 (1.1, 3.4)[e]
24–59 months	16 (09)	166 (91)	1	1
Gender				
Female	96 (17)	476 (83)	1.0	1.0 (0.7, 1.3)
Male	118 (17)	585 (83)	1	1
Season[f]				
Warm	155 (18)	692 (72)	1.4[e]	1.4 (1.0, 1.9)
Cold	59 (14)	369 (86)	1	1

[a] Cases with copathogens were not excluded.
[b] Odds Ratio (OR) adjusted for all other variables in the table (95% confidence intervals).
[c] *n* (%).
[d] $p<0.01$.
[e] $p<0.05$.
[f] Warm season from May to October; cold season from November to April.

TABLE 2

Distribution of co-pathogens detected with Cryptosporidium, *Hospital Based Study, (20 May 2000 to 19 May 2002)*

Copathogen	Number of cases (%)
Giardia	64 (30)
Rotavirus	41 (19)
ETEC	37 (17)
Campylobacter	6 (3)
Shigella	6 (3)

study sites in terms of age, sex distribution or socio-economic status of the children so the data was combined for presentation (Table 1). Diarrhea occurred most commonly during the summer with 847 (66 %) of the cases occurring between May and October. Of the 1275 enrolled children, 214 (17%) were *Cryptosporidium* positive by ELISA.

Of the 214 *Cryptosporidium* infections, *Cryptosporidium* was the sole pathogen in 90. *Giardia lamblia*, rotavirus, and ETEC were frequent copathogens (Table 2). *Cryptosporidium*-associated diarrhea was found to be more common in the younger children (Table 1). After controlling for confounders, children in the first year of life were 2.4 times more likely to be infected with *Cryptosporidium* than older children ($p<0.01$). Similarly, children 12 months to 23 months of age were 1.9 ($p<0.05$) times more likely to be infected with *Cryptosporidium* than children 24 to 59 months. Neither season nor gender appeared to influence the proportion of children infected with *Cryptosporidium*.

Controlling for age, gender, hospital, and season, children with *Cryptosporidium*-associated diarrhea were 3.3 times more likely to have persistent diarrhea than children with other enteropathogens ($p<0.01$). (Table 3) Vomiting and the need for hospitalization also occurred more commonly among children with *Cryptosporidium*-associated diarrhea, (Odds ratio 2.0 ($p<0.05$) and 1.6 ($p<0.05$), vomiting and hospitalization respectively). Children infected with *Cryptosporidium* also had a higher risk of being clinically dehydrated (Odds ratio 1.6) but the finding did not reach statistical significance ($p=0.06$).

Breast-feeding was common among children seeking treatment (Table 4). After adjusting for confounders, breastfeeding children were 40% (Odds ratio = 0.60) less likely to have *Cryptosporidium*-associated diarrhea than non-breastfeeding children which trended towards significance ($p=0.07$).

Discussion

The current project is the largest population based study of *Cryptosporidium* in a pediatric population of Northern Africa and the Middle East. Using this

TABLE 3
Crude and adjusted odds ratio for the association of selected clinical characteristics and presence of Cryptosporidium parvum, *Hospital Based Study, 20 May 2000 to 19 May 2002*

Clinical characteristics	*Cryptosporidium*			
	Positive[a] ($n=90$)	Negative ($n=804$)	Crude Odds Ratio	Adjusted OR (95%CI)[b]
Persistent[c]	6 (7)[d]	18 (2)	4.4[e]	3.3 (1.3, 8.7)[e]
Bloody Stools	8 (9)	34 (4)	1.9[f]	1.8 (0.8, 4.0)
Vomiting	76 (85)	764 (71)	2.3[e]	2.01 (1.1, 3.9)[f]
Fever	20 (22)	199 (19)	1.2	1.2 (0.7, 2.0)
Mucus	66 (73)	461 (58)	1.6[e]	1.5 (0.8, 2.8)
Convulsions	1 (1)	33 (3)	0.3	0.3 (0.0, 2.2)
Dehydration	37 (41)	296 (28)	1.8[f]	1.6 (1.0, 2.5)
Child Hospitalized	40 (44)	327 (31)	1.8[f]	1.6 (1.0, 2.7)[f]

[a] Cases with copathogens excluded.
[b] Odds Ratio (OR) adjusted for age, sex, hospital, and season (95% confidence intervals).
[c] Diarrhea of greater than 14 days duration.
[d] n (%).
[e] $p < 0.01$.
[f] $p < 0.05$.

TABLE 4
Crude and adjusted odds ratios for the association between breastfeeding and prevalence of Cryptosporidium, *Hospital Based Study, 20 May 2000 to 19 May 2002*

Breastfeeding	*Cryptosporidium*			
	Positive ($n=90$)[a]	Negative ($n=804$)	Crude Odds Ratio	Adjusted OR (95%CI)[b]
Yes	55 (10)	492 (90)[c]	0.96	0.6 (0.4, 1.0)
No	35 (10)	299 (90)	1	

[a] Cases with copathogens excluded.
[b] Odds Ratio (OR) adjusted for age, sex, hospital, and season (95% confidence intervals).
[c] Four cases were missing information on breastfeeding status.

model, we demonstrated that *Cryptosporidium*-associated diarrhea is common among Egyptian children with 16% of diarrhea attributed to this organism. Infections were found to be age related with significantly higher infection rates in the youngest children. Both the prevalence and age distribution of cases found in our study were comparable to reports from other areas of the developing world including Africa, Latin America and Bangladesh.[10–20]

In the current study, vomiting and persistent diarrhea was more likely to be associated with cases of *Cryptosporidium* as compared to other enteropathogens. Additionally, children with *Cryptosporidium*-associated diarrhea trended towards an increased likelihood of being clinically dehydrated. The combination of these findings likely explains the increased hospitalization among children infected with Cryptosporidium and is in agreement with previously published studies.[21–23]

Limitations

This study included only two research sites in Egypt. Therefore, we should be cautious to generalize these results to all of Egypt. It would seem improbable, however, that an organism of this importance is limited to these two areas. For many ill children, *Cryptosporidium* was identified with other copathogens. This may imply that *Cryptosporidium* infection is more common than *Cryptosporidium* diarrhea. Given this situation, it is possible that organisms other than *Cryptosporidium* induced the diarrhea that brought children to the hospital. We tried to rectify

this situation by removing an extensive list of copathogens associated with persistent diarrhea. By removing copathogens, we may also have underestimated the effect of *Cryptosporidium* on diarrhea, since there are controversies over if and when, for example, some phenotype of enterotoxigenic *E. coli* or *Giardia lamblia* induce diarrhea.

Implications

Our results suggest that *Cryptosporidium* is an important cause of diarrhea infection in Egyptian children. The prevention of this disease remains complicated. Cryptosporidial oocysts are protected by an outer cyst wall that allows it to survive outside the body for long periods of time and makes it resistant to disinfection including chlorine. Filtering this pathogen from the water supply is also problematic.[24] This implies that with high infection rates the disease will remain ubiquitous in the environment. Further complicating control of the organism is the fact that *Cryptosporidium* is a zoonotic disease, and transmission from animal to man is common.

Our study and other investigators have suggested that breastfeeding is protective for *Cryptosporidium* infection.[4,20,25,26] If this finding is substantiated, it strongly implies that breastfeeding in children may be the most important intervention for the prevention of this serious disease.

Nitazoxanide, a broad spectrum antihelminthic and antiprotozoan agent, has now been recommended for use against *Cryptosporidium* and, for the first time, offers physicians an effective treatment against the infection.[27,28] The drug has been found to be well tolerated and approved for use in children as young as one year of age.[27–29] The availability of an effective treatment has led some investigators to propose mass treatment campaigns with this drug.[28,29] However, information is lacking on the potential effectiveness of such a strategy in communities with a high prevalence of *Cryptosporidium*-associated diarrhea like Benha and Abu Homos where re-infection due to frequent animal exposure is likely.

In summary, we have found *Cryptosporidium parvum* to be a common infection in Egypt with clinical importance to young children. Fortunately, a treatment is now available which may lessen the burden of disease caused by the organism until effective preventive measures can be implemented.

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