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CRYPTOSPORIDIUM PARVUM INFECTION AMONG EGYPTIAN SCHOOL CHILDREN

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Abstract

The present study determined cryptosporidiosis among 120 randomly chosen school children aged 4-16years. Medical sheets were filled out on each child. The fresh stool samples were examined by using Sheather's sugar flotation stained with modified Ziehl Neelsen stain. Blood samples were examined by ELISA and IFA techniques.

The results revealed that, the prevalence rate was 13.51% with a peak among the age group (5-10) and. significant relation between males and females. There was a significant relation between infection and low socio-economic level in rural area. Also, a significant relation was obtained between the infection and the presence of animal contact. Watery and loose diarrhea was more significant among infected children. Positive stool samples were among 37 (30.8 %), while ELISA and IFA detected 30 (25%) and 33 (27.5%) respectively. The validity test of ELISA declared sensitivity andspecificity with 93.3% and 90% while IFA declared 90.9% and 91.1% respectively.

Keywords: Cryptosporidiosis, Children, Sheather sugar flotation, Ziehl-Neelsen, ELISA, IFA.

Introduction

Cryptosporidium species is a pathogen with a worldwide distribution and predicted to be the highest in developing countries especially in children. Diarrhea caused by *C. parvum* in childhood may be associated with subsequent impaired physical and cognitive development (Samie *et al,* 2006). The epidemiological studies have indicated that the main routes of *Cryptosporidium*transmission are human–animal contact, person-to-person and waterborne. Numerous reports provide strong evidence that contaminated water is a high risk factor for the cryptosporidiosis (Rimšelienė *et al,* 2011)

Cryptosporidium species are recognized globally as important causes of diarrhea in children and adults. The literature implicates these protozoan parasites in 3 main epidemiological scenarios-namely sporadic, often water-related, outbreaks of self-limiting diarrhea in otherwise healthy persons (Tumwine *et al,* 2005, Morse *et al,* 2007) chronic, life-threatening illness in immuno-compromised patients, most notably those with HIV/AIDS, and diarrhea and malnutrition in young children in developing countries. In industrialized nations, improved water-management practices have resulted in a decline in cryptosporidiosis in the general population (Abubakar *et al,* 2007; Lu *et al,* 2008).

In the developing countries cryptosporidiosis represent up to 15% of gastrointestinal diseases among children and sero prevalence rates are generally in the 25% to 35% range and often 2 to 3 times higher. Those rates suggest that infection can be more common than surveys of fecal oocysts excretion demonstrate, as oocysts may be shed sporadically (Al-Shamiri, 2010). Some studies on cryptosporidiosis have been conducted in countries such as Iraq (Mahdi *et al,* 1996), Kuwait (Iqbal *et al,* 2001, Sulaiman *et al,* 2005); South Africa (Samie *et al,* 2006), Iran (Mirzaei, 2007), Brazil (Bushen *et al,* 2007) and Yemen (Al-Tajand, Al-Shamiri, 2004, Baswaid, and Al-Haddad, 2008).

The rural and urban environments might expect zoonotic *Cryptosporidium*, because of disparities in animal exposure, access to

safe water and sanitation, and population density. No rural-urban differences in prevalence were observed in Malawi(Morse *et al,* 2007), and prevalence was likewise comparable in children residing in towns and peri urban and rural areas of Kenya (Gatei *et al,* 2006). Prevalence was also similar in rural and urban areas of Liberia, but, although a positive association existed between cryptosporidiosis and crowding in both areas, rural households with fewer children had a prevalence similar to that in more-crowded urban households (Kassi *et al,* 2004).

Seasonal peaks of cryptosporidiosis have been reported in all regions of sub-Saharan Africa, and, with Kenya and Rwanda as notable exceptions, peaks tend to occur in the wet months *(Gatei et al, 2006*). Seasonal patterns probably culminate from several environmental, environmental transport of feces, and wet, humid conditions favor parasite survival.(Morse *et al,* 2007) also note that replenished surface waters serve as both playing areas for children and water holes for animals, so bringing these host populations into close proximity. In West Africa and Zambia, human infections peak early in the season, perhaps because susceptible populations develop immunity after repeated exposure with the initial rains. The peak of cryptosporidiosis in the dry seasons in Kenya and Rwanda is intriguing and perhaps relates to the use of alternative, unsafe water sources during those months (Gay-Andrieu *et al,* 2007).

This study aimed to determine the prevalence of *Cryptosporidium parvum* in school children with diarrhea and to investigate some risk factors that can effect on the prevalence rate of infection andto compare the diagnosticsensitive and specific of microscopy, ELISA and IFA.

Subjects, Materials and Methods

The present study was performed on 120 school children complaining with severe, moderate and mild diarrhea. They were 64 males and 56 females, their mean age was (10.45±3.52), Patients were seen at Mansoura University Hospitals (Children; Internal Medicine Hospitals; Tropical Department).

All children were subjected to the following: Complete history taking, age, sex, residence and animal contact.- Morning stool samples were collected in a wide mouthed, clean, dry, properly labeled plastic container and freshly examined macroscopically for consistency, presence of mucus and blood. The stool samples were preserved in 10% formalin or frozen at 22°C until used. The samples were concentrated by Sheather's sugar flotation method. For detecting the *Cryptosporidium* oocysts, ordinary light microscope with 100 magnification power by oil immersion lens was used. A thin smear of the supernatant was prepared and left to dry for fixation and staining.

Sheather's sugar flotation method: 2ml of stool suspension was filtered through two layer of gauze into conical centrifuge tube. Sheather's sugar solution was added. Suspension was mixed well and more sugar solution was added to bring the fluid level within a few ml. of the tube rim. Suspension was centrifuged at 1000 X g for 10 minutes, a wire loop (diameter of 5-7 mm) was used to touch the surface film; several drops of the film were put on a slide, a coverslip was added and examined (Garcia and Bruckner, 2007). In feces *Cryptosporidium* oocysts were detected by using modified Ziehl-Neelsen stain. Fecal smears were air dried at room temperature, and fixed with absolute methanol for 5 minutes. Fixed smears were stained with Carbol-fuchsin (1:10) for 3-5 minutes, washed with tap water, decolorizedin 3% acid alcohol for 10-15 minutes and stained with 0.5% malachite green solution for a minute. Slides were washed with tap water, air dried, and examined with x400magnification. Samples were considered positive if at least one distinct oocyst was seen. Intensities of the oocysts were expressed as 1+<5 oocyst/slide, 2+<10 oocysts/slide and 3+ >10 oocysts/slide (Garcia, 2001).

Immunofluorescence staining (IFA) proce-

dure: C*ryptosporidium* antigen was detected in stool by commercial IFA following the manufacture instructions (Meridian Diagnostics, Inc., Cincemati, Ohio). Also, oocyts in stool were detected by commercial ELISA, following the manufacture instructions (Ridascreen *Cryptosporidium*, C1201, R-Biopharm AG, Germany).

Statistical analysis: A computer program, SPSS version 19 was used for data analysis. The Chi-square test was used for the analytical assessment. The differences were considered to be statistically significant when the p value obtained was less than 0.05.

Results

Table 1: Frequency of different age groups in *Cryptosporidium* infected children

Age of infant (years)	Positive (30) No.	%	Negative(90) No.	%	Statistical analysis
Up to 5 years	8	26.7	26	28.9	$\chi^2 = 4.34$
> 5 - 10 years	17	56.7	46	51.1	P <0.05*
>10 – 16 years	5	16.6	18	20	Sig

Prevalence significantly higher among children aged >5-10 year (P< 0.05*)

Table 2: S*ex*, residence, animals'contact and seasonality in infected children.

Data	Positive (30) No.	%	Negative (90) No.	%	χ^2	P value
Males	18	60	46	51.1	4.01	<0.05*
Females	12	30	44	48.9	0.78	>0.05
Urban area	10	33.3	60	67.7	0.45	>0.05
Rural area	20	67.7	30	33.3	4.78	<0.05*
Animals contact						
Yes	20	67.7	30	33.3	4.78	<0.05*
No	10	33.3	60	67.7	0.45	>0.05
Summer	7	23.3	34	37.8	0.46	>0.05
Autumn	11	36.7	36	40	0.32	>0.05
Winter	12	40	20	22.2	0.49	>0.05

Males commonly exposed to infection than females (P<0.05*), rural areas statistically significant than urban ones (P<0.05*), and animals contact statistically significant(P<0.05*). No significant differences association with seasonal infection.

Table 3: Clinical picture of children with gastroenteritis.

Clinical data	Positive (30)	Negative (90)	χ^2	P value
Duration ofDiarrhea in days mean ± SD	9.8 ±7	3.3±1.8	3.02	<0.05*
Frequency of bowlmovementsmean ±SD	6.1±2.1	5.8±1.9	1.08	>0.05
Consistency of stools	No %	No. %		
Watery	12 40	18 20	18.32	<0.001*
Mucoid	14 46.7	6 6.7	15.46	>0.05
Loose	4 13.3	24 26.7	0.69	<0.001 *

Clinically, *Cryptosporidium* gastroenteritis, long duration of diarrhea watery and loose stools (p<0.05)*, without significant difference in number of bowl movements.

Table 4: Other isolated co-infected enteropathogens

Enter pathogens	Positive No.	%.
Cryptosporidium (ELISA)	30	25
Gardia lamblia	28	23.3
Cryptosporidium+Entamoeba histolytica	14	11.7
Cryptosporidium+Ascaris	8	6.7
Entamoeba histolytica	8	6.7
Total positive infection	88	73.4
Total negative infection	32	26.6

Of 120 schoolchildren, 30(25%) had *Cryptosporidium*, 28(23.3%) had *G. lamblia*, 14 (11.7%) had *Cryptosporidium* & *E. histolytica*, 8 (6.7%) had *Cryptosporidium* &*Ascaris* and 8 (7.6%) had *E. histolytica*alone.

Table 5: Different methods of *Cryptosporidium*diagnosis

Methods	positive cases		Negative cases		Statistical analysis
	No.	%	No.	%	
MZN	37	30.8	83	69.2	χ^2= 0.5
IFA	33	27.5	87	72.5	P>0.5
ELISA.	30	25	90	75	Non sig

Of 120 samples, MZN gave positivity 37(30.8%), ELISA gave 30 (25%) and IFA gave 33 (27.5%), but without significant differences (P>0.5).

Table 6 : Validity results of ELIZA and IFA confirmed by Ziehl Neelsen stain

Method of investigation	Ziehl –Neelsen (37) +	-	Total	Method of investigation	Ziehl –Neelsen (37) +	-	Total
ELISA (30) +	28	9	37	IFA (33) +	30	7	37
ELISA (30) -	2	81	83	IFA (33) -	3	80	83
Total	30	90	120	Total	33	87	120

ELISA: Sensitivity 93.3 % Predictive value +ve 75.7%
Specificity 90 % Predictive value –ve 97.6%
Agreement 90.8 % Disagreement 9.2%
IFA: Sensitivity 90.9 % Predictive value +ve 81%
Specificity 91.1 % Predictive value –ve 96.3%
Agreement 91.7 % Disagreement 8.3%

Discussion

Cryptosporidiosis has a worldwide distribution and in most surveys is among the four major pathogens causing diarrheal diseases in children. It has major public health implications because infections can result from exposure to low doses of *Cryptosporidium* oocysts *(Xiao et al, 2000)*. In developing countries, *Cryptosporidium* is responsible for 8-19% of cases of diarrheal disease with a significant effect on mortality (Helmy *et al,* 2004). In the immunocompromised hosts, the impact of the disease is severe and includes respiratory problems, cholecystitis, hepatitis, and pancreatitis. On the other hand, in immunocompetent persons, infection is considered to be a self limited disease, and the subclinical infection rate is unknown (Bushen *et al,* 2007).

The present study declared that the prevalence of *Cryptosporidium* infection was significantly higher among children with young age of >5-10 years more than the other age groups (P< 0.05*). These findings were in accordance with (Al-Shamiri *et al,* 2010) who found that the highest positive rate of *Cryptosporidium* spp. in different groups of diarrheic children was in the preschool age group (48.1%), followed by the school age group (43.1%), whereas lowest positive rate was noticed in infant age group (20.2%).

Robin *et al*. (2001) explained that children aged 2-60 months, living under crowded conditions with steady contact among ages, showed elevated levels of *Cryptosporidium* antibodies. The sero prevalence of *C. parvum* antibodies was positively associated with the children ages and negatively associated with their socioeconomic status. The sero prevalence rates in this population were 13%, 38%, and 58% for the age groups <5 years, 5-13 years, and 14-21 years, respectively. Sero prevalence in adult populations showed the presence of detectable *Cryptosporidium* IgG antibodies in 32 % of volunteers before they traveled to developing countries.

The present study declared that male children in rural area and contact with animals were the most susceptible group of infection (P<0.05*). Non significant differences were

recorded according to association of infection with different season. these results were in accordance another one, where the men were infected at a higher rate (1.9%) than women (1.2%) (Park *et al,* 2006). The male higher infection rate of *C. parvum* than female was also observed in Guinea-Bissau (Molbak *et al,* 1994). Another study, in contrast, reported that the boys and girls had similar detectable positive rate (Lu *et al,* 2008).

Adjei *et al*. (2004) declared that the age distribution of persons with cryptosporidiosis differed between areas in their study with prevalence peaking at an earlier age in rural areas. Rural-urban variations in age predisposition have also been reported elsewhere for Costa Rica, where the variations were attributed to differences in breast-feeding habits This does not explain the findings in bottle-feeding, which was more common in the urban slum, was positively associated with cryptosporidiosis (Bushen *et al,* 2007). Also in another study (Yu *et al,* 2004) explained the prevalence of *Cryptosporidium* infection among rural area by the increasing contact with animals in these areas.

Al-Shamiri *et al.* (2010) in Yemen they found that 43.6% of cases were coming from rural areas and 25.1% from urban areas . This could be due to the social habits of the rural people in which keep the animals in their houses. Also they explained that the summer season was a rainy season, thus, the heavy rainfall could help the transmission of *Cryptosporidium* spp. by inducing a wider spread of the animal feces onto fields or water sources. In Malawi reported a peak prevalence between October and March, and this is the rainy season in this region *(Morse et al,* 2007). Similarly, Hoek *et al.* (2008) stated the same findings and focused on the effect of water and rainfall times in predisposition of *Cryptosporidium* infection.

The present study showed that the longer duration of diarrhea was due to *Cryptosporidium* infection ($p < 0.05$), whereas no statistically significant difference as regards the number of bowl movements was detected. Watery and loose stools were characteristic of *Cryptospori-dium* diarrhea.

The present study agreed with Robin *et al*. (2001) who found that 20% of infected children with *Cryptosporidium* had seven or more diarrheal episodes, and 40% had suffered from 3–6 events. Also, the infection with *Cryptosporidium* is characterized by watery diarrhea that persists for >2 weeks in a substantial proportion of children (Dlamini *et al,* 2005; Sopwith *et al,* 2005; Ajjampur *et al,* 2007).

The present study declared that among 120 school children30 (25%) were infected with *Cryptosporidium*, 28 (23.3%) were infected with *Gardia lamblia*, 14 (11.7%) were infected with *Cryptosporidium* & *Entamoeba histolytica*, 8 (6.7%) were infected with *Cryptospori-dium* & *Ascaris* and 8 (7.6 %) were infected with *Entamoeba histolytica*. This finding agreed with Samie *et al.* (2006) and Hoek *et al.* (2008). The most frequently found parasites were *Entamoeba histolytica, Giardia lamblia, Hymenolepis nana, Ascaris lumbricoids, Ancylostoma duodenale, Taenia saginata and Schistosoma mansoni* (Al-Taj and Al-Shamiri, 2004; Baswaid and Al-Haddad, 2008;Al-Shibani *et al,* 2009).

By using three methods, the present results declared thatpositive samples by Ziehl-Neelsen stain 37(30.8 %) while by ELISA and IFA 30 (25 %) and 33 (27.5 %) respectively. The validity test of ELISA was sensitivity and specificity with 93.3% and 90% while IFA declared 90.9% and 91.1% respectively. The accuracy of ELISA and IFA were 90.8% and 91.7%. These data agreed with Al-Shamiri *et al.* (2010) who found that 34.7% of samples were positive with Cryptosporidiosis by Ziehl-Neelsen stain, but by ELISA method 26.1% were infected. The sero-prevalence in developed countries is generally in the range of 25% to 35% compared to that of developing countries, which is 2-3 times higher.

Chalmers *et al.* (2011) reported that , ELISA positive reactions were 91.4-93.4%,

whilst the sensitivity of auramine phenol microscopy was 92.1% and that of immunofluorescence microscopy (IFM) was 97.4%, all with overlapping 95% confidence intervals. However, IFM was significantly more sensitive (P=0.01, paired test of proportions). The sensitivity of modified Ziehl-Neelsen microscopy was 75.4%, significantly lower than those for the other tests investigated. The sensitivity, specificity and positive predicative value of ELISA were 100%, 85% and 99%, respectively. They concluded that the widely used microscopy is a very specific, but less sensitive method for the laboratory detection of *C. parvum* in the feces but ELISA have good sensitivity. Also, El-Sibaei *et al.* (2003) found that ELISA was 100% sensitive.

Conclusion

The outcome data showed that *Cryptosporidium parvum* is a significant pathogen causing diarrhea among rural children who were contact with domestic animals, the main source of zoonotic cryptosporidiosis. The microscopic examination is a very specific but less sensitive and needs well trained technician, but ELISA and IFA are more sensitive and specific.

Consequently, the improving general hygiene and prevention of indoors animals is a must.

generally speaking, *Cryptosporidium* species is a significant pathogen among children especially less than 5 years. Urban versus rural habitation, sanitation and hygiene practices, animal exposures and seasonal variations provided to be a reasonable framework for understanding epidemiology of cryptosporidiosis.

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