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THE PRESENT SITUATION OF HUMAN *CRYPTOSPORIDIASIS* IN SOME CENTERS IN DAKAHLIA GOVERNORATE, EGYPT

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ABSTRACT

Background : Cryptosporidiosis is a zoonotic disease transmitted by the feco-oral route and results from the ingestion of Cryptosporidium spp. oocysts through the consumption of fecally contaminated food or water or through direct person-to-person or animal-to-person contact. Infection by the parasite accounts for up to 6% of all diarrheal diseases in immunocompetent patients, and presents in up to 24% of persons with both AIDS and diarrhea worldwide. In the literature, the prevalence of Cryptosporidium infection in Egypt varied significantly from 0% to 47%

Objective: The aim of the work was to assess the problem of cryptosporidiosis in some centers in Dakahlia Governorate, Egypt.

Subjects and Methodology: A total of 1786 individuals of different ages and both sexes were chosen randomly from May 2012 to August 2014 from rural and urban areas. All cases were subjected to history taking, clinical examination and laboratory investigations. Fecal specimens were examined by direct smear and concentration techniques using both formol-ether concentration and sheater's flotation methods. Microscopic examination of modified Ziehl-Neelsen, and aurmaine stained stool smears were also done as well as detection of coproantigens using ELISA technique .

Results: Out of 1786 collected stool samples from four centers, i.e. Mansoura, Talkha, Belqas and Aga, only 97(5.43%),109(6.1%) and 178(9.96%) were positive according to Ziehl-Neelsen, Auramine and Coproantigen techniques. The highest prevalence of infection was recorded in Talkha center and Coproantigen was the most sensitive technique. Rural areas showed non significant higher prevalence of Cryptosporidiosis, while females showed relatively significant higher infection when using auramine stain. The infection was non significantly higher among the age group less than 5 years. Mixed infections were found in 43 cases (39.45%) and distributed as 22 cases (20.18%) having one parasite, 11 cases (10.09%) having two parasites while those infected with more than two parnsites were 10 cases (9.17%). Asymptomatic cases represented 15 cases (22.73%), while symptomatic cases were 51 cases (77.27%) and disributed as having abdominal pain (43 cases-5.15%), jaundice (8 cases-12.12%), abdominal distesion (39 cases -59.09%), diarrhea (42 cases-63.43 %), tender abdomen (44 cases-86.27%), hepatomegaly (13 cases-25.49 %), dehydration (1 cases- 41.18 %) and with ascites (5 cases-11.36%) .

Conclusion: The high prevelance of Cryptosporidium infection in the studied areas must be taken in mind of laboratory personell and as a routine examination of all diarrhiec samples.

INTRODUCTION

Cryptosporidium spp. is a coccidian parasite which infects humans and animals **(Xiao, 2010)**. Diarrhea caused by parasites accounts for more than 3.1 million deaths each year among children less than 15 years of age, mostly in developing countries **(Colford *et al.*,**

2005).

Cryptosporidiosis causes chronic and life threatening diarrhea among immuno-compromised individuals as well as self-limiting diarrhea in immunocompetent individuals **(Paul & Gordon., 2002 and Banwat e*t al*., 2004)**. Infection by the parasite accounts for up to 6% of all diarrheal diseases in immunocompetent persons and presents in up to 24% of persons with both AIDS and diarrhea worldwide **(Bialek *et al*., 2002).**

Transmission occurs through the feco-oral route, following direct or indirect contact with *Cryptosporidium* oocysts via person-to-person, zoonotic waterborne, foodborne or airborne contact **(Fayer *et al*., 2000).** In developing countries, the parasite is endemic and significantly associated with moderate-to-severe diarrhea in infants, a finding that highlighten the need to develop resources to diagnose, treat, and prevent cryptosporidiosis in resource-poor settings. Under this situation, routine diagnosis and effective reporting of Cryptosporidium to local and national surveillance organizations remain of key importance in understanding the epidemiology of this important, but often underestimated, pathogen **(Caccio and Putignani, 2013).**

The impact of Cryptosporidium on children has been demonstrated also in Arab countries such as Egypt, Jordan, Kuwait, Libya, Palestine, Saudi Arabia and Tunisia. Prevalence rates ranged from 1–43 % (mean 8.7 %) in diarrheic immunocompetent children, and from 1–82 % (mean 41 %) in immunocompromised children and adults were reported **(Ghenghesh et al., 2012).**

In Egypt, *Cryptosporidium* has been identified as a prevalent and virulent agent of childhood diarrhea in the Nile Delta **(Antonios *et al*., 2001).** Methods to detect *Cryptosporidium* spp. in feces usually involve microscopic examination of stained fecal smears (modified Ziehl-Neelsen, safranin methylene blue, auramine phenol), antigen detection (immunofluorescence, enzyme-linked immunosorbent assay [ELISA]), or genome detection (polymerase chain reaction [PCR] amplification of the 185 rRNA gene). Each varies in sensitivity and specificity, and there is no universally accepted "gold standard technique" **(Areeshi *et al*., 2007).**

There are big differences in the prevalence of cryptosporidiosis disease. In 19 studies carried out on immuno-competent individuals with diarrheal diseases, the prevalence rate varied between 0% and 47% **(Youssef et al., 2008).** The aim of the present work was to asses the problem of cryptosporidiosis in Dakahlia Governorate, Egypt.

SUBJECTS AND METHODS

A total of 1786 cases of different ages in both sexes with written consents from them or their gardeners were obtained. Dakahlia Governorate is the largest agricultural district in the Nile Delta, we selected Mansoura city the Capital of Dakahlia Governorate and centers around it to carry out the field and laboratory studies on human *Cryptosporidiasis.* Four centers were choosen, i.e. Mansoura, Talkha, Aga and Belqas between the period from May 2012 to August 2014. The work was developed in the Department of Parasitology, Al-Azhar Faculty of Medicine.

Cases were randomly taken from rural

and urban areas, i.e. houses, schools, health units and hospitals. All of them were subjected to :

1- History taking of clinical examination including name , age, sex, occupation, residence, complain taking (if present), nausea, vomiting, dyspepsia, constipation, diarrhea, abdominal distension or enlargement, abdominal tenderness, hepatomegaly, signs of dehydration and ascites.

2- Laboratory examination:

a- Urine examination **(Garcia, 2007).**

b- Stool examination : Fecal specimens were examined by direct smear or concentrated by both formol-ether concentration and sheater's flotation **(Ridley *& Hawgood*., 1956 and Markell *et al*., 1999).**

c- Microscopic examination according to modified Ziehl-Neelsen and aurmaine stained smears were done for positive samples **(Paik, 1980)**

d- Detection of coproantigens using Ridascreen *Cryptosporidium (*Art. No: C1201, Lot: 12131 R-Biopharm AG, Darmstadt, Germany according to the manufacturer's method). **(Ridley *and Hawgood,* 1956) .**

Statistical analysis: The collected data were organized, tabulated and statistically analyzed using SPSS, version 13 (USA) . For quantitative data, number and percent distribution, the mean and standard deviation were calculated. Chi (X^2) square was used as a test of significance. Significance was adopted at $P < 0.05$ for interpretation of results of tests of significance.

RESULTS

Out of 1786 collected stool samples from the four centers, only 97(5.43%), 109(6.1%) and 178(9.96%) were positive according to Ziehl-Neelsen, Auramine and Coproantigen techniques respectively. The difference was significant using Z/N ($\chi2$ = 9.034 and p= 0.001), not significant using ELISA technique ($\chi2$ = 16.113 and p= 0.128) and Auramine stain ($\chi2$= 10.381 and p = 0.115). The highest prevalence of infection was recorded in Talkha center . Non statistically significant difference of prevalence was detected among the centers ($\chi2$ = 3.711 and p= 0.294), and coproantigen was the most sensitive technique (Table 1). The infection was relatively higher among the age group less than 5 years. The prevalence was significantly higher using Ziehl-Neelsen stain ($\chi2$ = 9.034, p= 0.028), Auramine stain ($\chi2$ = 10.381, p=0.015) and by Coproantigen detection ($\chi2$ = 16.113, p= 0.001- Table 2). Females showed relatively significant higher infection when using auramine stain($\chi2$ = 4.046 and p= 0.044). However, using Coproantigen detection by ELISA and Z/N the difference showed no statistical significance ($\chi2$ = 0.36 and p= 0.548) and ($\chi2$ = 7.36 and p= 0.321) respectively (Table 3). Rural areas showed higher prevalence of infection statistically: The difference was significant using Auramine stain ($\chi2$ = 7.716 and p= 0.005. However, neither by Z/N stain nor by ELISA the difference was statistically significant, i.e. ($\chi2$= 1.247, p= 0.264) and ($\chi2$= 2.247, p= 0.133) respectively (Table 4). Mixed infections was found in 43 cases (39.45%) and distributed as one parasite in 22 cases (20.18%), two parasites in 11 cases (10.09%), while those infected with more

than two parasites were 10 cases (9.17%- Table 5). Asymptomatic cases represented 15 cases (22.73%), while symptomatic cases were 51 cases (77.27%), and distributed as having abdominal pain (43 cases- 65.15%), jaundice (8 cases- 12.12%), abdominal distesion (39 cases- 59.09%), diarrhea (42 cases- 63.43%), tender abdomen (44 cases-86.27%), hepatomegaly (13 cases-25.49 %), dehydration (21 cases- 41.18 %) and ascites (5 cases-11.36%). The patients complained of more than one symptom and sign (Table 6).

Table (1): Diagnosis of *Cryptosporidium* cases by Ziehl-Neelsen, Aurmaine and coproantigen (ELISA) in the selected centers.

Locality / **Methods**	**Mansoura** (No = **442)**	**Talkha** (No = **663)**	**Belqas** (No = **294)**	**Aga** (No = **387)**	**Total** (No =**1786)**
Ziehl-Neelsen	17(17.53%)	35(36.08%)	25(25.77%)	20(20.62%)	97(5.43%)
Auramine	20(18.35%)	38(34.87%)	28(25.69%)	23(21.1%)	109(6.10%)
Coproantigen	37(20.79%)	54(30.34%)	46(25.84%)	41(23.03%)	178(9.96%)

Table (2): Age distribution of cryptosporidiosis.

Methods / **Age**	**Ziehl-Neelsen**	**Auramine**	**Coproantigen**
Up to 5 (325)	26(8%)	29(8.92%)	42(12.92%)
> 5- 20 (566)	34(6.01%)	37(6.54%)	70(12.37%)
> 20 – 40 (559)	19(3.40%)	21(3.76%)	34(6.08%)
> 40 (336)	18(5.36%)	22(6.55%)	32(9.52%)
Total (1786)	97(5.43%)	109(6.10%)	178(9.96%)

Table (3): Sex distribution of Cryptosporidiosis.

Methods / **Sex**	**Ziehl-Neelsen**	**Auramine**	**Coproantigen**
Male(912)	52(5.70%)	44(4.82%)	85(9.32%)
Female (874)	45(5.15%)	65(7.44%)	93(10.64%)
Total (1786)	97 (5.43%)	109 (6.10%)	178 (9.97%)

Table (4): Residence distribution of Cryptosporidiosis.

Methods / **Residence**	**Ziehl-Neelsen**	**Auramine**	**Coproantigen**
Urban (581)	43(7.40%)	40(5.88%)	79(13.60%)
Rural (1205)	54(4.48%)	69(5.73%)	99(8.22%)
Total (1786)	97 (5.43%)	109(6.10%)	178 (9.97%)

Table (5): Co- existing of *Cryptosporidium* and other parasites.

Parameters / **No. of parasites**	**No. of patients = 109 (%)**	**Other detected parasites**
Single	66 (60.55%)	-
Mixed:	43 (39.45%)	-
One parasite	22 (20.18%)	- *With Entameba histolytica (6)* - *Isosopra(3)* - *Chilomastix(4)* - *Entrobious vermicularis(5)* - *Hymenolepyis diminuta(1)* - *Giardia lamblia* (1) - *Blastocysts hominis*(2)
Two parasites	11 (10.09%)	-With *Giardia lamblia+ Blastocysts hominis(5)* - *Strongyloides spp + Entameba histolytica (1)* - *Blastocysts hominis + Giardia lamblia* (5)
More than two parasites	10 (9.17%)	-With *Hymenolepyis nana+ Giardia lamblia + Blastocysts hominis(1)* - *Entrobious vermicularis+ Isospora + Endolimax nana(2)* - *Isospora+ Chilomastix+ Myxoplus(3)* - *Entameba histolytica+ Isospora+ Chilomastix (*4)

Table (6): Clinical presentations among patients.

Clinical presentations	**No = 66 (%)**
Asymptomatic	**15 (22.73%)**
Symptomatic	**51 (77.27%)**
Abdominal pain	43 (65.15%)
Jaundice	8 (12.12%)
Abdominal distension	39 (59.09%)
Diarrhea	42 (63.64%)
Signs	**No=51 (%)**
Tender Abdomen	44 (86.27%)
Hepatomegaly	13 (25.49%)
Dehydration	21 (41.18%)
Ascites	5 (11.36%)

DISCUSSION

In the present study, 97 (5.43%) of the individuals were positive for *Cryptosporidium* spp. infection using Ziehl-Neelsen (Z/N) stain, and by Auramine-phenol stain, 109 (6.10%) were positive. Using ELISA technique to detect Crypto-coproantigen infection was revealed in 178 (9.96%). The difference was significant using Z/N, non significant using ELISA technique and Auramine stain. These results were in accordance with previous results by **El-Shazly *et al*. (2002)** who diagnosed *C. parvum* in stool samples by Z/N stain as 5.3% and ELISA as 8.3%. Also, **Helmy et al. (2014)** detected that the prevalence of *Cryptosporidium* was 2.4, 6.7 and 49.1% in children using EIA, ICT and PCR, respectively. However, it was higher than **Yilmaz *et al*. (2008)** who recorded that only 1.95% of 2000 children were positive on microscopy of acid fast stained smears, and 4.9% were positive by ELISA. On the other hand, the present results were lower than **Al-Shamiri *et al*. (2010)** in Yemen who recorded that 34.7% were positive by microscopy, and 26.1% were positive by ELISA.

Among the centers examined, the highest infection prevalence recorded was in Talkha by Z/N, Auramine and Coproantigen detection respectively followed by Belqas, Aga and the lowest prevalence was detected in Mansoura center. However, non statistically significant difference of prevalence was detected among the centers .

Cryptosporidiosis recorded in the present study was relatively higher in the age group up to 5 years old by Z/N, Auramine and Coproantigen detection respectively. The prevalence was significantly higher using Ziehl-Neelsen stain, followed by Auramine stain and lastly by Coproantigen detection . The second group was >5- 20 years, then the group > 40 and lastly the group >20-40, by Z/N, Auramine and Coproantigen detection respectively . This agreed with a study in Zagazig (Egypt) by **Abou-El-Magd and Abou-Shady (1986)** who stated that cryptosporidiosis was more common in the age of 2-12 years old. Also, **Al-Shamiri *et al*. (2010)** stated that the highest rate of infection was observed in preschool age group between 2 - 6 years, and the highest percentage seroprevalence was observed among infant group. **Youssef *et al*. (2008)** detected an infection prevalence of 18.9% of children < 2 years old. In Korea, also the peak of infection was in children aged 1-5 years **(Casemore, 1990).** Cryptosporidiosis occurs mainly in children aged 1–9 years, with the onset of infection peaking in the summer in association with communal swimming venues and recreational water use **(Barry et al., 2013).**

Outbreak investigations have also put into focus the role of food handlers as a source of food contamination and subsequent transmission of cryptosporidiosis **(Robertson and Chalmers, 2013).** Clinical infection is less common after the age of 40 years, and there is apparently no evidence of elevated incidence rates in the elderly **(Casemore, 1988 and Abd El-kader et al., 2011)**. However, incidence in adults may increase dramatically during waterborne outbreaks of infection and, therefore, may provide an early indication

of the likely route of transmission of *Cryptosporidium* to the community **(Casemore, 1995).**

Cryptosporidiosis was found to be relatively higher in females than males using Auramine stain with statistically significant difference results. However, using Coproantigen detection by ELISA was 10.64% and 9.32% in females and males respectively. The difference showed no statistical significance . This agreed with **Park *et al*. (2006)** who recorded that the infection reached 1.9% in females and 1.2% in males with no statistical difference. Also, **Al-Shamiri *et al*. (2010)** recorded that cryptosporidiosis was 36.2% in females and 32.7% in males. **Abd-Al Kader *et al*. (2011)** reported a slight increase of cryptosporidiosis infection in males (5.1%) and in females (4. 9%). Higher prevalence in females could be attributed to higher sample size of females in the study. This is on contrary to other studies that showed higher prevalence in males due to the presence of males in outdoor areas as farms, and contact with animals more than females. However, other studies suggested that distribution of cryptosporidiosis cases by sex indicates that males and females appear to be equally susceptible to infection **(Fayer and Ungar, 1986).**

The present study revealed that increased infection with cryptosporidiosis in rural areas were more than urban areas. the difference was significant using Auramine stain . **Soliman (1992)** detected a significant correlation between presence of animals and close contact with soil and *Cryptosporidium* spp. infection. The prevalence was 49.1% where *C. hominis* was dominated (60.5%), followed by C. parvum (38.3%). Living in villages, drinking underground water and having contact with animals were risk factors **(Fathy et al., 2014)**. Higher infection rates were found in children living in rural and semi-urban areas than in those residing in urban areas **(Ghenghesh et al., 2012). Youssef *et al*. (2008)** reported 54.3% of *Cryptosporidium* spp. cases who revealed a history of animal contact. In addition, **Al-Shamiri *et al*. (2010)** reported 43.6% of cases coming from rural areas, and 25.1% from urban areas. Residence in rural area appears to be a contributing factor to increase *Cryptosporidium* spp. infection risk with increased exposure to zoonotic infection from peridomestic animals **(Abdel-Wahed, 1999 and Yu *et al*., 2004).**

Mixed infection of *Cryptosporidium* spp. with other parasites was detected in about 39.45% of cases . Co-existing parasites varied between *H. nana, G. lamblia, B. hominis, E. vermicularis, Isospora belli, Endolimax nana, Chilomastix mesnili, E. histolytica,* and *Strongyloides stercoralis*. Mixed infection with one parasite was detected in 20.18%, two parasites were detected in 10.09%, and more than 2 parasites in 9.17%.

Obiajuru *et al*. (2008), in Imo State, Nigeria, showed that most of the patients (74.04%) had mixed infections with *E. histolytica*, *G. lamblia* and *E. coli,* while 10.90% had single infection of cryptosporidiosis. **Certad *et al*. (2005)** in Venezuela reported that 34% of *Cryptosporidium*-infected patients had mixed infections with other parasites, mostly with *B. hominis* in 19% and *S. stercoralis* in 7% of cases .

In the present study, 15 (22.73%) of the infected cases were asymptomatic, and 51 (77.27%) were symptomatised. These results were in agreement with **Kirkpatrick et al. (2008)** who reported that in study on Ninety-six Bangladeshi children (42.5 %) were diagnosed with Cryptosporidium infection. A total of 51 (22.6 %) had asymptomatic infection, whereas 58 (25.7 %) had symptomatic cryptosporidiosis, of whom 17 (29.3 %) had recurrent disease. Also, **Sajjad et al. (2014)** showed in study in Pakistan on 105 patients with acute diarrhoea, Fifty three (50.4%) were males. The mean age was 34±8.4 years. Of 105, 58 (55%) patients had Cryptosporidium isolated in stool examination. Infected patients had statistically significant greater stool frequency per day, abdominal pain , vomiting, low grade fever, fatigue and dehydration and a shorter duration of illness with more watery diarrhea. Also, **Mirzaei (2007)** recorded 25.6% of cryptosporidiosis cases had diarrhea. According to **Abd El-Messeh *et al*. (2005)** vomiting and persistent diarrhea are important clinical findings associated with *Cryptosporidium* spp., and they need hospitalization. **Hassan *et al*. (1995)** recorded that 91.7% of children suffering from diarrhea were positive for *Cryptosporidium* coproantigen by ELISA.

The most frequently recognized clinical picture of cryptosporidiosis involves chronic watery diarrhea. However, asymptomatic infection is also relatively common. A nonspecific low-grade fever, malaise, anorexia, abdominal discomfort, and nausea may accompany the diarrhea. These symptoms, if chronic, can lead to weight loss, dehydration, and malnutrition**. (Derouin et al., 2010).**

CONCLUSION

There was a high prevelance of *Cryptosporidium* infection in the studied areas and must be taken in mind as a routine examination in all diarrhia samples especially in children .

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خلفية البحث : الكريبتوسبوريديوم هو طفيل حيواني المنشأ يصيب البشر عن طريق تناول الأغذية أو المياه الملوثة ويسبب إسهال مزمن قد يهدد الحياة خاصة في الأشخاص ذوى المناعة الضعيفة ، بينما في الأشخاص ذوى المناعة القوية يسبب إسهال محدود. وهناك فروق كثيرة في نسبة إنتشاره في العالم ونسبة إنتشاره في مصر تختلف بشكل كبير من 0٪ إلى 47٪ في دراسات مختلفة.

هدف الدراسة : تقييم مشكلة طفيل الكربتوسبوريديوم في محافظة الدقهلية - مصر.

الأشخاص ومنهجية البحث :أجريت الدراسة على 1786 شخص من مختلف الأعمار ومن كلا الجنسين حيث تم إختيارهم بشكل عشوائي في الفترة من مايو 2012 وحتى أغسطس 2014 من المناطق الريفية والحضرية .وتم أخذ التاريخ المرضى والفحص السريري والفحوص المختبرية، وتم فحص عينات البراز عن طريق اللطخة المباشرة وطريقة الترسيب بالفورمالين والكحول ، وتم صبغهم بصبغة الأورامين والزيل نلسن وتحديد الأنتيجينات في البراز بإستخدام إختبار الإليزا.

النتائج: بينت النتائج أن نسبة إنتشار الطفيل تتراوح بين 5.43٪ - 10.06٪ ، وهناك فروق ذات دلاله إحصائية بين مختلف الطرق التشخيصية، وبمعدلات أعلى في المناطق الريفية عن المناطق الحضرية وفى الإناث عن الذكور .وقد سجل نسبة عالية في الفئة العمرية من 1: 5 سنوات من العمر.وبالنسبة للإصابة بأكثر من طفيل بينت الدراسة وجود طفيل واحد فى 22 حالة (20.18٪) ، ووجود إثنين من الطفيليات فى 11 حالة (10.09٪) ، في حين أن المصابين بأكثر من إثنين من الطفيليات كانت 10 حالات (9.17٪). و كانت أعراض المرض ظاهره على 51 حالة (77.27٪) وكانت كالتالي: وجود آلام في البطن 43 (65.15٪) ، واليرقان فى8 حالات (12.12٪) ، وانتفاخ في البطن فى 39 حالة (59.09٪) ، إسهال فى 42 حالة (63.43٪) ، زيادة حساسية البطن للمس في 44 حالة (86.27٪) ، تضخم الكبد فى 13 حالة (25.49٪) ، 21 حالة يعانون الجفاف (41.18٪) والذين يعانون من حالات الإستسقاء كانوا 5 حالات (11.6٪).

الإستنتاج :أوضحت الدراسة أن طفيل الكريبتوسبوريديوم لابد أن يكون في ذاكرة كل أخصائى المختبرات وكتشخيص متغاير وإختبار روتيني لكل عينات البراز المصحوبة بإسهال خاصة بين الأطفال.