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Cryptosporidiosis Among Children Attending Al-Nasser Pediatric Hospital, Gaza, Palestine

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Aim: The present study was undertaken to determine the prevalence of cryptosporidiosis in patients with diarrhea who attended Al-Nasser Pediatric Hospital in Gaza.

Materials and Methods: From January to May 2005, single stool specimens from each of 416 children attending Al-Nasser Hospital were examined by Ziehl Neelsen (ZN) stain and ELISA for the presence of *Cryptosporidium* oocysts.

Results: *Cryptosporidium* oocysts were detected in 62 (14.9%) of the tested specimens by acid-fast staining technique and in 68 (16.3%) using ELISA kit. The number of infected females was found to be significantly higher than males (P = 0.03). The highest number of infected children was observed in March. *Entamoeba histolytica/dispar, Giardia lamblia, Ascaris lumbricoides* and *Hymenolepis nana* were the most common parasites detected (14.4%, 7.2%, 0.7% and 0.7%, respectively).

Conclusions: It is concluded that cryptosporidiosis still exists among children in Gaza. In addition, the diagnosis of protozoal parasites such as *Cryptosporidium* should draw more attention and should not be neglected due to its clinical importance.

Key Words: Antigen, children, *Cryptosporidium*, diarrhea, prevalence

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Al-Nasser Çocuk Hastanesine Başvuran Çocuklar Arasında Cryptosporidiosis Sıklığı

Amaç: Bu çalışma, Gazadaki Al-Nasser çocuk hastanesine ishal yakınması ile başvuran çocuklardaki Cryptosporidiosis sıklığını araştırmak amacıyla yapıldı.

Yöntem ve Gereç: 2005 yılı ocak ve mayıs ayları arasında başvuran 416 çocuktan alınan tek bir dışkı örneğinde direk boyama ve ELISA yöntemleri kullanılarak *Cryptosporidium* ookistleri araştırıldı.

Bulgular: Test edilen örneklerin 62'sinde (%14.9) boyama yöntemi ile ve 68'inde (%16.3) ELISA kiti kullanılarak *Cryptosporidium* ookistleri tespit edildi. Enfekte kız çocuklarının sayısı erkenlerden daha yüksekti (P = 0.03). En fazla sayıda enfekte çocuk mart ayında saptandı. Diğer en sık saptanan parazitler sırasıyla *Entamobea histolytica/dispar* (%14.4), *Giardia lamblia* (%7.2), *Ascaris lumbricoides* (%0.7) and *Hymenolepis nana* (%0.7) idi.

Sonuç: Gaza'daki çocuklarda Cryptosporidiosis halen mevcuttur ve klinik önemi nedeniyle bu durum göz ardı edilmeden tanı için daha dikkatli davranılmalıdır.

Anahtar Sözcükler: Çocuk, ishal, antijen, prevalans, cryptosporidium

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Introduction

Cryptosporidium parvum, an intestinal protozoan parasite, is considered as one of the major diarrheagenic pathogens in humans throughout the world (1-4). There are several reports of hospital-based studies from different countries on the prevalence of *Cryptosporidium* in children with diarrhea. However, little information is available on asymptomatic carriage of *Cryptosporidium* in an apparently healthy population (5), and community-based studies carried out in depth are insufficient in the case of developing countries. Acute *Cryptosporidium* infection in children is usually associated with both acute and persistent diarrhea and various other gastrointestinal symptoms in immunocompetent persons and life-threatening illness in immunocompromised persons,

e.g. in acquired immunodeficiency syndrome (AIDS) patients (6). Asymptomatic cryptosporidiosis, which represents potential reservoirs of unrecognized, infected individuals who are capable of transmitting the infection to other individuals, was also documented (7-9). Asymptomatic individuals, livestock such as cattle and drinking water are known to be important vehicles in the epidemiology of the pathogen.

Cryptosporidium parvum appears to have become a threat to public health as it is ubiquitous and highly resistant to disinfectants and there is no effective therapy. The oocysts are shed in large quantities with the feces of infected individuals and are spread into the environment contaminating surface and ground waters. In a watery milieu, the parasite can survive for several months. It is completely resistant to the drinking water disinfection with chlorine. Cryptosporidiosis is endemic in developing and neighboring countries, such as in Fayoum (15%) (10) and among young children of the Nile River Delta in Egypt (17%) (11); in Israel (3.4-7.4%) (12); and among children in Jordan (37.3%) using direct immunofluorescence (13) as a result of poor sanitation and crowded living conditions (14). Gaza Strip dwellers have potentially high rates of parasitic infestation due to poor sanitation, groundwater contamination, infiltration of uncontrolled discharge of untreated sewage into the ground, contaminated water supply, and high population density (15).

In the Gaza Strip, a few unpublished reports mentioned the infection status of *Cryptosporidium*. The data resulting from this work would be of great importance in revealing the baseline prevalence of this parasite in Gaza and Palestine, and may help in undertaking any further interventions for its prevention and management.

Materials and Methods

The study population: The population included children attending Al- Nasser Pediatric Hospital in Gaza city from different ages and sex in the period from January to May 2005.

Description of study area: Al Nasser Hospital has four divisions in addition to the blood disease department (leukemia, hemophilia and thalassemia), intensive care unit, immature infants department, reception pharmacy, laboratory, and X-ray department. The total number of inpatient beds is 151, with 33 beds for daily care (reception and emergency). The reception department receives 3,500–4,000 patients monthly. The general department receives 950-1,000 patients monthly, and inpatients normally stay for three days and are then discharged.

Sample size: A total of 416 stool samples (one sample for each child) were randomly collected from children attending Al Nasser Pediatric Hospital in the period of 15 January to 7 May 2005. The collection of stool samples was performed twice per week from 8 a.m. - 12 p.m.

Ethical considerations: Permission was obtained from the director of Al-Nasser Pediatric Hospital, the Director of the hospital's laboratory and the Helsinki ethical committee to facilitate the collection of stool samples from children attending the hospital. Families of all children were informed verbally about the purpose of the study.

Questionnaire: All children or their guardians were interviewed. The designed questionnaire was reviewed by a consultant in public health and was piloted before starting the study. Age, sex, residence, clinical information, and family information data were recorded.

Parasitological Methods

Direct smear microscopy: Each stool sample was subjected to a systematic scanning after being processed onto a clean slide, where the stool sample was diluted with a drop of normal saline, and a cover slip was placed on this preparation. In the present study, one stool sample was used due to difficulty in collection of three samples.

Formal ether concentration technique: The present study employed just one method of concentration, formal ether sedimentation technique, to detect the parasite or oocyst.

Modified Ziehl-Neelsen (ZN) technique: This method was used in the present study. After spreading the sediment onto a slide, it was stained with Ziehl-Neelsen (ZN) and examined under light microscope.

Enzyme Linked Immunosorbent Assay (ELISA): Stool samples were extracted and processed according to the manufacturer's recommendations (International Immuno-Diagnostics, 2003). Absorbance was read at "wavelength 450nm" using StatFax ELISA reader.

Statistical Analysis

Data generated from the sampling program along with those from the questionnaire were uploaded to Statistical Package for Social Sciences SPSS (version 11). Frequency and cross-tabulation were performed.

Results

During the study period from January to May 2005, stool specimens from 416 children were examined; 122 (29.3%) children had diarrhea. Sixty-two (14.9%) were confirmed as true positive for *Cryptosporidium* (Table 1). Two protozoan types (*Entamoeba histolytica/dispar and Giardia lamblia)* but only one type each of round worms and tapeworms (*Ascaris lumbricoides* and *Hymenolepis nana*, respectively) were observed during the present study, as shown in Table 2.

It was found that the rate of infection by both types of detected protozoa was higher in boys than in girls, while the rate of infection by both helminths was higher in girls than in boys, as indicated in Table 2, but there was no statistical difference.

The clinical features and other variables with relation to *Cryptosporidium* are summarized in Table 3. The age group with the highest rate of positivity for cryptosporidiosis was 1-4 years. Twenty-eight of cases (20.3%) positive for *Cryptosporidium* were girls, while 34 cases (12.2%) were boys. There was a significant difference in the distribution of cases between boys and girls. Prevalence of *Cryptosporidium* infection was similar in March and April.

Discussion

Cryptosporidium parvum cause a persistent diarrhea in developing countries (16). In the present study, *Cryptosporidium* oocysts were detected by modified ZN in 62 (14.9%) out of 416 examined stool specimens. Slightly higher prevalence (19%) was reported by Sallon et al. (17) among children attending the same institution as in the present study, Al-Nasser Pediatrics Hospital. Osman et al. (18) reported a similar prevalence (14.19%) among Egyptian children and attributed the high prevalence to lack of specific treatment and public health awareness of cryptosporidiosis. Our poor hygiene practices and lack of public health awareness among the community also contributed to such a prevalence. This has been only the second study since 1990 to investigate the prevalence of cryptosporidiosis in Gaza, and the prevalence is still high. We found apparent significant difference among females (20.3%) and males (12.2%) positive for *Cryptosporidium* ($P = 0.03$), which may be explained by the fact that girls are more exposed to the source of infection than boys.

Table 1. Diagnostic techniques for *Cryptosporidium* by staining and antigen detection.

Examination technique	*Cryptosporidium* positive n = 416	Rates of detection
Ziehl-Neelsen staining	62	14.9
Antigen detection	68	16.3

Table 2. Parasite distribution according to sex.

Parasite	Infected boys n = 276		Infected girls = 138	
	No.	%	No.	%
Entamoeba histolytica/dispar	40	(14.5)	12	(8.7)
Giardia lamblia	20	(7.2)	4	(2.9)
Ascaris lumbricoides	2	(0.7)	4	(2.9)
Hymenolepis nana	2	(0.7)	4	(2.9)

Table 3. Different variables associated with *Cryptosporidium* infection (n = 416).

Variables		Positive for *Cryptosporidium*		Negative for *Cryptosporidium*		P-value
		No.	%	No.	%	
Age	< 1 year	42	13.2	276	86.8	>0.05
	1-4 years	12	22.2	42	77.8	
	>5 years	8	18.2	36	81.8	
Sex	Boys	34	12.2	244	87.8	0.030
	Girls	28	20.3	110	79.7	
Patient	In-patient	32	12.4	226	87.6	0.067
	Out-patient	30	19.0	128	81.0	
Constipation	yes	8	30.8	18	69.2	0.019
	no	54	13.8	336	86.2	
Abdominal pain	yes	12	19.4	122	34.5	0.012
	no	50	80.6	232	65.5	
Loss of appetite	yes	8	7.1	104	92.9	0.007
	no	54	17.8	250	82.2	
Diarrhea	yes	6	4.4	130	95.6	0.001
	no	56	20.0	224	80.0	
Seasonal variation	Jan	12	9.7	112	90.3	0.017
	Feb	14	11.1	112	88.9	
	March	22	21.2	82	78.8	
	April	14	22.6	48	77.4	
Years of education (father)	<12 y	26	13.3	170	86.7	0.043
	12-18 y	2	4.8	40	95.2	
	>18 y	34	19.1	144	80.9	
Parents' occupation	Laborer	48	15.7	258	84.3	0.627
	Employed	10	11.6	76	88.4	
	Unemployed	4	16.7	20	83.3	

P < 0.05: significant.
P > 0.05: not significant.

When coproantigen was used for the same stained specimens by ZN in the present study, six specimens were found to be positive for *Cryptosporidium*. Regarding coproantigen and acid-fast staining, similar numbers and similar percentages were shown using both techniques. It seems there was no statistical or observational difference. Staining of stool specimens for *Cryptosporidium* and other protozoa is not performed in the local laboratories of private and/or governmental hospital clinics and hospitals in Gaza Strip. Unfortunately, the dependence on direct microscopy using wet mount saline is the only method, which may affect the estimation of the prevalence of detected protozoa and/or epidemiological studies based on the Ministry of Health records. Most studies recommend that up to three separate, sequentially collected stool specimens should be examined in the laboratory to accurately diagnose enteric parasitic infection (19,20). In contrast, other studies have stated

that immunological-based detection methods are not significantly more sensitive than conventional microscopy (21,22).

In the present study, 22.2% of children positive for cryptosporidiosis were aged 1-4 years. Most studies have indicated that in developing countries, children may be most susceptible and those less than 2 years old may demonstrate the greatest prevalence (23,24). In another study, *Cryptosporidium* oocyst was found more among children aged below 2 years. MacPherson and Stephenson (25) reported that 30% of infected cases were aged 1-4 years, while Adegbola et al. (26) and Chacin-Bonilla et al. (27) reported that *Cryptosporidium* was frequent in children below 2 years. This high prevalence in children may be attributed to immature immunity and malnutrition (28) and the overall pattern suggests a decrease in prevalence after 12 months of age, which is most probably related to the development of immunity (29,30).

Despite age associations with *Cryptosporidium*, there was no clear trend in the prevalence of cryptosporidiosis with age in this study. In addition, it is known that children <5 years attend nurseries and kindergartens, where there is a little inspection for public health. The present finding indicated that 16.7% of infected children lived in houses with open sewage system versus 10.5% in houses with closed sewage system, with the higher rate due to more exposure to infection and contamination. The present study showed that diarrhea (4.4%) and other clinical features like constipation (30.8%), abdominal pain (9.0%), and loss of appetite (7.1%) were associated significantly with positive *Cryptosporidium* infection.

According to Al-Braiken et al. (31), the prevalence of mono-infection with *Cryptosporidium* among children with diarrhea (who presented to pediatric outpatient clinics) was 32% in Saudi Arabia. In Gaza, diarrheal disease is one of the greatest concerns among children. Another study carried out by Sallon et al. (17) showed that 19% of children with diarrhea were found to excrete *Cryptosporidium* oocyst. Inability of laborers and the unemployed to purchase medication may explain the high prevalence of *Cryptosporidium* among their children (15.7% and 16.7%, respectively) when compared to children of those employed (11.6%), which may be associated with the difficult socioeconomic conditions in which they live. Level of education was found to be associated with infection with *Cryptosporidium*. In our study, prevalence of cryptosporidiosis was observed in both March and April (21.2% and 22.6%, respectively), when there is transition from the cooler season to the start of the hot season. According to Sallon et al. [17], there was a striking difference in the prevalence of cryptosporidiosis between September 1989 (28%) and November 1989 (9.0%). Regarding seasonal activity, the hot season is more suitable for protozoal activity. It is concluded that cryptosporidiosis still constitutes a concern in Gaza and the diagnosis of *Cryptosporidium* should be improved in the governmental and private sector.

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