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EVALUATING THE FREQUENCY OF CRYPTOSPORIDIOSIS IN CHILDREN UNDER FIVE YEARS OF AGE PRESENTING WITH DIARRHEAL DISEASE

By

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Abstract

Cryptosporidium species are the second leading cause of diarrheal disease and death in children of developing countries. This study aimed to determine the prevalence rate of *Cryptosporidium* among children under 5 years old with diarrhea, who were admitted to a big tertiary hospital in Egypt.

This was a hospital-based cross-sectional study involving children 200aged <5 years old in the gastroenterology causality at Cairo University Pediatric Hospital between February 2021 and October 2021. Morning stool samples were microscopically examined as fresh smears and modified Ziehl-Neelsen stained materials.

The results showed that all participants suffered from diarrhea <30 months old. A high prevalence 21/200 (10.5%) of *Cryptosporidium* was detected. But, there was no significant difference between cryptosporidiosis positive or negative cases with respect to stain technique, age, sex, residence, Z-score of weight, symptoms, or stool consistency.

Keywords: Children, diarrhea, Prevalence, treatment

Introduction

Generally, the diarrheal disease is the second predisposing cause of death in children <5 years old, with ~525000 deaths annually (Levine *et al,* 2020). Gargala *et al.* (2017) in France reported that cryptosporidium is the most important diarrhea-causing protozoan parasite, with severe health consequences for very young, malnourished children living in endemic areas and for immunocompromised ones widely transmitted through person-to-person or animal-to-person contact, or contaminated food or water drinking or swimming. The recent occurrence of large water- and foodborne outbreaks in several European Countries, as well as the results of many surveys of human and animal cryptosporidiosis, indicate that this parasite is widespread (Steiner *et al,* 2021). In low middle-income countries, a mild infection is characterized by watery diarrhea, but a severe infection led to diarrhea-related deaths (Yang *et al,* 2021). Ben Abda *et al.* (2011) in Tunisia reported that cryptosporidiosis with serious clinical symptoms was in children, particularly those with CMH class II deficiency syndrome, and that clinical and laboratory diagnosing causing agent of diarrhea was a must. Abdel Wahed *et al.* (2021) in Egypt reported that most studies on gastroenteritis focused on the viral and bacterial infections, while gastroenteritis, whereas *Cryptosporidium* infection among children caused acute gastroenteritis They concluded that the clinical picture of cryptosporidiosis infected children didn't significantly different from those presented with gastroenteritis due to any other causes. Collinet-Adler and Ward (2010) in USA reported that the *Cryptosporidium* spp. were responsible for endemic and epidemic disease worldwide with severity ranged from asymptomatic or mild to severe, intractable diarrhea with wasting depending on immune status, nutrition, and age. They added that although it is often self-limited in healthy persons, yet therapy remained a challenge in the immune-compromised, and prevention depended on appropriate hygiene and proper water management and treatment. Hanieh *et al.* (2021) in Austral reported that *Cryptosporidium* infection played a contributory role in the childhood malnutrition, having been

linked to the impaired physical fitness in late

This study aimed to determine the prevalence rate of *Cryptosporidium* spp. among children suffering from diarrhea < 5 years of age admitted to a Tertiary Hospital in Egypt.

Subjects and Methods

This cross-sectional study was conducted in the Gastroenterology Causality, Cairo University Pediatric Hospital between February 2021 and October 2021. It included 200 infants and children of both sexes with ages <5 years. All suffered from acute gastroenteritis, with associated comorbidities as congenital heart disease, inborn error of metabolism, and chronic renal or hepatic illness.

The study protocol was approved by the Research Ethics Committee of Pediatrics Department, Cairo Faculty of Medicine (approval: n-57). All research procedures were carried out according to the Helsinki Declaration (2000). All children were enrolled in the study after having informed consent from their parents/guardians. Patients beyond the age limit were excluded, and medical sheets were filled out on each child

All patients underwent a full clinical history assessment, emphasizing the history of present acute gastroenteritis attack (onset, course, nature of diarrhea consistency, and with or without vomiting) and history of fever, cough, and chronic illness. Also, they were subjected to anthropometric measurements, and weight was measured without diapers in younger children using an electronic digital calibrated scale, older ones were measured wearing only underwear and no shoes, following the Pedi-Tools electronic growth chart calculators (Chou *et al,* 2020)

Stool examination: Morning stool samples without any urine contamination were collected in labeled disposable carton boxes. Stools examined were examined macroscopically for consistency, pinworm, gravid segments, mucus and blood. Then, examined microscopically by direct wet smears, concentration techniques **(**Cheesbrough, 2000**)**, staining smears by using Trichrome stain and Modified Ziehl-Neelsen stain (El Shazly childhood and perhaps youth.

et al, 2007). Safety measures against potenti al infection during handling and dealing and disposal of samples were applied (Sewell, 2004). Microscopic identification of *Cryptosporidium spp*. was done by correlating the morphology with a control slide and measured by eyepiece and slide micrometer. All reagents were labeled with (i) content, (ii) concentration, (iii) date prepared, (iv) date placed in service, and expiration date. Each new batch of stain was routinely checked with control specimens for correct staining procedures before use.

Statistical analysis: Data values were revised for completeness and logical consistency. Data were computerized and analyzed using the Microsoft Office Excel Software Program 2017. Recoded data were then transferred and entered into the Statistical Package of the Social Science Software program, version 26 (SPSS), for statistical analysis. Quantitative variables were described as mean, standard deviation, median, and interquartile range. These results were compared using the Mann–Whitney U test, with a significant p-value = <0.05. Qualitative variables were described as frequency and percentage. These results were compared using the Chi-square test or Fisher exact test, accordingly, where a significant p-value is <0.05.

Results

Children (200) were 124 (62%) were males and 76 (38%) were females, of whom 153 (76.5%) were from Cairo, 11 (5.5%) from Giza, and 36 (18.0%) from other governorates.

All the positive cases (21) were equal to or <30 months old. Three were > 30 months (P= 0.55). The winter season rate of *Cryptosporidium* infections was significantly higher than other seasons.

Children average weight was 7.2±2.5kg with a mean Z-score of -1.7±2.01, 83 of them (91.2%) had a normal weight Z-score and 96 patients (88.1%) were underweight, but without significant difference between the normal and underweight cryptosporidio-

sis patients as positive stain concentration.

All the patients were presented with diarrhea, 153 (76.5%) with fever, among whom 95 (47.5%) suffered from vomiting, 137 (68.5%) had dehydration with mean of (8±3) diarrhea episodes of the 78 (39.2%) suffered from cough associated with diarrhea, two children had associated comorbidities of the cerebral palsy or hydrocephalus.

Macroscopic examination showed different stool consistencies; 10.5% with formed one, 47.5% with semi-formed, and 42% were loose, 78 (39%) had mucous in stools, but none showed bloody stool.

The microscopic examination showed four intestinal protozoa: *Blastocystis hominis* (7), *Giardia lamblia* cyst (1), *Entamoeba coli* cyst (1), and *Cryptosporidium parvum* oocysts were 11/200 (5.5%). The child was considered cryptosporidiosis *parvum* infected patient when the stool sample was positive with stain concentration method. Thus, *Cryptosporidium parvum* was identified in 21 (10.5%), which were also positive cases by direct stain method with a significant value ($P < 0.05$).

Details were given in tables (1 & 2) and figure (1).

Table 1: Epidemiological and clinical data as to positive and negative children

Variable	Negative cases	Positive cases	P-value
Age in months ≤30 months	176 (89.3%)	21(10.7%)	0.55
>30 months	3 (100%)	0(0.0)	
Male	109(87.9)	15(12.1%)	0.347
Female	70 (92.1%)	6 (7.9%)	
Residence: Urban	146 (89%)	18 (11%)	0.64
Rural	33 (91.7%)	3 (8.3%)	
Fever: No	44 (93.6%)	3 (6.4%)	0.292
:Yes	135 (88.2%)	18 (11.8%)	
Vomiting: No	97 (92.4%)	8 (7.6%)	0.162
: Yes	82 (86.3%)	13 (13.7%)	
Dehydration: No	57 (90.5%)	6 (9.5%)	0.76
: Yes	122 (89.1%)	15 (10.9%)	
Comorbidity: No	177 (89.4%)	21 (10.6%)	0.626
: Yes	2 (100%)	0 (0.0)	
Stool color: Brown	30 (81.1%)	7 (18.9%)	0.31
: Green	40 (90.9%)	4 (9.1%)	
: Orange	1 (100%)	0 (0.0)	
: Whitish	10 (76.9%)	3 (23.1%)	
: Yellow	58 (90.6%)	6 (9.4%)	
: Yellowish brown	25 (96.2%)	1 (3.8%)	
: Yellowish green	14 (100%)	0 (0.0)	
Stool consistency: Formed	18 (85.7%)	3 (14.3%)	0.384
: Semi-formed	88 (92.6%)	7 (7.4%)	
: Loose	73 (86.9%)	11 (13.1%)	
: Watery	0 (0.0)	0 (0.0)	
Blood in stool: No	179 (89.5%)	21 (10.5%)	0.67
: Yes	0 (0.0)	0 (0.0)	
Mucous in stool: No	112 (92.6%)	9 (7.4%)	0.127
: Yes	67 (85.9%)	11 (14.1%)	
Another parasite: No	171 (89.5%)	20 (10.5)	0.952
: *B. hominis*	6 (85.7%)	1 (14.3%)	
: *E. coli* cyst	1 (100%)	0 (0.0)	
: *Giardia* cyst	1 (100%)	0 (0.0)	
Z-score: underweight (Z< −1)	96 (88.1%)	13(11.9%)	0.471
: normal (Z-score >−1)	83(91.1%)	8 (8.8%)	

Table 2: Stain technique results

Test	Positive	Negative	P-value
Direct stain	4 (36.4%)	175 (92.6%)	<0.001
Stain concentration	7 (63.6%)	14 (7.4%)	

Discussion

Cryptosporidium is an opportunist disease, causing diarrhea and intestinal disorders in immune-deficient and immuno-compromised individuals, but it is self-limiting in immuno-competent hosts (Liu *et al,* 2012). Guerrant *et al*. (1999) in Brazil reported that the early child- hood cryptosporidiosis was associated with growth retardation, cognitive deficits, and a higher overall risk of mortality. Sarkar *et al.* (2014) in India reported that factors associated with acquisition of cryptosporidiosis in children were assessed over a range of demographic, socio-economic, nutritional, hygiene, and environmental variables. Checkley *et al.* (2015) in USA reported that cryptosporidiosis is well documented cause of diarrheal disease during waterborne epidemics and in immunocompromised hosts. They added that infection incrimination increased as an important cause of morbidity and mortality worldwide, and that partial immunity after exposure suggested the potential for successful vaccines, and several were in development; however, surrogates of protection are not well defined. Also, Murphy *et al.* (2015) in USA reported that an infected swimmer excreted 107-108 oocysts in a diarrheal incident in water that can survive >7 days at CDC-recommended concentrations of >1ppm free available chlorine. Chalmers and Cacciò **(**2016**)** in United Kingdom concluded that increased dependable *Cryptosporidium* diagnostic practices were fundamental to the meaningful interpretation of surveillance data, species distribution and genotypes. They added that a web-based database for interpretation of genotype and distribution in an epidemiological context is indicated. El-Bahnasawy *et al.* (2018) in Egypt reported endemic zoonotic cryptosporidiosis, and Ibrahim *et al.* (2019) in Egypt added that in infected children *Helicobacter pylori* supported colonization by *Giardia intestinalis* and *Cryptosporidium parvum* or vice versa. Ahmed and Karanis (2020) in the Gulf Countries reported that water contamination, imported food, animal contact, and air transmission were discussed in detail, to address their significant role as a source of infection, and so, their impact on disease epidemiology among the populations.

In the present study, ages of cryptosporidiosis were not significant although the highest prevalence was among children aged up to 30 months old. This agreed with Tombang *et al.* (2019**)** in Cameron who showed the *Cryptosporidium* rate was 8.9% among hospitalized children aged up to five years, and that cryptosporidiosis was the etiologic agent of diarrhea among them.

In the present study, *Cryptosporidium* oocysts were slightly more in females 6 (7.9%) than in males 15 (12.1%), but without significant difference (P = 0.347). Tamomh *et al.* (2021) in Sudan reported a prevalence of 19/70(27.1%) of *Cryptosporidium* among children both sexes with diarrhea than those without diarrhea 7/80 (8.8%), and the main source in children was the consumption of piped-water sources. Of these patients, 11.8% were from Cairo and 8.3% lived in other rural areas. The difference in residences may be related to the hospital location. Meanwhile, the treatment and cleaning of water treatment plants in the communities were highly irregular, which could certainly be a contributing factor. Also, the rate of malnourished children <5 years of age in this study population (00%) was lower than the national rate of 36% found at the national level **(**Kabayiza *et al***,** 2014). This may explain why *Cryptosporidium* infection appears to be delayed until six months and beyond when complementary foods are introduced. The infection was common in children, but decreased with increasing in age, suggested the development of immunity from frequent exposure to infective agents in the environment (WHO, 2017).

In the present study, among 200 children <5 years with diarrhea, 21 were cryptosporidiosis positive (10.5%). This agreed with Goodgame *et al.* (1993**)** who in 10.4% Tanzania and 9.4%, Ethiopia. Abdel Gawad *et al*. (2018) in Egypt among diarrheic immunocompetent patients by PCR reported high

rate (21.0%), compared to ELISA (12.5%) and MZN staining (9.5%).

In the present study, positive cases were treated according to CDC (2021) who declared that the anti-diarrheal medicine may help slow down diarrhea, but a healthcare provider should be consulted before such medicine is taken. Nitazoxanide is FDA-approved to treatment diarrhea caused by *Cryptosporidium* in people with healthy immune systems and is available by prescription.

Conclusions

Cryptosporidium is paediatric infection co mmon in preschool, and infants with gastrointestinal manifestations mainly diarrhoea.

This study showed an overall prevalence of 10.5% for cryptosporidiosis among children of ages <5 years that attended the gastro enterology causality at Cairo University Pediatric Hospital. The study clearly proved that *Cryptosporidium* is the protozoan etiologic agent affecting the small children in the tertiary hospital.

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Fig. 1: Bar chart showed relationship between seasons and Cryptosporidiosis positive cases