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ORIGINAL PAPER

Detection of *Cryptosporidium parvum* and *Cryptosporidium hominis* in human patients in Cairo, Egypt

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Abstract *Cryptosporidium* is a significant cause of diarrheal disease in developing and industrialized nations. *Cryptosporidium hominis* and *Cryptosporidium parvum* are the main agents of cryptosporidiosis in humans. In Egypt, very little is known about genetic structure of *Cryptosporidium* spp. Therefore, this study was designed to examine samples from sporadic cases of cryptosporidiosis in Egyptians in order to identify the species involved in infection as well as the transmission dynamics and distribution of the parasite in the Great Cairo area. A total of 391 human faecal samples were collected, between May 2008 and March 2009, from ten public hospitals in Great Cairo. Initial screening by immunochromatographic detection kit "the Stick Crypto-Giardia; Operon" showed 23 possible positive cases. Twenty of them were confirmed by microscopic examination. PCR was performed by amplification of the oocyst wall protein (COWP) gene where 18 out of 23 samples were positive, one not detected by microscopy. *Cryptosporidium* genotyping was performed by RFLP analysis of PCR products of the diagnosis PCR. Only 15 samples rendered a digestion pattern. The genotyping distribution was nine cases showing *C. hominis* genotype, three showing *C. parvum* genotype and three showing mixed infection by *C. hominis* and *C. parvum*. The data showed an elevated prevalence of *C. hominis* (80.0%), the most anthroponotic species, suggesting a human–human transmission. Furthermore, the presence of up to 40% of samples infected with *C. parvum* shows that further investigations are required to determine the subgenotypes of C. *parvum* to clarify the mode of transmission in order to improve the control measures.

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Introduction

Cryptosporidium is an enteric parasite that infects a wide range of hosts including humans, domestic and wild animals. In humans, *Cryptosporidium* infection can result in severe diarrhoea, which is usually self-limiting in immunocompetent individuals, but may be chronic and life threatening to those that are immunocompromised (Xiao 2010). In developing countries, diarrhoea caused by *Cryptosporidium* early in childhood may be associated with subsequent impaired physical and cognitive development (Guerrant et al. 1999). Furthermore, the risk of infection may be high and the disease probably exerts most of its impact on neonates and infants (Hunter et al. 2009). During the last decades, *Cryptosporidium* has emerged as an important enteric pathogen and has defied water and health authorities by its ability to withstand chlorine disinfection and filtration. It has been the cause of multiple diarrhoea outbreaks in developed and developing countries (Mac Kenzie et al. 1994; Insulander et al. 2005; Samie et al. 2006; Ng et al. 2010).

The diagnosis and genetic characterization of the different species and population variants (usually recognized as "genotypes" or "subgenotypes") of *Cryptosporidium* is

central to the prevention, surveillance and control of cryptosporidiosis (Jex and Gasser 2008).

Currently 21 species of *Cryptosporidium* are recognized as valid and at least 8 of them have been reported in humans: *Cryptosporidium hominis*, *Cryptosporidium parvum*, *Cryptosporidium meleagridis*, *Cryptosporidium felis*, *Cryptosporidium canis*, *Cryptosporidium muris*, *Cryptosporidium suis* and the *Cryptosporidium* cervine genotype (Xiao 2010). *C. hominis* and *C. parvum* are responsible for the majority of human infections. *C. hominis* is found almost exclusively in humans, whereas *C. parvum* is found also in domestic livestock and wild animals (Xiao et al. 2001). The presence of several *Cryptosporidium* species in human infections has provided evidence that anthroponotic and zoonotic infection cycles are infection routes for humans (Spano et al. 1998).

In Egypt, *Cryptosporidium* has been identified as a prevalent and virulent agent of childhood diarrhoea in the Nile River Delta (Antonios et al. 2001) but limited studies had been performed to ascertain cryptosporidiosis in human and animals (Amer et al. 2010). The literature review shows big differences: (1) in the prevalence of cryptosporidiosis disease; in 19 studies carried out in immunocompetent individuals with diarrhoeal diseases, the prevalence varied between 0% and 47% (Youssef et al. 2008); and (2) in the *Cryptosporidium* species involved in the infection (Eida et al. 2009). This report is aiming to improve the knowledge of the cryptosporidiosis situation in the Great Cairo, including the determination of the transmission routes in order to improve the control measures against the disease.

Materials and methods

Samples

A total of 391 faecal samples were collected from Egyptian patients in ten hospitals of the Great Cairo area between May 2008 and March 2009. Informed consent was obtained from all patients or their parents when patients were under 18 years old. Samples were collected from either in-hospital patients suffering from diarrhoea or outpatients requesting stool analysis due to gastrointestinal discomfort associated with diarrhoea. Samples were kept fresh or frozen for 1 or 2 days before being transported to the laboratory for analysis. The patient's gender distribution was 224 males, 158 females and 9 samples from patients with unavailable data. Patient age ranged from 1 month to 70 years old; by age group, 130 patients were under 2 years old, 86 between 2 and 5 years, 87 between 6 and 14 years and 65 older than 14. In 23 samples, the patient's age was unknown.

Parasite screening

Stool samples were screened for *Cryptosporidium* and *Giardia intestinalis* using the Stick Crypto-Giardia immune-chromatographic kit (Operon). Microscopic examination of modified Ziehl–Neelsen-stained smears was done for *Cryptosporidium*-positive samples.

Molecular diagnosis of species

DNA extraction was performed using the QIAamp DNA stool kit (QIAGEN, Germany) following the manufacturer's instructions. Species identification was carried out by nested amplification of the *Cryptosporidium* oocyst wall protein (COWP) gene followed by *Rsa* I restriction enzyme digestion (Spano et al. 1997; Pedraza-Díaz et al. 2001). The digestion pattern of the amplicon fragment (543–554 bp) is different for each characteristic *Cryptosporidium* species infecting humans: *C. parvum* (34, 106 and 413 bp fragments) and *C. hominis* (34, 106, 130 and 284 bp fragments).

Statistical analysis

The association between variables was determined using χ^2 and Fisher's exact test analysis. Values of $P<0.05$ were considered statistically significant.

Results

Twenty three (5.88%) and 89 (22.76%) out of 391 samples were immune reactive for *Cryptosporidium* and *Giardia* respectively with the Crypto-Giardia immunochromatography kit. Five of the positive cases (1.28%) were mixed infection.

The microscopic examination of the Crypto-Giardia immunochromatography kit-positive samples confirmed only 20 samples that *Cryptosporidium* oocysts were present after repeated examination (Table 1).

The COWP PCR was successful in 18 samples, 1 of them being a negative microscopy sample. The three positive-microscopy samples negative by PCR correspond with three samples with low oocysts load (Table 1).

Positive patients were just reported in five of the hospitals where samples were collected. Nineteen out of 23 positive cases were collected in the Cairo University Paediatric Hospital which count for 7.5% of the samples collected in the hospital. Only one positive patient was found in the Centre for Social and Preventive Medicine (7.1% of total hospital samples), in the National Cancer Institute (5.0% of total hospital samples), in the Al-Abbasia Fever Hospital (1.4% of total hospital samples) and in the ASU Medical Camp in Cairo where just one sample was analysed.

Table 1 Data for positive *Cryptosporidium* samples, including collection month, their age, gender, results of different methods carried out and genotyping

Sample no.	Collection month	Age	Gender	IC kit	mZN	COWP	Genotype
1	May	>14	M	+	+	+	*C. hominis*
2	June	>14	M	+	+	+	*C. parvum*
3	July	6–14	M	+	+	+	*C. parvum*
4	August	ND	ND	+	+	+	*C. hominis*
5	August	2–5	F	+	+	+	*C. hominis*
6	August	2–5	F	+	+	+	*C. parvum*
7	August	<2	M	+C&G	+	+	*C. hominis*
8	August	ND	ND	+	+	+	*C. hominis*
9	August	6–14	F	+	+	+	*C. hominis*
10	August	<2	F	+	+	+	*C. hominis*
11	August	2–5	M	+C&G	+	+	*C. hominis*
12	August	<2	M	+	+	+	*C. hominis*+*C. parvum*
13	August	2–5	F	+C&G	+	+	*C. hominis*+*C. parvum*
14	August	<2	M	+	+	+	*C. hominis*
15	February	6–14	M	+/−	+/−	−	
16	February	2–5	M	+/−	+/−	−	
17	February	<2	F	+C&G	+	+	*C. hominis*+*C. parvum*
18	February	<2	F	+C&G	+/−	+	
19	March	2–5	F	+/−	+/−	−	
20	March	<2	M	+/−	+/−	+	
21	March	6–14	M	+/−	−	−	
22	March	2–5	M	+/−	−	−	
23	March	6–14	M	+/−	−	+/−	

ND no data, *F* female, *M* male, *IC* immunochromatographic kit result, *mZN* microscopy results, *COWP* PCR results, *+C&G* mixed *Cryptosporidium* and *Giardia* infection, + positive, − negative, +/− weak positive or low parasitaemia

The age group with most of the positive *Cryptosporidium* cases was infants less than 2 years old and the group between 2 and 5 years with seven cases each, followed by the group of 6–14 years old with five cases (Table 2).

There is a slight increase of cryptosporidiosis infection in males (11) as compared to females (8), but the frequency of positive cases for sex, 4.9% for males and 5.1% for females, shows that there are no differences of infection by sex. In two positive cases, the sex of the patients was unknown. Positive samples were found in all months of collection, with a higher prevalence in August (11 cases) and in February to March period with 9 (Table 3).

Fifteen samples out of 18 positive COWP gene PCR were typed by RFLP analysis. Nine isolates were *C. hominis* genotype, three *C. parvum* and three mixed infections.

Discussion

In the literature, the prevalence of *Cryptosporidium* infection in Egyptian patients varied significantly from 0% to 47% (Youssef et al. 2008). In this report the prevalence of *Cryptosporidium* infection in patients suffering from diarrhoea was 5.9%, 5.1% or 4.6%, by immune chromatography, microscopy or PCR of COWP gene. This was consistent

Table 2 Samples collected by age group and percentage of positive samples by age group and by total

Age group	<2 years	2–5 years	6–14 years	>14 years	ND
Samples collected	130	86	87	65	23
Positive samples	7	6	4	2	2
Percent positive within group	5.38	6.98	4.60	3.08	8.70
Percent of all positive samples	33.33	28.57	19.05	9.52	9.52

Table 3 Samples distribution by collection date and percentage of positive samples by month and total

Month	February	March	May	June	July	August
Samples collected	100	104	33	36	47	71
Positive samples	4	3	1	1	1	11
Percent by month	4.00	2.88	3.03	2.78	2.13	15.49
Percent by total	19.05	14.29	4.76	4.76	4.76	52.38

with previous reports done in Egypt in children (5.6%; Rizk and Soliman 2001), among US troops deployed near Alexandria (5–7%; Sanders et al. 2005) or in immunocompetent patients with diarrhoea (8.3%; Rezk et al. 2001). The broad range in prevalence may be attributed to many causes as immune state of the patients, patient's age, environmental habitats or seasonal variation (Derouin et al. 2010).

The prevalence of *Giardia* infection, detected by the immuno-test in this study, was 22.8%, which represented a higher infection rate compared to *Cryptosporidium* infection. These data were in concordance with other studies in Egypt (Abdel Hameed et al. 2008). *Giardia* spp. cysts are more abundant than *Cryptosporidium* spp. oocysts in drinking water, but *Giardia* cysts are less resistant to conventional chemical disinfectants than are *Cryptosporidium* oocysts (Smith and Grimason 2003). *Cryptosporidium* is deemed to be a greater public health threat because of its insensitivity to disinfection regimens used in water purification plants leading to an increased likelihood of infectious oocysts being present in conventionally treated drinking water (Smith et al. 2006).

The number of mixed infections with *Giardia* represented 21.7% (5/23) of the *Cryptosporidium*-positive patients. *Cryptosporidium* is a common pathogen present in cases with mixed infections (Sanders et al. 2005).

The three different *Cryptosporidium* diagnosis methods used in this report show differences in the detection of cases. The most sensitive method was the Stick Crypto-Giardia immunochromatographic kit which detected 23 positive cases of *Cryptosporidium*. The golden standard diagnosis method is the microscopic detection of the pathogen. This method identified 20 positive cases, 5 with low parasite load. The third method used was a nested PCR for the COWP gene. Just 18 samples were positive by PCR; 1 of them was a microscopy-negative sample. Three positive-microscopy samples with low parasitic load were negative by this method. This assay is described as the most sensitive method (Pedraza-Díaz et al. 2001; Yu et al. 2009), but in our case, COWP PCR sensitivity was unexpectedly low for unknown reasons.

Only five hospitals had positive *Cryptosporidium* cases. The Cairo University Paediatric Hospital is the one with more cases (17 out of 21), while the rest of hospitals only reported a positive case each. The percentage of positive cases by patients suffering diarrhoea in each hospital is similar (6.8%, 7.1% and 5.0%), and the differences are not statistically significant (P=0.29), except with the Al-Abbasia Fever Hospital (1.4%) which shows a percentage deviation on the expected frequency of minus 77%. This hospital is generally related with acute febrile diseases that together with the diarrhoea symptom seem to be more related to virus and bacterial infection or some immunocompromised diseases than to cryptosporidiosis. The fifth hospital to have a positive case was the ASU medical camp in Cairo which confirmed the only case analysed.

Cryptosporidiosis particularly affects children under 4 years of age. A high incidence of the disease in this age group has been reported in Canada, Unites States, New Zealand, England and Wales and France (Derouin et al. 2010). In Egypt, our data confirm that 61.9% of cases are attributable to this age group but without clear differences between the 2 and 5 years old as previously reported for Egypt. Children aged 12–23 months were 1.9 times more likely to be infected with *Cryptosporidium* than children 24–59 months (Abdel-Messih et al. 2005). The reason for this high incidence in children is unknown, but it may be related to lacking immunity, more exposed due to water play games together with unawareness or due to the fact that diarrhoeic children attend a physician more frequently, thus increasing the probability of *Cryptosporidium* detection (Derouin et al. 2010).

Most cases were recorded in August (52.4%), which is in accordance with other studies in different regions in Egypt (El-Shazly et al. 2007). The summer seasonal diarrhoeal peak could be due to the coincidence with the recreational water season, reflecting the increased use of rivers, lakes, swimming pools and water parks (Yoder and Beach 2007). In this study, there is a second infection peak in February and March with four (19.1%) and three (14.3%) cases respectively. The second peak is observed in the short precipitation period in Cairo (November to March). In Ethiopia a peak occurs between February to April (60.7%) which is the end of the dry season and beginning of the light rainy season (Adamu et al. 2010). Seasonal variation has been related to cycle of transmission and to *Cryptosporidium* species involved. The spring peak was almost exclusively due to *C. parvum*, while *C. hominis* was identified in patients infected during the late summer–autumn peak or in those with a history of foreign travel to tropical countries (McLauchlin et al. 2000; Pedraza-Díaz et al.

2001; Chalmers et al. 2009). In our study, using the COWP gene locus for genotyping, 10 out of 12 *C. hominis* infections were diagnosed in August while *C. parvum* occurs along the year in concordance with the seasonal variation found in other countries (Table 3).

The prevalence of cases by species was 80.0% for *C. hominis* and 40% for *C. parvum*, including three mixed infections. Mixed infections have previously been reported (Leoni et al. 2006; Chalmers et al. 2009). It has been reported that when hosts are coinfected with both species, *C. parvum* predominates and rapidly displaces *C. hominis* (Akiyoshi et al. 2003).This may explain the difference in the intensity of RFLP banding patterns between *C. parvum* and *C. hominis* observed in our study.

Genotyping results in our study showed predominance in the *C. hominis* distribution over *C. parvum*. This result is contradictory to the results of other studies in Egypt, where the predominant species was *C. parvum* (66.7%) followed by *C. hominis* (27.7%) and *C. meleagridis* (5.6%; Eida et al. 2009). In cases of human cryptosporidiosis, *C. hominis* predominates in industrialized nations such as Australia (76%), Canada (76%), Japan (68%), USA (67%), Spain (65.7%) and in developing countries such as Peru (79%), Thailand (83%) and South Africa (82%), showing that anthroponotic species are the leading cause of human cryptosporidiosis. However, in other countries, the relation of *C. parvum*/*C. hominis* is more balanced as in France (51%), Belgium (54.2%), England and Wales (49.2%) and *C. parvum* dominates in the Netherlands (72%) or Italy (90%, McLauchlin et al. 2000; Xiao et al. 2001; Cacciò et al. 2002; Ong et al. 2002; Samie et al. 2006; Llorente et al. 2007; Geurden et al. 2009). In these countries the anthroponotic transmission is not clear and subgenotyping of the *C. parvum* isolates is necessary to characterise zoonotic or anthroponotic genotypes. In Great Cairo, against more rural Egypt, our data suggest a clear anthroponotic transmission due to *C. hominis* which occurs in the summer period but further study is necessary to resolve the possible zoonotic transmission of *C. parvum* and its role in the spread of the disease in the spring season.

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