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ORIGINAL ARTICLE

Prevalence of *Cyclospora cayetanensis* among symptomatic and asymptomatic immune-competent children less than five years of age in Alexandria, Egypt

Naguib M. Massoud [a], **Doaa E. Said** [b,*], **Ahmed R. El-Salamouny** [a]

[a] *Department of Pediatrics, Faculty of Medicine, Alexandria University, Alexandria, Egypt*
[b] *Medical Parasitology, Faculty of Medicine, Alexandria University, Alexandria, Egypt*

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KEYWORDS

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Abstract *Background:* The objective of the present work is to determine the prevalence of *Cyclospora cayetanensis* in symptomatic and asymptomatic immune-competent children less than five years in Alexandria, Egypt.
Subjects and methods: This study was conducted on two groups: Group I: 100 children suffering from acute diarrhea for less than 14 days. Group II: 100 apparently healthy children without diarrhea. All patients were subjected to history taking, physical examination and stool examination by: direct smear examination, concentration using formol ether sedimentation and Sheather's sugar floatation technique and staining using modified Ziehl–Neelsen and modified trichrome stains.
Results: There was a significant difference between *Cyclospora* infected children in symptomatic (17%) and asymptomatic (6%) groups. *Cryptosporidium* was detected in 10 diarrheic children (10%), five cases were combined with *Cyclospora* infection and not detected in any of the asymptomatic group. *Microsporidia, Giardia lamblia and Hymenolepis nana* were also detected in the symptomatic group. There was no significant difference as regards age and residency of *Cyclospora* positive and negative cases in both groups. In asymptomatic group, *Cyclospora* infected cases were males while in negative cases, 50% were males. This was statistically significant. There was no significant difference between the type of feeding and the *Cyclospora* infected cases in the two groups. As regards weight for height standard deviation (SD), there was no significant difference between the number of cases below normal in infected and noninfected diarrheic children. All

Abbreviation: *C*. cayetanensis, *Cyclospora cayetanensis*.

* Corresponding author.
E-mail address: drdoaa_1995@hotmail.com (D.E. Said).

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asymptomatic cases were within the normal range without a significant difference. There was no significant difference between symptomatic *Cyclospora* infected and noninfected cases as regards the duration of diarrhea and clinical presentations.

Conclusion: *Cyclospora* infection in immune-competent symptomatic and asymptomatic children in Alexandria is common. Physicians should request a routine fecal examination for this parasite in any case with diarrhea or gastrointestinal troubles.

1. Introduction

Diarrhea is defined as the passage of three or more loose or liquid stools per day or more frequent passage than is normal for the individual.[1] For infants and children, this would result in stool output more than 10 g/kg/24 h, or more than the adult limit of 200 g/24 h.[2]

Diarrheal disease is the second leading cause of death in children under five years of age.[1] It accounts for a large proportion (18%) of childhood deaths, with an estimated 1.8 million deaths per year globally. The World Health Organization (WHO) suspects that there are more than 700 million episodes of diarrhea annually in children less than five years of age in developing countries. While global mortality may be declining, the overall incidence of diarrhea remains unchanged at about 3.2 episodes per child per year.[3]

Egypt Demographic and Health Survey (EDHS) 2008 noted that the prevalence of diarrhea among children under five years of age was nine percent. Children under age 24 months, particularly those age 6–11 months, were more likely to suffer from diarrhea than older children. Looking at the residential differentials, diarrheal episodes were more common among children living in Upper Egypt and the Urban Governorates than in Lower Egypt and the Frontier Governorates.[4]

Diarrhea is caused by a variety of bacterial, viral, and parasitic pathogens. In developed countries, the vast majority of episodes of diarrhea are caused by viral pathogens.[5–7] In developing countries with poor hygiene and sanitation, enteric bacteria and parasites are more prevalent.[8–10]

In Egypt, parasitic agents that most commonly cause acute diarrheal illness in children are *Cryptosporidium parvum, Giardia lamblia* and *Entamoeba histolytica*.[11]

Coccidian protozoa of genus *Cyclospora* are obligate intracellular apicomplexan parasites that infect the mucosal epithelium of the small intestine or bile duct of a variety of hosts, mostly vertebrates.[12] *Cyclospora* was first identified as a human pathogen in three patients from Papua, New Guinea but it was thought to be a coccidium, probably a new species of *Isospora*.[13] The parasite was described from human fecal material in Peru and was identified as a coccidian of the genus *Cyclospora* because when the oocysts were induced to sporulate, they yielded two sporocysts, each containing two sporozoites. The human species was named *Cyclospora cayetanensis*. It differs significantly from all other *Cyclospora* species not only in its host but also in its oocyst stage, which is much smaller and spherical rather than oblong.[14,15]

Cyclospora is an important emerging cause of diarrhea worldwide that leads to significant morbidity and mortality. In immune-competent hosts, mild-to-moderate, self-limiting diarrhea is common while in immune-compromised hosts, severe intestinal injury and prolonged diarrhea is observed.[16] The clinical presentation of *C. cayetanensis* also includes gastrointestinal (GI) symptoms such as loose or watery diarrhea, nausea, vomiting, abdominal cramps and loss of appetite; or constitutional symptoms such as unintentional weight loss, fever, chills, muscle aches, joint aches, generalized body aches, headache, or fatigue.[17] In developed countries, the disease has been associated with cases of travelers. The parasite is commonly isolated from travelers to Latin America, the Indian subcontinent and South East Asia.[18–20]

Direct person-to-person transmission of *Cyclospora* is highly unlikely because of the period needed by the oocysts outside the host to sporulate and become infectious.[19] Thus, a transmission vehicle must be involved. *Cyclospora* oocysts can be transmitted in humans through exposure to fecally contaminated environmental water, food or soil. In areas where environmental sanitation may be compromised, such as disadvantaged community settings, the frequency of transmission may be high.[21] Waterborne oocysts are a common source of infection, but definitive documentation is lacking.[19] A study designed to address the prevalence and risk factors for infection in El-Sharkia showed that water was an important source of infection. *Cyclospora* oocysts were detected in several water sources suggesting water as the main vehicle of transmission. The densities of water contamination by the oocysts indicated sewage contamination.[22] In Alexandria, the parasite was identified in different water sources, including swimming pools.[23] In the developing world, cyclosporiasis has been associated with eating vegetables in Nepal[24] and Jordan.[25] In Egypt, the coccidium was isolated from lettuce[26] and bivalves (shell fish) collected in markets from Alexandria.[27] Contact with soil has been a risk factor for cyclosporiasis. Studies from Peru,[28] Guatemala[29] and Egypt[22] showed this factor as an important source of infection among children.

Variations in prevalence of *Cyclospora* infections in endemic countries may be influenced by study design, geographic area, age, immunologic status of the population studied, seasonal variability of the parasite, methods of detection used and expertise of the microscopist.[21] From a review of 47,642 apparently immune-competent individuals attending health care centers in Peru, most of them with diarrhea, infection rates ranged from 0% to 13% (average 1.7%), whereas the isolation rates from matched asymptomatic controls varied from 0% to 4.2% (average 0.4%). Among 3340 immune-compromised persons, mostly HIV/AIDS patients with diarrhea, the percentages of *Cyclospora* infections ranged from 0% to 36% (average 4.5%). It appears that in endemic areas in Indonesia, the situation at the general population level is quite different than that observed

in health center populations in whom a strong association of the parasite with diarrhea has been recognized.[30–33]

The diagnosis of *Cyclospora* infection is based on microscopic detection of oocysts in fecal specimens. Examination of wet mounts of fresh, unpreserved stool by means of bright-field microscopy reveals nonrefractive spheres that are 8–10 μm in diameter and contain numerous refractive globules enclosed within membrane.[14] Because of potential low oocyst numbers, we routinely use a concentration procedure before examination. The standard formalin-ethyl acetate (FE) sedimentation (centrifugation) concentration procedure has been routinely used and found to be efficient.[34] Floatation procedures for the concentration of *Cyclospora* oocysts also can be used. A variety of solutions have been used to float parasite oocysts.[34] Sheather's sugar floatation procedure, as recommended for detection of *Cryptosporidium* oocysts, is also the preferred procedure for *Cyclospora*. The use of acid-fast-stained smears serves as the standard for detecting *Cyclospora* oocysts. Other stains include routine trichrome modified trichrome, Giemsa, chromotrope, Gram-chromotrope, Kinyoun acid-fast, auramine–rhodamine and safranin stains.[35] Polymerase chain methods (PCR) have also been developed for diagnosis and detection in the environment, but the primers appear to cross react with *Eimeria* spp.[36–38]

The objective of the present work is to determine the prevalence of *C. cayetanensis* in symptomatic and asymptomatic immune-competent children less than five years of age attending Alexandria University Children's Hospital.

2. Subjects and methods

The present study was conducted on 200 children less than five years of age presented to the outpatient clinic of Alexandria University Children's Hospital in the period from July through December 2010. Any case suffering from a chronic disease or receiving treatment leading to immune-suppression was excluded. They were classified into two groups: Group I: 100 children aged from one to 59 months old and suffering from acute diarrhea persistent for less than 14 days. Group II: 100 apparently healthy children attending the outpatient clinic due to other causes than diarrhea. All children were subjected to the following:

(1) Thorough history taking, stressing upon: demographic data (age, sex, residency), feeding history (breast, formula or cow's milk feeding), duration of illness, presence of blood in stool, associated symptoms as nausea, vomiting, or fever.
(2) Complete physical examination, including: anthropometric measures (weight, height, weight for height) temperature and degree of dehydration according to WHO guidelines (no signs of dehydration, some and severe dehydration). Systemic examination was done to detect any associated illness.

2.1. Investigations

2.1.1. Collection of stool samples

Stool was collected in a clean container: (1) for the potty-trained child, the child was instructed to have a bowel movement in the container without urination. (2) Child in diapers, an urine bag was placed on the child to prevent urine from coming into contact with the stool specimen. Specimens were collected in a disposable diaper by turning the diaper inside out with the plastic side facing the skin. Specimens collected on the absorbent side were not acceptable. Specimens were placed in the appropriate container. Patient name, date, & time of collection were written on the container. The specimens were transported to the Lab as soon as possible.[39]

2.2. Stool examination

(a) Direct smear examination (saline and iodine smear).[40]
(b) Concentration of each sample using Sheather's sugar floatation technique and formol ether sedimentation technique.[40]
(c) Staining using modified Ziehl–Neelsen[41] and modified trichrome[42] stains

Cyclospora oocysts were identified by their size measured by ocular micrometer and morphological criteria of different stains.[41–43] Other pathogenic parasites found in the samples were also detected.

2.2.1. Data analysis

Statistical analysis was performed with SPSS (Version12). It was presented as mean (X) ± standard deviation (SD) for each subgroup and compared using Fisher Exact test (FEp) to assess the difference of means among the two groups and Mann–Whitney nonparametric test (MWp) and Monte Carlo test (MCp) to assess differences in two independent groups. Probability level of $\leqslant 0.05$ was considered significant.

2.2.2. Ethical considerations

Ethical approval was obtained from the committee of research, Publications and Ethics of Alexandria University. All procedures were explained to parents or guardians of the participating children and written informed consent was obtained. Infected patients were informed of their diagnosis. Concerned physicians were also informed in order to prescribe suitable treatment and follow-up the patients.

3. Results

3.1. Analysis of symptomatic and asymptomatic groups

The age of the diarrheic cases ranged between 1 month and 59 months, with a mean age of 13.01 ± 15.23 months. Whereas the age of the asymptomatic group ranged between 1 month and 42 months with mean age of 16.47 ± 12.80 months. This difference was statistically significant ($p = 0.020$). Regarding sex, the percentage of males among symptomatic cases was 65%, whereas in asymptomatic cases, it was 56%; however, this was not statistically significant ($p = 0.193$). As regards residency, 68% of symptomatic and 62% of asymptomatic cases were from rural areas; this also was not statistically significant ($p = 0.374$). 52% of symptomatic cases were breast fed, 47% were formula fed, and 1% was cow milk fed, whereas 64% of asymptomatic cases were breast fed and 36% were formula fed, with no statistical significance between the two groups

($p = 0.120$). As regards the nutritional state of the two studied groups (weight percentile, height percentile and weight for height standard deviation) one third of diarrheic cases were below 3rd percentile. However in the nondiarrheic cases, all children were in the normal range (between 3rd and 97th percentile). This difference was statistically significant ($p \leqslant 0.001$). At the time of presentation, 88% of cases presented with vomiting, 73% with fever, 3% with dysentery and 61% came with moderate or severe dehydration.

3.2. Analysis of *Cyclospora* infected cases

3.2.1. Results of stool analysis

Cyclospora was detected in stool of 17 children among symptomatic (17%), and of 6 children among asymptomatic cases (6%) (Fig. 1). This was statistically significant ($p = 0.015$). The pure *Cyclospora* infected cases were 12 only (12%), but this was not statistically significant ($p = 0.387$). *Cryptosporidium* was detected in 10 diarrheic children (10%), five cases were combined with *Cyclospora* infection (these cases were excluded from the total number of *Cyclospora* infected cases as shown above) and the other five cases were pure *Cryptosporidium* infection while it was not detected in any of control group, which was statistically significant when compared with the control group ($p = 0.031$). *Microsporidia* was detected in five diarrheic children (5%) and not in the nondiarrheic group. *G. lamblia* was also detected in two cases of diarrheic children (2%) and one case of control group (1%). H*ymenolepis nana* eggs were detected in one case of diarrheic children (1%) and not in any of the control group (Table 1).

3.2.2. Demographic data

The results were summarized in Table 2. There was no significant difference as regards age and residency of *Cyclospora* positive and negative cases in the symptomatic and asymptomatic groups. Ten out of twelve (83.3%) symptomatic *Cyclospora* infected cases were less than two years of age. Two out of six (33.3%) patients of asymptomatic infected cases were less than two years of age.

As regards the gender, *Cyclospora* positive cases in the symptomatic group are not statistically significant when compared with negative cases ($p = 0.606$). However in the asymptomatic group, all *Cyclospora* infected cases were male while in negative cases, 50% were males. This was statistically significant.

3.2.3. Feeding

There was no significant difference between the type of feeding and the *Cyclospora* infected cases in the two groups as shown in Table 3.

3.2.4. Anthropometric measures

In the symptomatic group, Weight percentile of 25% of *Cyclospora* cases was below third percentile. Among *Cyclospora* negative cases, 28.4% were below third percentile in growth curves. There was no significant difference between the two groups ($p = 0.175$). As regards height percentile, 33.3% of *Cyclospora* cases in symptomatic group were below third percentile. While in *Cyclospora* negative cases, 21.6% were below third percentile. Also there was no significant difference ($p = 0.107$). Weight for height standard deviation (SD) for 33.3% of *Cyclospora* cases with diarrhea was below -2 SD. Whereas in *Cyclospora* negative cases in the same group, 17% of them were below -2 SD which is the normal value for this age group. There was no significant difference between the two groups ($p = 0.192$). As regards the asymptomatic cases, all of them were between 3rd and 97th percentiles of weight and height. Also, weight for height standard deviation (SD) of all these cases was within the normal range. There was a significant difference between nondiarrheic and diarrheic cases (Table 4).

3.2.5. Duration of diarrhea

The duration was selected to be less than 14 days to fulfill the criteria of acute infection. The difference between the duration

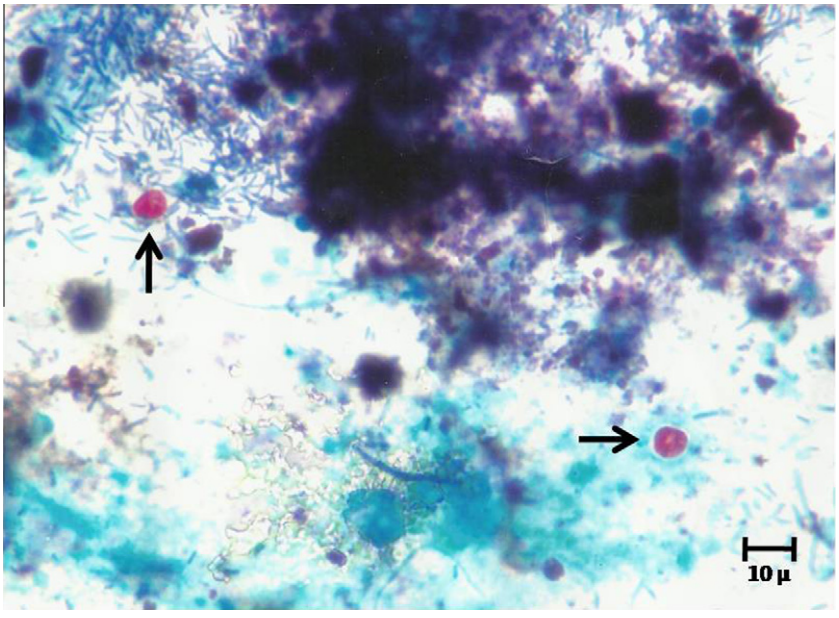

Figure 1 *Cyclospora cayetanensis* oocyst (arrows) in stool samples stained with Modified Ziehl–Neelsen stain ×1000.

Table 1 Different parasites detected in stool samples from diarrheic and nondiarrheic children included in this study.

	Group I		Group II		Total		Test of sig.
	No.	%	No.	%	No.	%	
Cyclospora cases							
Pure	12	12.0	6	6.0	18	9.0	$p = 0.138$ FEp = 0.059
Mixed (*Cyclospora* + *Cryptosporidia*)	5	5.0	0	0.0	5	2.5	
Cryptosporidia	5	5.0	0	0.0	5	2.5	FEp = 0.059
Giardia lamblia	2	2.0	1	1.0	3	1.5	FEp = 1.000
Microsporidia	5	5.0	0	0.0	5	2.5	FEp = 0.059
Hymenolepis nana	1	1.0	0	0.0	1	0.5	FEp = 1.000
No parasite	70	70.0	93	93.0	163	81.5	$p < 0.001$*
Total	100	100.0	100	100.0	200	200.0	

Group I: cases with diarrhea Group II: nondiarrheic cases.
FEp: Fisher Exact test.
* Statistically significant at $p \leqslant 0.05$.

Table 2 Demographic data of *Cyclospora* positive and negative cases among symptomatic and asymptomatic groups.

	Group I				Group II			
	−ve		+ve		−ve		+ve	
	No.	%	No.	%	No.	%	No.	%
Sex								
Male	58	65.9	7	58.3	50	53.0	6	100.0
Female	30	34.1	5	41.7	44	46.8	0	0.0
Test of sig.	$p = 0.606$				FEp = 0.033*			
Age								
< 2 years	75	85.2	10	83.3	60	63.8	2	33.3
⩾2 years	13	14.8	2	16.7	34	36.2	4	66.7
Test of sig.	1.000				FEp = 0.179			
Range of age	1.0–59.0		2.0–36.0		1.0–42.0		3.0–35.0	
Mean ± SD	13.05 ± 15.82		12.75 ± 10.44		16.18 ± 12.64		21.0 ± 14.64	
Median	7.0		8.0		12.0		25.0	
Test of sig.	0.523				MWp = 0.581			
Residency								
Urban	29	30.0	3	25.0	94	100.0	6	100.0
Rural	59	67.0	9	75.0	0	0.0	0	0.0
Test of sig.	0.747				–			

Group I: cases with diarrhea Group II: nondiarrheic cases.
MWp: p value for Mann–Whitney test.
FEp: p value for Fisher Exact test.
* Statistically significant at $p \leqslant 0.05$.

Table 3 Relation between *Cyclospora* positive and negative cases and type of feeding in symptomatic and asymptomatic groups.

	Group I				Group II			
	−ve		+ve		−ve		+ve	
	No.	%	No.	%	No.	%	No.	%
Feeding								
Breast feeding	48	54.5	4	33.3	60	63.8	4	66.7
Formula feeding	39	44.3	8	66.7	34	36.2	2	33.3
Cow milk	1	1.1	0	0.0	0	0.0	0	0.0
Test of sig.	MCp = 0.309				FEp = 1.000			

Group I: diarrheic cases Group II: nondiarrheic cases.
MCp: p value for Monte Carlo test.
FEp: p value for Fisher Exact test.

of diarrhea in *Cyclospora* infected and noninfected cases of symptomatic group was not statistically significant ($p = 0.141$), as shown in Table 5.

3.2.6. Clinical presentation

As shown in Table 6, there was no significant difference between symptomatic *Cyclospora* infected and noninfected cases as regards vomiting, fever, dysentery and dehydration.

4. Discussion

Diarrheal disease is the second leading cause of death in children under five years old, and is responsible for killing 1.5 million children every year. Globally, there are about two billion cases of diarrheal disease every year, and mostly results from contaminated food and water sources.[1] In Egypt, enteric pathogens were identified in 46% of children less than five years of age.[44]

C. cayetanensis is a coccidian protozoan that has emerged as an enteric pathogen.[14] Numerous recent reports implicating *Cyclospora* in diarrheal disease have suggested that the organism has a wide geographic distribution, however, most of these reports have been among adults with a history of travel to developing countries or among human immune-deficiency virus-infected individuals.[45–47]

In the present work, there was a significant difference between age of diarrheic, and nondiarrheic groups. Also, there was a significant difference between the two groups as regards the weight for age, the height for age and weight for height standard deviation. This means that diarrhea affect the nutritional state of children in this age group significantly. These findings may have important implications for the effects on growth at age less than 24 months. These results were supported by previous studies that have shown deleterious effects on growth and development after symptomatic infections in children who acquired the infection at less than one year of age. Also boys were affected more significantly rather than girls.[48]

At the time of presentation, 88% of cases presented with vomiting, 73% with fever, 3% with dysentery and 61% came with moderate or severe dehydration. In accordance with several studies in the same age group, in Fayoum in 2006, 84% had fever, 54% had vomiting, 9% had dysentery, and 48% came

Table 4 Relation between *Cyclospora* and anthropometric measures in the two studied groups.

	Group I				Group II			
	−ve		+ve		−ve		+ve	
Wt for age								
Range	0.50–97.0		2.90–41.0		3.50–91.0		39.0–78.0	
Mean ± SD	31.75 ± 30.53		13.33 ± 11.30		48.08 ± 23.48		61.33 ± 17.99	
Median	20.0		12.50		51.0		67.0	
p	0.180				0.172			
Wt percentiles								
<Third percentile	25	28.4	3	25.0	0	0.0	0	0.0
3–97 percentile	63	71.6	9	75.0	93	100.0	6	100.0
FEp	1.000				–			
Ht for age								
Range	2.90–99.0		2.90–38.0		3.30–86.0		19.0–69.0	
Mean ± SD	33.13 ± 28.85		16.58 ± 14.34		40.46 ± 21.72		49.0 ± 23.66	
Median	29.0		9.0		40.0		59.0	
p	0.107				0.257			
Ht percentile								
<Third percentile	19	21.6	4	33.3	0	0.0	0	0.0
3–97 percentile	67	76.1	8	66.7	94	100.0	6	100.0
>97	2	2.3	0	0.0	0	0.0	0	0.0
MCp	0.592				-			
Wt for Ht								
Range	−8.80–2.66		−2.69–0.39		−1.80–2.21		0.26–0.84	
Mean ± SD	−0.66 ± 1.74		−1.11 ± 1.06		0.15 ± 0.78		0.47 ± 0.29	
Median	−0.37		−0.98		0.24		0.30	
p	0.192				0.172			
SD								
Normal	73	83.0	8	66.7	94	100.0	6	100.0
Abnormal	15	17.0	4	33.3	0	0.0	0	0.0
FEp	0.234				–			

Group I: cases with diarrhea Group II: nondiarrheic cases.
Wt: weight Ht: height.
MWp: *p* value for Mann–Whitney test.
MCp: *p* value for Monte Carlo test.
FEp: *p* value for Fisher Exact test.

Table 5 Relation between *Cyclospora* positive and negative cases and duration of diarrhea in the symptomatic group.

	Cyclospora		Test of significance
	−ve (n = 88)	+ve (n = 12)	
Duration of diarrhea			
Range	1.0–13.0	1.0–7.0	MWp (p = 0.141)
Mean ± SD	4.52 ± 2.71	3.33 ± 1.67	
Median	4.0	3.0	

MWp: p value for Mann–Whitney test.
* Statistically significant at $p \leqslant 0.05$.

Table 6 Relation between *Cyclospora* positive and negative cases and clinical presentations in the symptomatic group.

	Cyclospora				Test of significance
	−ve (n = 88)		+ve (n = 12)		
	No.	%	No.	%	
Vomiting					
No	12	13.6	0	0.0	FEp = 0.351
Yes	76	86.4	12	100.0	
Fever					
No	22	25	5	5.7	FEp = 0.222
Yes	66	75	7	58.3	
Dehydration					
No	35	39.8	4	33.3	MCp = 0.767
Mild	40	45.4	7	58.3	
Severe	13	14.8	1	8.4	

FEp: p value for Fisher Exact test.
MCp: p value for Monte Carlo test.
* Statistically significant at $p \leqslant 0.05$.

with dehydration.[44] In a study in Nepal in 2004, 67% had abdominal pain, 38% had vomiting, and 9% were reported to have had blood in their stools.[49]

The current study findings revealed that *Cyclospora* infection was common among immune-competent children with 23% prevalence. Prevalence of cyclosporiasis in diarrheic children was 17% while of asymptomatic nondiarrheic children it was 6%. Because of the absence of follow-up information it is not known if the six infections in controls represented true asymptomatic carriage, as has been documented in Peru,[28] or infection during the incubation period prior to developing symptoms. In comparison with previous studies, it was detected in 12.1% of cases and 5.7% of controls in Nepal,[50] 11% of cases and 4.2% of controls in Saudi Arabia,[31] in 9% of cases in El-Menoufia[51] and Jordan,[25] with no available data about the asymptomatic control in the last two studies. Higher prevalence rates were reported in Ismailia, Egypt (19.6% of immune-competent and 34.4% of immune-compromised children with diarrhea)[52] and outside Egypt in Peru (14.3% of cases and 13.7% of controls).[53] Lower prevalence rates were reported in Cuba (4.4% in cases with no infection in controls),[32] and in another study in Nepal in 1995 (5% of cases and 2% in controls).[49]

Detection of *Cryptosporidium* in 10% of cases but not in any control, in the present study, was in accordance with previous studies in Ismailia (11.6% of immune-competent diarrheic children and 0% in the controls)[52] and Cuba (11.5% of cases and 0% in the controls),[32] and it was less prevalent in Nepal (5%),[49] and Peru (3.4%)[53] of cases and also 0% in the controls. Five cases out of ten in diarrheic children infected with *Cryptosporidia* were co-infected with *Cyclospora*. These five co-infected cases were excluded from the 17 cases infected with *Cyclospora* because the cause of diarrhea may be due to one or both parasites. *Cryptosporidium* was the only associated parasite with *Cyclospora* among the symptomatic group. The high rate of co-infection in cyclosporiasis patients was also found in Peruvian children with a prevalence rate of 13%, and frequent multiple parasitism in 45.6%.[54] The high rate of co-infection between cyclosporiasis and cryptosporidiosis may cause nonspecific symptoms, including abdominal pain, loose or watery stool, which could easily be confused with other common intestinal diseases. In the present study, this finding was expected as cryptosporidiosis in most surveys was among the four major pathogens causing diarrheal diseases in children.[55]

The age of *Cyclospora* positive cases ranged from 2 to 36 months. Ten of twelve cases (83.3%) of *Cyclospora* diarrhea were less than two years of age; a finding similar to that of Ortega et al.[14] Abdel-Wahab et al.[52] revealed that the infection rate was significantly higher in the age group 1–4 years and that infection rate decreased with age.[52] On the other hand, it was suggested that the development of partial immunity in older age groups protects them from the pathogenic effects of the coccidium, but not from re-infection.[56] Studies from Peruvian towns suggest that immunity becomes complete by adolescence.[57] Bern et al.[54] stated that after an initial episode of cyclosporiasis, the likelihood of diarrhea decreased significantly with each subsequent infection.[54] There was no significant difference between the number of males and females among positive *Cyclospora* cases as many previous studies reported.[49,52,58]

In agreement with other studies,[52,56] most of the infected children (75%) were living in rural areas. The authors related their data to personal hygiene and living environmental condition. In rural areas, plenty of simple toilets, deficiency of sanitary facilities and diffusing feces contamination were commonly seen, and most people were unaware of health knowledge and good hygiene habits.

In the present study, 66.7% of *Cyclospora* cases were formula fed, whereas 33.3% were breast fed, so breast-feeding showed a trend toward being protective against *Cyclospora* infection. Similar finding was also observed by Hoge et al.[49]

Regarding nutritional state, 33.3% of *Cyclospora* cases were malnourished (they had Z-scores of less than −2 SD for weight-for-height). The same percentage was detected by Nimri et al.[25]while it was 20% in Abdel-Wahab et al. study.[52] Rizk et al. detected *Cyclospora* among 5.6% of malnourished children.[59] Moreover, severe malnutrition was associated with *C. cayetanensis* and *Cryptosporidium* spp. causing persistent diarrhea in Nepal.[60]

At the time of presentation, duration of diarrhea among *Cyclopspora* cases ranged from 1 to 7 days, with mean duration of 3.33 ± 1.67 days. Also it was ranged from 3 to 18 days with a mean of 7.2 ± 6.14 days in Nepal.[49] Abdel-Wahab et al.[52] reported that 83% of *Cyclospora* diarrhea had duration of 3 days or less.[52] However, there were reports of a longer duration of diarrhea in children with *Cyclospora* in El-Menoufia (mean duration of illness was 28 ± 8 days).[51]

Among *Cyclospora* diarrhea, 100% of cases presented with vomiting, 58.3% with fever, no case presented with dysentery. One third of cases came with no dehydration, 58.3% with mild dehydration, and 8.4% with severe dehydration. In Ismailia, 96% of cases were associated with abdominal pain, 80% with fever, 76% with nausea, 35% with flatulence, 17% with vomiting and 1.6% with dysentery.[52] In Cuba, clinical characteristics associated with *Cyclospora* were abdominal pain (80%), vomiting (60%), fever (40%) and anorexia (20%).[32] In Peru, two types of clinical manifestations were found; an acute type that can cause dehydration, and a chronic condition with several digestive signs and/or symptoms, particularly abdominal pain.[61] Puente et al.[20] detected heart burn as a frequent symptom, a finding not often previously described. On the other hand, dehydration was reported as the only significant manifestation in infected patients from Colombia.[62]

5. Conclusion

In conclusion, *Cyclospora* infection in immune-competent symptomatic and asymptomatic children in Alexandria is common. Further studies in indigenous populations are needed to determine the relative rates of symptomatic and asymptomatic infection among persons of all ages (including older children and adults), assess natural sources of the parasite and routes of transmission as well as seasonality as risk factor for infection. Physicians should request a routine fecal examination of this parasite in any case with diarrhea or gastrointestinal troubles.

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