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Genetic Diversity of *Cryptosporidium* Causing Infections from Diarrheic Cases in Egypt and Co-infections with Other Intestinal Protozoan Parasites

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Abstract

CRYPTOSPORIDIOSIS is endemic worldwide; the highest rates are found in low- and middle-income countries. *Cryptosporidium* is transmitted via the fecal–oral route. *Cryptosporidium* spp. are important parasites in the small intestines of humans and animals. Cryptosporidiosis is a significant cause of diarrhea among humans in Egypt; however, data on *Cryptosporidium* genotypes in symptomatic patients from Egypt is scarce. The purpose of the current study was to identify the prevalence and various genotypes of *Cryptosporidium* species that circulate among diarrheal children in Egypt. A total of 185 stool samples from diarrheic children at the Hospital of Abu El Rish, Cairo were collected and examined using the modified acid-fast (AF) stain. *Cryptosporidium*-positive samples were conducted to multilocus genotyping using three genetic markers (COWP, Hsp90, and SSU rRNA) in various n-PCR-RFLP reactions. Co-infections with the other intestinal protozoa were detected using the direct wet mount as well as formol-ether concentration procedures. Of the 185 diarrheic subjects, 50 were confirmed positive for intestinal parasites (27.0%), including 18 that tested positive for *Cryptosporidium* either microscopically or molecularly. The other detected protozoa were Blastocystis hominis (24.0%), Giardia intestinalis (22.0%), and Entamoeba histolytica (18.0%). Two *Cryptosporidium* species were identified; *Cryptosporidium* hominis was the predominant species (83.33%) followed by *Cryptosporidium* parvum (16.66%). This study updates the status of cryptosporidiosis between children in Egypt and highlights the urgent need for establishing effective control strategies against this ubiquitous protozoon.

Keywords: Coccidian Parasite, RFLP, COWP, Hsp90, SSU rRNA.

Introduction

Cryptosporidiosis is an infection caused by the coccidian parasite *Cryptosporidium* spp. This parasite can be transmitted between animals and humans, resulting in health problems and economic losses. Infections have been documented in various animals, including horses, cattle, sheep, goats, and deer. One of the most significant symptoms of this infection is diarrhea, which is especially common among affected animals [1].

Members of the genus *Cryptosporidium* are widespread, obligate intracellular protist parasites that infect all groups of vertebrates. This parasite is closely linked to human poverty and can significantly impact the socio-economic status of infected individuals living in endemic areas [2].

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Cryptosporidiosis disease was present in the Neglected areas reported by the World Health Organization in the year of 2004 [3]. *Cryptosporidium* is an enteric pathogen known to cause diarrheal disease. It is transmitted through the fecal-oral route, either indirectly by consuming contaminated water or food, or through direct contact with infected humans or animals [2]. The symptoms associated with *Cryptosporidium* infection can vary widely and are influenced by multiple factors, including the presence of co-infecting enteropathogens, the composition of gut microbiota, the age of the host, the host's immune status, and nutritional factors [4].

Cryptosporidiosis is a significant source of global morbidity and mortality, affecting both veterinary and public health [5]. After rotavirus, this widespread parasite has been identified as the second most significant cause of diarrheal infections in humans [6]. Diarrhea, stomach pain, nausea or vomiting, and low-grade fever are signs of human cryptosporidiosis. While healthy individuals may experience limited effects, cryptosporidiosis can be fatal for immunocompromised patients and young children. [7]. There are several transmission pathways for human infections, including person-to-person, zoonotic, foodborne and water-borne [8,9].

Cryptosporidium spp. and *G. duodenalis* are parasitic protozoa that can infect a variety of hosts, including domestic animals, wild animals, and humans. This makes them significant from both medical and veterinary perspectives. To confirm the presence of *G. duodenalis* cysts and *Cryptosporidium* spp. oocysts, a direct immunofluorescence test was conducted. Molecular confirmation was performed using Polymerase Chain Reaction (PCR) to target the *G. duodenalis* S*SUrRNA* gene and the *Cryptosporidium* spp. *SSUrRNA* gene. The detection of species and genotypes with zoonotic characteristics in asymptomatic lambs and kids indicates that the oocysts and cysts shed by these animals are important for the health of both domestic animals and humans. [10].

To determine the parasitic burden accurately, various techniques are used, including microscopy scanning and copro-antigen detection methods such as immunoassay, immunofluorescence assay, and immune chromatography tests. Additionally, serological examination and nucleic acid amplification techniques are employed [11]. There is currently no clear immunological or gold-standard diagnostic method for differentiating between all types of oocysts. [12]. However, the introduction of molecular technology has enhanced our understanding of the epidemiology, biology, and transmission of this widespread parasite, including aspects of speciation and subtyping [8,13].

There are 120 genotypes and 44 valid species of *Cryptosporidium*, which is a significant concern for public health due to its zoonotic nature. Cryptosporidiosis is associated with severe outcomes, particularly for immune-compromised individuals. The disease can cause severe diarrhea and, in some cases, can be fatal or lead to persistent symptoms. In immune-compromised humans, it may also result in weight loss and acute malnourishment. In neonatal animals, Cryptosporidiosis can cause severe diarrhea, while in adult animals, the infection tends to manifest and acts as a primary source of infection [14].

The epidemiology and genotypes of *Cryptosporidium* isolates have been determined using many genetic markers, such as Small subunit (SSU) rRNA, *Cryptosporidium* oocyst wall protein (COWP), heat shock protein 90 (hsp 90), and gp60 [15]. The prevalence of Cryptosporidium spp. in both humans and animals has been documented across various provinces in Egypt. Previous microscopic and serological studies have shown that *Cryptosporidium* spp. are widely found in both diarrheic and non-diarrheic humans, as well as in different animal species, including cattle, rabbits, sheep, and goats [16]. However, there have been only a few studies that examined the molecular characteristics of *Cryptosporidium* spp. in human and animal specimens [17]. From 1.4 to 49.1%, the frequency of *Cryptosporidium* infection in Egyptian children varies substantially by governorate [18].

Recent studies utilizing restriction fragment length polymorphism (RFLP) and partial sequencing of various genetic markers have identified several species of *Cryptosporidium*, including *C. parvum*, *C. hominis*, and *C. meleagridis*. While there has been progress in researching *Cryptosporidium* species in Egypt, alarming evidence has emerged regarding the diversity and epidemiology of this parasite in humans. Our analysis aims to provide an updated overview of cryptosporidiosis infections among different stages of the human population and to offer essential information for developing effective control strategies against this parasite. Additionally, this study seeks to identify the intestinal protozoan parasites that contribute to diarrhea in children in Egypt.

Materials and Methods

The study design and population

The samples of this study were conducted at the Faculty of Medicine (Kasr Al Ainy), Cairo University. A total of 185 stool samples were collected from children aged 1 month to 5 years with diarrhea at the outpatient clinics of Abu El Rish Hospital, Cairo University. All ethical guidelines were strictly followed during the sample collection, and verbal consent was obtained from the parents of the participants. Information regarding age, gender, place of residence, and whether the children belonged to the majority or minority population was recorded for each stool sample container. Children with any bacterial infections (such as *Salmonella* and *Shigella*) or viral infections (such as Rotavirus) as causes of diarrhea were excluded from the study.

Methods of Coprological Diagnosis

All samples were kept at 4 °C without any preservation and delivered right away to the lab for additional parasitological testing. Each stool sample was 10 - 15 gm in volume. Sample were macroscopically inspected for tap worm proglottids, then per sample was subjected to direct wet mount with Lugol's iodine staining followed by formalin-ethyl acetate concentration technique [19]. For detection of oocyst of Cryptosporidium, the smears fecal were made and stained by a modified acid-fast (AF) stain [20].

Molecular Diagnostic Methods

Extraction of DNA

After the initial thermal shock (10 cycles of freezing in liquid nitrogen and thawing at 95°C) of fecal specimens, DNA was extracted directly from fresh stool samples using the QIAamp DNA Stool Mini Kit (cat. no. 51504) following the manufacturer's instructions.

Species and genotype identification

A nested PCR procedure was tested to evaluate *Cryptosporidium* samples that tested positive using MLGs at the loci for the small subunit-18S rRNA (SSU rRNA), heat shocking of protein (Hsp90), and *Cryptosporidium* oocyst wall protein (COWP), which are frequently used for Cryptosporidium genotyping research. The primer sequences, reaction conditions, and the restriction enzymes are given in Table 1. PCR reactions for amplification of the three loci under investigation were run in an Applied Biosystems 9700 thermal cycler. PCR products were subjected to RFLP using four restriction enzymes (RasI, StyI, HphI, and SspI) (Thermo Scientific); fragments of ~553, ~ 676 and ~850 bp in length corresponding to COWP, Hsp90, and 18S rRNA r were detected, respectively. For restriction digestion (37 °C for 2 h), 10 µl of secondary product in a 31-µl (total volume) reaction were used, and after staining with ethidium bromid, the products were fractionated on 3% Metaphor agarose medium [21].

Statistical analysis

IMB SPSS version 20 (Chicago, IL, USA) is a statistical tool for social science that was used to analyze the questionnaire data. Both all quantitative and qualitative data were presented, in addition to, when appropriate, the Fisher's exact test and the chi-squared test were employed to compare treated groups. The features of probable patients, clinical symptoms, and seasonality associations of infection with different the species of *Cryptosporidium* were identified by using the univariate analysis, and their significance was evaluated to determine appropriateness for multivariate logistic regression studies. The connections were described using odds ratios, and a p-value $\leq$ 0.05 was considered significant

Results

Demographic and clinical Data for Participants

Of the 185 diarrheic subjects, 50 (27%) had GIT parasites, including 18 (36%, 18/50) tested positive for Cryptosporidium either by AF staining or nested PCR. Demographic data for all participants such as age group, sex, residence, and clinical symptoms (vomiting, fever, dehydration, constipation, and abdominal pain) are listed in Table 2. The association between the clinical data and demographic of the study participants in Table 3. The mean age of the infected individuals with parasites was (3.6± 1.4 years); 45% were females and 55% were males and 67.6% were urban residents

Protozoa detected in the collected samples and Cryptosporidium PCR positive cases

Four intestinal parasites species were scanned in the diarrheic fecal samples out of 50 children cases (27.0%) as follows: *Cryptosporidium* spp. (18 cases), *Blastocystis hominis* (12 cases), *Giardia intestinalis* (11 cases), and *E. histolytica* complex (9 cases). Among the 50 cases of parasitic infection, 41 had a single parasitic infection (82%), and 9 cases had mixed infection (18%). *Cryptosporidium* spp. coinfected with *Giardia intestinalis* in 2 cases as well

as with *E. histolytica* complex in a single case. In addition, 3 cases of B. hominis coinfected with *E. histolytica* complex, 2 cases of *B. hominis* coinfected with *G. intestinalis* and one case of *E. histolytica* complex coinfected with *G. intestinalis*.

Genotyping of Cryptosporidium from fecal samples

Eighteen *Cryptosporidium*-confirmed in identification for samples by nested PCR (Figure 1) were genotyped using restriction enzymes (*RasI, StyI, HphI, and SspI*). Two *Cryptosporidium* species were identified; *C. hominis* was the predominant and detected in 15 (83.33%) samples. *C. parvum* was detected only in 3 (16.66%) samples

Discussion

Diarrhea is resource of illness and mortality in young children worldwide [22]. Intestinal protozoan parasites can show a diarrhea disease in children particularly in developing countries, with *Cryptosporidium* spp. being the most prevalent in children with diarrhea symptom, followed by *Giardia lamblia* and *Entamoeba histolytica*; respectively [23,24]. In this study, four parasites were detected in 50 fecal samples from children with diarrhea: *Cryptosporidium* spp. (36%), *Blastocystis hominis* (24%), *Giardia intestinalis* (22%), and *Entamoeba histolytica* complex (18%). The presence of these parasites validates the possible causes of children's diarrhea in this study. Likewise, those four parasites were also detected in diarrheic children from Minia and Ain-Shams districts, Egypt [25,26]. Intestinal parasitic infections among Egyptian individuals have different health socio-demographic and clinical data with the four parasites [27], and infections with *G. intestinalis, Cryptosporidium* spp., *E. histolytica* [28-31] have been documented in various governorates.

The infection with *Cryptosporidium* was reported among humans prevalence in Egypt ranged from 0.06 to 50% (Table 4). The prevalence of Cryptosporidium spp. among diarrheic patients in this study was 9.27% (18/185) coinciding with that has been recorded in other governorates [32-34]. The high prevalence of cryptosporidiosis symptom in Egypt could be attributed to the nature of human practices in the region, their close associations with animals, lack of hygienic conditions, and access of livestock animals to water sources which may increase the infection of the risk [18].

Different diagnostic tools that used in the site studies from Egypt to identify *Cryptosporidium* oocysts, of which, molecular tools were relayed on different genetic markers including the COWP, Hsp90, SSU rRNA and TRAP-C2 genetic loci. Even though they were not genotyped, some of these studies recognized *C. parvum* oocysts recovered from positive clinical samples. However, based on the process of (RFLP) restriction fragment length polymorphism and partial sequencing of a few genetic markers, and a few recent papers identified *C. hominis, C. parvum* and *C. meleagridis* species and surprisingly *C. bovis* which has been recorded in one study [35]. Over 90% of the global human infections are caused by the two main species, *C. parvum,* and *C. hominis,* with *C. hominis*, also being noted as the predominant species in the developing world [36-37]. *Cryptosporidium meleagridis* is the third species most commonly confirmed in the human cases which is largely seen in developing countries [32,34]. Nevertheless, only four studies reported *C. bovis* in humans worldwide including a study from Egypt [35,38].

Cryptosporidium species identified in this study were *C. hominis* (83.33%) and *C. parvum* (16.66%). Similarly, earlier studies in Egypt demonstrated the dominance of *C. hominis* over *C. parvum* among genotyped samples [34, 39-41]. Contrastingly, *C. parvum* was dominant in other studies [32, 35]. These results indicate a complex transmission pattern of cryptosporidiosis in Egypt with the dominance of anthroponotic over zoonotic transmission.

For different groups, there were not significant variations in the prevalence of *Cryptosporidium* spp. among based on gender, clinical symptoms and residence, which is consistent with earlier findings in individuals from various Egyptian governorates [39,42,43]. *Cryptosporidium* prevalence in this study was nearly equal for children from rural and urban areas (37.5% and 35.3%, respectively). In Egypt, children in rural areas are more likely to have animal contact, which is one of the most commonly documented risk factors for cryptosporidiosis in low and middle-income nations [44]. In contrast, larger population densities in metropolitan settings may result in increased person–to–person transmission of *C. hominis* [45]. This idea was validated in Egypt, where *C. hominis* was discovered primarily in urban individuals, but *C. meleagridis C. parvum* and were frequently found in rural residents [41]. Furthermore, the frequent detection of both species *C. parvum* and *C. hominis*, as well as distinct subtypes, suggest the transmission of cryptosporidiosis by both anthroponotic and zoonotic patterns, which increases

the risk of infections throughout the country, both in rural and urban regions.

Conclusion

The data included in this study suggest the potential risk of cryptosporidiosis in the Egyptian population with evidence of complex transmission patterns with both anthroponotic and zoonotic transmission cycles. The current knowledge of *Cryptosporidium* infections among humans is largely lack and nationwide disease surveillance is required for setting up effective control strategies. Investigations of children's diarrheal-causing agents are important for both treatment and control. Better hygienic measures and disinfection are required especially for those having chronic disease conditions; as well as fencing of livestock away from water sources and good sanitation are also required to provide safe drinking water.

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Declaration of Competing Interest

The authors declaring that they have no personal relationships or known competing financial interests that could have appeared to influence the work reported in this study.

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Ethical of approval

This study was approved by the Ethical Committee of Research during the faculty of Veterinary Medicine, Mansoura University, Egypt with the No. Ethical code: MU-ACUC (VM.R.23.10.127).

Availability of data and material: NA

Contributions of Authors: The study concept and design as following: AI, EE and MG acquisition of data: AI, EE and MG analysis and interpretation: AI, EE and MG manuscript writing and revision: AI, EE, AD, DN, MG

to Consent participate

All were informed cases of the purpose of the collection of sharing and samples in the current study was selective.

Consent for publication

All authors affirm that human participants of research provided informed consent for publication.

TABLE 1. Primers, targets, reaction conditions, and restriction enzymes

Primer	Sequence (5'-3')	Gene	Reaction Conditions	Restriction	Ref.
BcowpF	ACC GCT TCT CAA CAA CCA TCT TGT CCT C	COWP	35 cycles of 94°C for 60 s, 63°C for 60 s, and 72°C for 60 s	RasI	46, 47
BcowpR	CGC ACC TGT TCC CAC TCA ATG TAA ACC C				
Cry-15	GTA GAT AAT GGA AGA GAT TGT G		35 cycles of 94°C for 60 s, 54°C for 30 s, and 72°C for 60 s		
Cry-9	GGA CTG AAA TAC AGG CAT TAT CTT G				
Hsp90-F3	CTA GTG AAA GCT ACG AGT TCC AA	Hsp90	35 cycles of 94°C for 45 s, 50°C for 45 s, and 72°C for 60 s	StyI and HphI	48
Hsp90-R3	TCT ATTTCA CCT TCG GCG GAA AA				
Hsp90-F4	GGA TAT TAT TAT TAA CTC TCT CTA TTCTCA		35 cycles of 94°C for 45 s, 55°C for 45 s, and 72°C for 60 s		
Hsp90-R4	CCA TAT TGC CTT TTC TAC ATT AAC				
rRNA F	TTCTAGAGCTAATACATGCG	18s rRNA	35 cycles of t 94°C for 45 s, 60°C for 45 s, and 72°C for 60 s	SspI	49
rRNA R	CCCATTTCCTTCGAAACAGGA				
Nest	GGAAGGGTTGTATTTATTAGATAAAG		35 cycles of 94°C for 45 s, 54°C for 45 s, and 72°C for 30 s		
Nest	CTCATAAGGTGCTGAAGGAGTA				

TABLE 2. Socio-demographic and clinical data of positive cases for Parasites

Variables			Positive N (%)	Negative N (%)	Total N (%)	P-value
Age group (Year)	<1		8 (4.3%)	38 (20.5%)	46 (24.8%)	0.19
	>1-5		42 (30.2%)	97 (45.0%)	139 (75.2%)	
Sex	Male		29 (28.7%)	72 (71.3%)	101 (55.0%)	0.724
	Female		21 (25.0%)	63 (75.0%)	84 (45.0%)	
Residence	Urban		32 (25.6%)	93 (74.4%)	125 (67.6%)	0.56
	Rural		18 (30.0%)	42 (70.0%)	60 (32.4%)	
Clinical Symptoms	Vomiting	Yes	26 (22.0%)	92 (78.0%)	118(63.8%)	0.92
		No	24 (35.8%)	43 (64.2%)	67 (36.2%)	
	Abdominal pain	Yes	38 (30.4%)	87 (69.6%)	125 (67.6%)	0.315
		No	12 (20.0%)	48 (80.0%)	60 (32.4%)	
	Fever	Yes	15 (26.3%)	42 (73.7%)	57 (30.8%)	0.428
		No	35 (27.3%)	93 (72.7%)	128 (69.2%)	
	Dehydration	Yes	29 (41.4%)	41 (58.6%)	70 (37.8%)	0.724
		No	21 (18.3%)	94 (81.7%)	115 (62.2%)	
Total			50 (27.0%)	135 (73.0%)	185 (100%)	

P-value is statistically significant at <0.05

TABLE 3. Socio-demographic and clinical data of nPCR positive cases for Cryptosporidium

Variables			Positive N (%)	Negative N (%)	Total N (%)	P-value
Age group (Year)	<1		2(16.6%)	10 (83.4%)	12 (24.0%)	0.125
	>1-5		16(42.1%)	22 (57.9%)	38 (76.0%)	
Sex	Male		11(36.6%)	19 (63.4%)	30(60.0%)	0.636
	Female		7 (35.0%)	13 (65.0%)	20 (40.0%)	
Residence	Urban		12(35.3%)	22 (64.7%)	34 (68.0%)	0.50
	Rural		6(37.5%)	10 (62.5%)	16 (32.0%)	
Clinical Symptoms	Vomiting	Yes	9 (28.1%)	23(71.9%)	32(64.0%)	0.562
		No	9 (50.0%)	9(50.0%)	18 (36.0%)	
	Abdominal pain	Yes	14(41.2%)	20(58.8%)	34 (68.0%)	0.285
		No	4(25.0%)	12(75.0%)	16 (32.0%)	
	Fever	Yes	4 (28.6%)	10 (71.4)	14 (28.0%)	0.388
		No	14(38.9%)	22 (61.1%)	36 (72.0%)	
	Dehydration	Yes	10(52.6%)	9(47.4%)	19 (38.0%)	0.81
		No	8(25.8%)	23(74.2%)	31 (62.0%)	
Total			18(36.0%)	32(64.0%)	50(100%)	

P-value is statistically significant at <0.05

TABLE 4. Study characteristics of *Cryptosporidium* infections among humans* in Egypt.

Region	Age range	Status	No. tested	No. positive (%)	Diagnostic tools	*Cryptosporiduim* genotypes and subtypes	Ref.
Benha	<5 yr	diarrheic patients	1275	214 (17)	commercially ELISA kit	*C. parvum*	50
Alexandria							
Fayoum	< 60 mo	diarrheic patients	253	39 (15)	commercially ELISA kit	*C. parvum*	51
Cairo	1 mo-70 yr	diarrheic patients	391	23 (5.88)	immunochromatography kit	*C. hominis* and *C. parvum*	52
			23	20	MZN		
			20	18	n-PCR-RFLP (COWP gene)		
Minia	Children	immunosuppressed #	200	100 (50)	MZN	*C. parvum*	53
		immunocompetent	250	60 (24)			
Ain Shams	2–15 yr	children with ALL	25	6 (24)	MZN	NS	54
		healthy control	30	1 (3)			
Damietta	< 12 yr	Patients with or without diarrhea	330	25 (7.57)	MZN	NS	55
	> 12 yr						
Cairo; Fayoum and Benha	6 mo-60 yr	diarrheic patients	396	8 (2)	real-time PCR	*C. parvum*	56
				4 (1)	MZN		
		healthy controls	202	3 (1.5)	real-time PCR		
				0 (0)	MZN		
Ismailia	1 d-10 yr	diarrheic patients	165	11 (6.7)	RIDA®QUICK test	*C. parvum* (60.5%) (IIdA20G1; IIaA15G1R1; IIaA15G2R1) ©, C. hominis (38.2%) and C. bovis (1.2%)	57
				81 (49.1)	n-PCR-RFLP (18S rDNA)		58
Mansoura	Variable	CLD patients	150	45 (30)	MZN, ELISA	*C. hominis* and *C. parvum*	59
		diarrheic patients	50	7 (14)			
Cairo	13–59 yr	GI patients	104	7 (7)	RIDA®QUICK test	*C. parvum/hominis*	60
Cairo	Variable	diarrhoeic patients	862	64 (7.4)	MZN	*C. hominis* (95.8 %) and *C. parvum* (3.0 %)	61
				168 (19.5)	n-PCR-RFLP (COWP gene)		
Cairo	< 5 yr	diarrheic patients	356	14 (3.9)	commercial ELISA kit	NS	62
Ain-Shams	Children	children with CLD	50	5 (10)	MZN	NS	26
		GI patients controls	50	6 (12)			
Qalubyia	1 - 14 yr	diarrheic patients	430	50 (11.63)	MZN n-PCR-RFLP (TRAP-C2 gene)	C. hominis (12%) and *C. parvum* (82%)	32
Alexandria	Variable	mentally handicapped patients	200	47 (23.5)	MZN	NS	63
Assiut	1-6 yr	GI patients	300	35 (11.7)	MZN	NS	64
Alexandria	21-59 yr	municipality solid-waste workers	346	81 (23.4)	MZN	NS	65
Beni-Suef	1 mo – 60 yr	healthy and diarrheic	290	55 (19)	MZN	*C. hominis* (n=15) and *C. parvum* (n=5) (IIdA20G1) ®	66
Cairo	4 – 12 yr	diarrhoeic patients	182	24 (13.2)	ELISA	C. parvum	67
				47 (25.8)	n-PCR-RFLP (COWP gene)		
		apparently healthy	100	8 (8)	ELISA		
				16 (16)	n-PCR-RFLP (COWP gene)		
Sharkia	6-60 yr	GI patients	71	18 (19.8)	MZN	*C. parvum*	68
				19 (20.9)	RIDA®QUICK test		
El-Wadi El-Gadded	6-16 yr	School children	1615	1 (0.06)	MZN	*C. parvum* (authors didn't report genotyping method)	69
Aswan	6-12 yr	School children	300	5 (1.7)	MZN	*C. parvum* (authors didn't report genotyping method)	70
Mansoura	2-58 yr	GI patients	185	41 (22)	commercially ELISA kit	NS	29
				28 (15)	MZN		
Cairo		diarrhoeic patients	150	14 (9.33)	AF stain	NS	33
				15 (10)	ICT RIDA®QUICK test		
				16 (10.66)	ELISA		
					n-PCR (Hsp90 gene)		
		apparently healthy	100	35 (23.33)	AF stain		
					ICT RIDA®QUICK test		
				0 (0)	ELISA		
				1 (1)	n-PCR (Hsp90 gene)		
				2 (2)			
				6 (6)			

TABLE 4. Study characteristics of *Cryptosporidium* infections among humans* in Egypt. [Continue……]

Region	Age range	Status	No. tested	No. positive (%)	Diagnostic tools	*Cryptosporiduim genotypes and subtypes*	Ref.
Cairo	1 - 12 yr	diarrhoeic patients	331	78 (23.6)	n-PCR-RFLP (Hsp90 gene)	*C. hominis* (89.3%) and *C. parvum* (7.1%)	39
		apparently healthy	100	6 (6)			
Beni-Suef	Variable	diarrheic patients	200	42 (21.0)	n-PCR (COWP gene)	NS	42
				25 (12.5)	ELISA		
				19 (9.5)	MZN		
Giza	<10 yr	diarrheic patients	173	6 (3.5%)	MZN	NS	71
El-Dakahlia	2 - 8 yr	healthy and diarrheic	272	5 (1.8)	n-PCR-RFLP (SSU rRNA)	*C. parvum* (IIaA15G2R1; IIcA5G3), *C. hominis* (IbA6G3; IdA17; IdA24) ©	16
El-Gharbia		healthy and diarrheic	189	2 (1.1)	n-PCR-RFLP (SSU rRNA)	*C. parvum* (IIdA20G1) *C. hominis* (IbA6G3) ©	
Damietta		healthy and diarrheic	124	1 (0.8)	n-PCR-RFLP (SSU rRNA)	*C. hominis* (IfA14G1R5) ©	
Qena	38 - 73 yr 20-66	CKD patients	150	60 (40.00)	MZN	NS	72
		healthy control	50	3 (6.00)			
Sharkia	2–12 yr	healthy and diarrheic	100	35 (35)	MZN	*C. hominis* (n=7) and *C. parvum* (n=4) and mixed (n=3)	73
				14 (14)	n-PCR (COWP gene)		
Alexandria	Variable	HD patients	120	39 (32.5)	MZN	NS	27
		apparently healthy	100	11 (11.0)			
Cairo	NS	CLD patients	60	2 (3.3)	n-PCR- RFLP	*C. parvum*	74
		apparently healthy	60	0 (0)			
Cairo	3mo-12yr	diarrheic patients	100	5 (5)	MZN	*C. hominis*	43
				19 (19)	n-PCR-sequencing		
Gharbia	1 –<5yr 5–15 yr	healthy and diarrheic	996	15 (1.5)	Modified Kinyoun's Acid-Fast Stains	*C. parvum*	30
Alexandria	<10yr	childern with malignancies	100	6 (6)	MZN	*C. parvum* type II	34
				3 (3)	COWP (PCR-RFLP)		
Sharkyia	1-15 yr	diarrheic patients	97	27 (27.8)	MZN	*C. hominis* (65.2%) and *C. parvum* (30.4%)	40
			27	23 (85.2)	COWP (PCR-RFLP)		
Alexandria	> 18 yr	NAL patients	40	2 (5)	MZN	NS	75
				2 (5)	RIDA®QUICK test		
Alexandria	19-65 yr	HIV patients	100	16 (16)	COWP (PCR-RFLP)	*C. hominis* (45.4%), *C. parvum* (27.3%), and *C. meleagridis* (18.2%)	41
				15 (15)	MZN		

*Human samples were mainly collected from hospitals which are visited by patients from different provinces and the mentioned regions are the hospitals location.
#60 cases (30%) with severe malnutrition, 50 cases (25%) with chronic diseases, 65 cases (32.5%) were receiving corticosteroids, and 25 cases (12.5%) with malignancies.
® nested PCR analyses of COWP and gp60 genes was used for typing and subtyping analysis of 20 human positive samples.
© Subtyping analysis was conducted based on gp60 gene.
Abbreviations: ALL: acute lymphoblastic leukemia; CLD: chronic liver diseases; CKD: Chronic Kidney Disease; HD: Hemodialysis patients; NAL: neutropenic acute leukemia patients; HIV: human immunodeficiency virus; GI, patients with gastrointestinal complaints; MZN, modified Ziehl–Neelsen-stained smears; d, day old; wk, week; mo, month; yr, year; NS, not stated.

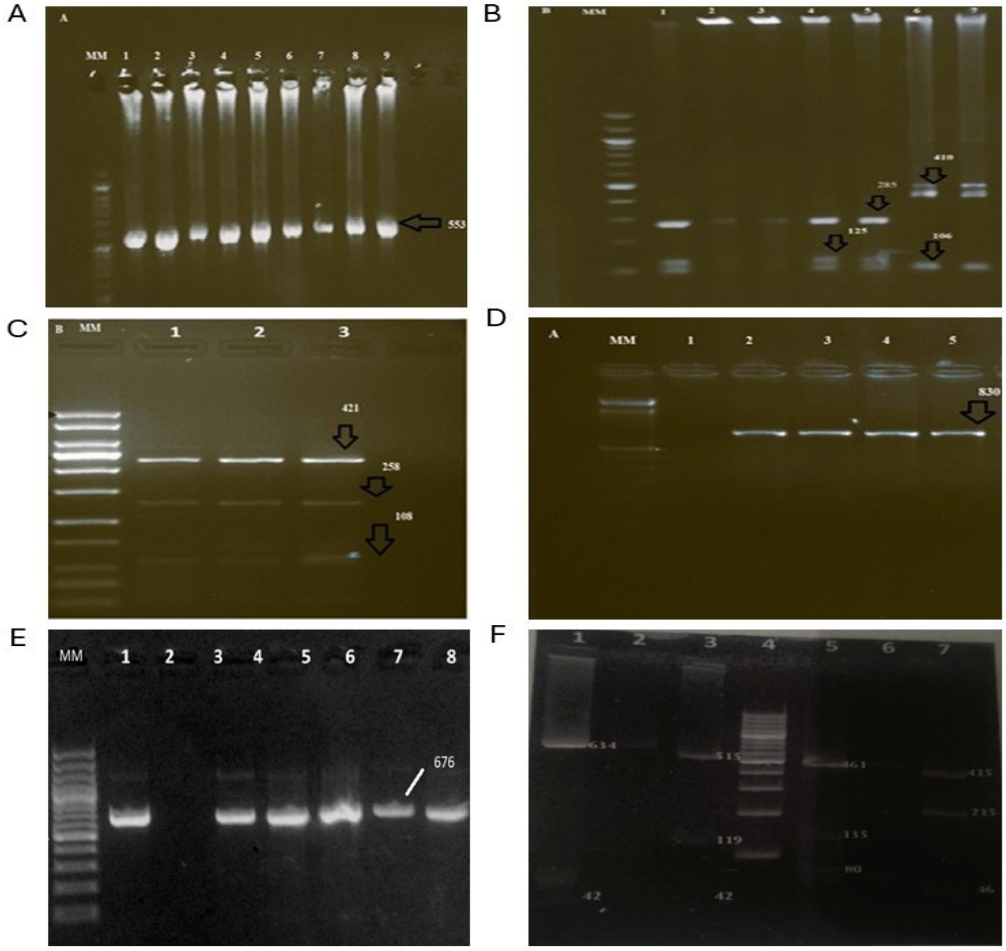

Fig. 1. Agarose gel electrophoresis for the products of the nPCR targeting A: COWP gene of Cryptosporidium spp. at 553bp. LaneMM: 100 bp DNA molecular weight marker "ladder". Lane 1 Positive control at 553bp. Lanes2-9: positive control. B: Hsp90 gene of Cryptosporidium spp. at 850bp. Lane MM: 100 bp DNA. C: Lane MM: 100 bp DNA. Lane 1 Negative sample, Lanes 2-5 Positive samples, D: After digestion, Lanes 1-3 positive (*C. hominis*/ *C. parvum*) samples (421,258,108). E: Lane MM: 100 bp DNA. Lane positive sample, Laen 2 Negative sample. Lanes (3-8) positive samples. F: Lane MM: 100 bp DNA. Lane 1 Positive sample, Lane 2 Negative sample, Lanes (3-5) Positive sample, Lane 6 Negative sample, Lane 7 Positive sample.

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التنوع الوراثي لطفيل الكريبتوسبوريديوم المسبب للعدوي من حالات الإسهال في مصر والعدوي المصاحبة للطفيليات المعوية الأخري

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الملخص

يعتبر مرض الكريبتوسبوريديوسيس مستوطن في جميع أنحاء العالم. وتوجد أعلى المعدلات في البلدان المنخفضة والمتوسطة الدخل. فقد وجد ان طفيل الكريبتوسبوريديوم ينتقل عبر المجري البرازي الفموي وهذا الطفيل منشأه في الأمعاء الدقيقة للإنسان والحيوان. يعد داء خفيات الأبواغ أحد الأسباب المهمة للإسهال بين البشر والحيوانات في مصر؛ ومع ذلك، فإن البيانات المتعلقة بالأنماط الجينية للكريبتوسبوريديوم في المرضى الذين تظهر عليهم الأعراض من مصر نادرة ولذالك كان الغرض من هذه الدراسة هو التعرف على مدى انتشار وأنماط وراثية مختلفة لأنواع الكريبتوسبوريديوم المنتشرة بين الأطفال المصابين بالإسهال في مصر حيث تم الحصول على إجمالي 185 عينة براز من الأطفال المصابين بالإسهال في مستشفى أبو الريش بالقاهرة وصبغها باستخدام صبغة معدلة للحمض السريع (AF). تم إجراء عينات إيجابية للكريبتوسبوريديوم على التنميط الجيني متعدد البؤر باستخدام ثلاث علامات وراثية (COWP ، Hsp90، و SSU rRNA) في تفاعلات n-PCR-RFLP المختلفة وايضا تم الكشف عن حالات العدوى المصاحبة مع الأوليات المعوية الأخرى باستخدام التركيب الرطب المباشر بالإضافة إلى إجراءات تركيز الفورمول إيثر ايضا وجد انه من بين 185 شخصًا مصابًا بالإسهال، تم التأكد من إصابة 50 شخصًا بالطفيليات المعوية (27.0%)، بما في ذلك 18 شخصًا تم اختبارهم إيجابيًا للكريبتوسبوريديوم إما مجهريًا أو جزيئيًا. وكانت البروتوزوا الأخرى المكتشفة هي (Blastocystis hominis البلاستوسيتس هوميناس 24.0٪)، (والجيارديا المعويةEntamoeba histolytica 22.0٪)، (ومركبEntamoeb histolytica complex 18٪). تم التعرف ايضا على نوعين من انواع جنس الكريبتوسبوريديوم؛ وهم كالتالي: Cryptosporidium hominis وهو النوع السائد (83.33%) يليه Cryptosporidium parvum 16.66%) وهو النوع الاقل . ولذالك فإن تعمل هذه الدراسة تعمل على تحديث حالة داء خفيات الأبواغ بين الأطفال المصابون في مصر، وايضا تسلط الضوء على الحاجة الضرورية لوضع استراتيجيات مكافحة فعالة ضد هذا انواع هذا الطفيل الأولي المنتشر في كل مكان.

الكلمات الدالة: COWP ،جين Hsp90, SSU Rrna بروتين، طفيل الكوكسيدا ،الحمض النووي الريبوزومي ، طريقة تحديد التغيرات في توالیات DNA.