Original Article

Cryptosporidium spp. and *Helicobacter pylori* in a hospital-based study of diarrheic immunocompromised Egyptian children: Insight into risk factors, and co-infection

Asmaa Ibrahim[1], Yasser BM Ali[1], Amal Abd El-Aziz[1], Ayman A El-Badry[2]

Genetic Engineering and Biotechnology Research Institute, University of Sadat City, Egypt[1], Department of Microbiology, Faculty of Medicine, Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia[2]

ABSTRACT

Background: Immunocompromised children are more susceptible to a diversity of pathogens including norovirus, rotavirus, diarrheagenic *Escherichia coli*, *Cryptosporidium* spp. and *Helicobacter pylori*.
Objective: This study aimed to determine the detection rate of *H. pylori* and *Cryptosporidium* spp.; their co-infection in a hospital-based study of diarrheic immunocompromised children and their predictive risk factors; and to evaluate the diagnostic performance of the used assays.
Subjects and Methods: Fecal specimens were collected from 102 immunocompromised diarrheic children, with ages ranging from few months old to 16 years. All fecal samples were examined microscopically for detection of parasites, as well as immunologically and molecularly for detection of *H. pylori* and *Cryptosporidium* spp. Copro-antigens of *Cryptosporidium* and *H. pylori* were detected immunologically using rapid chromatographic copro-immunoassay tests. Amplification of *H. pylori* and *Cryptosporidium* copro-DNA was performed using the nPCR assay targeting genes encoding *H. pylori* urease A and *Cryptosporidium* oocysts wall protein (COWP). Amplified *Cryptosporidium* PCR products were digested by a restrictive enzyme to detect genotype.
Results: *H. pylori* copro-DNA and copro-antigen were detected in 56 (54.9%) and 18 (17.6%) patients, respectively. *Cryptosporidium* copro-DNA, and copro-antigen were detected in 22 (21.6%), and 16 (15.8%) patients, respectively, while microscopy detected *Cryptosporidium* oocysts in only 6 patients (5.9%), with a clear predominance of anthroponotic *C. hominis* (81%). *Cryptosporidium* spp. and *H. pylori* co-infection occurred in 15.8% of patients. None of the studied variables had a significant association with any of the tested pathogens, neither separately nor combined.
Conclusion: There was a high detection rate of *H. pylori* and *Cryptosporidium* spp. and their co-existence in diarrheic immunocompromised children. Our study results highlight that PCR increased the sensitivity for the diagnosis of *Cryptosporidium* spp. and *H. pylori*. More research is needed to establish their relevance.

Keywords: children; co-infection; copro-antigen; copro-DNA; *Cryptosporidium*; genotyping; *H. pylori*; immunocompromised.

Received: 25 May, 2022; **Accepted:** 14 August, 2022.

Corresponding Author: Ayman A. El-Badry, **Tel.:** +966 538045488, **E-mail:** aelbadry@kasralainy.edu.eg

Print ISSN: 1687-7942, **Online ISSN:** 2090-2646, **Vol. 15, No. 2, August, 2022.**

INTRODUCTION

Several disorders can compromise the immune systems of pediatric populations, making them more susceptible to a diversity of infections. These disorders may be inborn (primary) or acquired (secondary) deficiencies of immunity. Secondary immunodeficiency is more prevalent than primary immunodeficiency. The latter are caused by genetic defects affecting cells of the immune system. Infectious agents, medications, metabolic illnesses, and environmental variables can all influence a host with an innately normal immune system, resulting in secondary immunodeficiencies[1]. Infections in immunocompromised children remain a substantial cause of morbidity and mortality[2].

Cryptosporidium spp. and *H. pylori* pathogens are prevalent in pediatric populations in the first five years of life. Awareness about the parasitic protozoa *Cryptosporidium* spp. increased following several epidemics in developed countries[3]. Cryptosporidiosis commonly causes acute self-limiting diarrhea in immunocompetent individuals. In neonate and young infants. and immunocompromised individual's, infection becomes chronic with severe diarrhea leading to dehydration[4]. Cryptosporidiosis is one of the most prevalent illnesses among immunocompromised children presenting with diarrhea and was recognized by the WHO as one of the major causes of child and infant mortality in many developing countries[5]. Nevertheless, cryptosporidiosis is still miss-diagnosed and

 DOI: 10.21608/PUJ.2022.140818.1170

neglected, particularly in developing countries[6]. Routine detection of *Cryptosporidium* oocysts commonly relies upon both microscopic examinations of stool samples stained with modified acid-fast (AF) stain[7] and immunoassays[8]. *Cryptosporidium* copro-antigen detection is the most common immunoassay, either using immunochromatography test (ICT) or direct fluorescent antibody assay or sandwich-ELISA[8]. Molecular assays, particularly PCR-based assays, accurately detect and characterize *Cryptosporidium* spp.[9].

On the other hand, *H. pylori* is one of the most prevalent pathogens in gastro-duodenal diseases. The infection is usually acquired during early childhood and causes chronic gastritis in children[10]. It can also cause extra–gastrointestinal diseases, especially in immunocompromised individuals[11]. Several diagnostic tests for the detection of *H. pylori* infection are available; each has its own set of benefits and drawbacks. None of them can be regarded a gold standard because of their low sensitivity or specificity. Combining multiple tests, such as urease enzyme production test[12], microscopy, bacterial isolation, and PCR, typically results in an acceptable diagnosis. These methods, on the other hand, are invasive methods that require endoscopy and biopsy. They are costly, and only appropriate for tertiary level laboratories. Because invasive procedures have their drawbacks, numerous non-invasive diagnostics such as urea breath test, serology, and detection of *H. pylori*-antigen and *H. pylori*-DNA in stool were developed to identify *H. pylori*[12].

Cryptosporidiosis and *H. pylori* infections share the same transmission route of infection and are associated with socioeconomic hygienic habits[13]. In general, chronic *H. pylori* infection in childhood increases stomach acidity due to the inflammation of antral cells. Urease produced by *H. pylori* decreases stomach acidity that helps intestinal parasites and bacteria to colonize the gastrointestinal tract[14].

Our study aimed to determine the detection rates of *Cryptosporidium* spp. and *H. pylori* and their co-infection in diarrheic immunocompromised children; evaluate the diagnostic performance of different diagnostic methods and highlight the predictive risk factors for *Cryptosporidium* spp. and *H. pylori* as well as their co-infection.

SUBJECTS AND METHODS

This hospital-based study was conducted at the Laboratory of Molecular Medical Parasitology (LMMP) and the Diagnostic and Research Unit of Parasitic Diseases (DRUP), Kasr Al-Ainy Faculty of Medicine, Cairo University, during the period from December 2016 to October 2017.

Study design: Stool samples collected from immunocompromised diarrheic children were examined for detection of *Cryptosporidium* spp. Rapid chromatographic copro-immunoassay tests, and PCR assays were used for immunological and molecular detection of *Cryptosporidium* spp. and *H. pylori*. Amplified *Cryptosporidium* PCR products were genotyped.

Target population: The target group for the present study was children between the ages of less than one year to 16 years old suffering from diarrhea. Our study populations were either primary or secondary immunodeficiency disorders from Pediatric Allergy and Immunology Department, at Abu El Reesh Cairo University Pediatrics Hospital, Kasr-Al–Ainy Faculty of Medicine, Cairo University. A total of 102 diarrheic stool samples were collected.

Stool specimen and data collection: Fresh single fecal specimens were collected from each child and the related sociodemographic and clinical data were collected using a questionnaire.

Stool specimen processing: Collected fecal specimens were examined microscopically, then processed using immune and molecular assays to detect copro-antigen and copro-DNA for both *Cryptosporidium* and *H. pylori*.

Copro-parasitological microscopy: All collected stool specimens were microscopically examined for intestinal parasites by using direct wet mount before and after double concentration of fecal specimens[15]. Fecal smears were stained by modified acid-fast (AF) stain for detection of *Cryptosporidium* oocyst[16].

Copro-immunoassay: Rapid chromatographic copro-immunoassay tests (ICT) were utilized for the detection of *Cryptosporidium* copro-antigen (RIDA QUICK Art. No. N1203)[17] and *H. pylori* copro-antigen (CTK Biotech, USA) according to the directions of the manufacturer[18].

Copro-nested PCR assay: Copro-DNA was extracted using the Favor Stool DNA Spin Columns Isolation Mini Kit (Favorgen Biotech Corporation, Taiwan) according to the manufacturer's directions[18], after the initial thermal shock composed of cycles of freezing and thawing of fecal specimens at 95°C, to disrupt the *Cryptosporidium* oocyst wall[17]. The samples were placed in liquid nitrogen in a foam tube rack for 5 min then transferred to a water bath at 95°C for 5 min (10 cycles).

Amplification of *H. pylori* and *Cryptosporidium* copro-DNA was performed using the nPCR assay targeting genes encoding *H. pylori* urease A and *Cryptosporidium* oocysts wall protein (COWP) (Table 1). Each reaction was performed using a PCR mix: 12.5 µl PCR Master Mix (Thermo Scientific, UK), 1 µl of 200 nmol/l of each forward and reverse primer,

Table 1. Used primers targeting genes encoding *H. pylori* urease A and *Cryptosporidium* COWP genes.

Pathogen	Primer	Sequence	Product size
H. pylori	**1ry**	**Forwaed:** 5'-ATATTATGGAAGAAGCGAGAGC-3' **Reverse:** 5'-ATGGAAGTGTGAGCCGATTTG-3'	293 bp
	2ry	**Forwaed:** 5'-CATGAAGTGGGTATTGAAGC -3' **Reverse:** 5'-AAGTGTTGAGCCGATTTGAACCG-3'	200 bp
Cryptosporidium **spp.**	**1ry**	**Forwaed:** 5´-ACCGCTTCTCAACAACCATCTTGTCCTC-3´ **Reverse:** 5´-CGCACCTGTTCCCACTCAATGTAAACCC-3´	769 bp
	2ry	**Forwaed:** 5´-GTAGATAATGGAAGAGATTGTG-3´ **Reverse:** 5´-GGACTGAAATACAGGCATTATCTTG-3´	553 bp

2.5 µl of template DNA, 0.1 µl Taq polymerase (5 U/µl) and 7.9 µl of sterile distilled water to complete a total volume of 25 µl. Reactions were performed in a gradient thermal cycler (thermo-cycler, Biometra; Applied Biosystems, California, USA) after adjusting the thermal profile to initial denaturation at 95°C for 4 min, followed by 30 cycles of amplification. Each cycle consisted of denaturation at 94°C for 60 sec, annealing at 65°C for 60 sec and extension at 72°C for 60 sec. Final elongation was performed at 72°C for 10 min. The second-round PCR was identical to the first-round PCR except for denaturation at 94°C for 50 sec, annealing at 54°C for 30 sec, and extension at 72°C for 50 sec for *Cryptosporidium*[19,20].

For *H. pylori*, each reaction was performed after adjusting the thermal profile to initial denaturation at 95°C for 3 min, followed by 35 cycles of amplification. Each cycle consisted of denaturation at 94°C for 60 sec, annealing at 57°C for 60 sec and extension at 72°C for 1 min and 30 sec. Final elongation was performed at 72°C for 5 min for both rounds[21]. The nPCR-based assays were done using two sets of 2 primer pairs, where the first set of the primer pairs was used to produce the DNA template for the nested reaction. The second set of primer pairs was used to anneal the previously obtained amplicon (293 and 769 bp for *H. pylori* and *Cryptosporidium*, respectively), to increase the specificity of *H. pylori* and *Cryptosporidium* detection. Products of 2ry PCR (200 and 533 bp for *H. pylori* and *Cryptosporidium*, respectively) were electrophorized on 1.5% agarose gel after ethidium bromide staining and observed on a UV transilluminator.

***Cryptosporidium* genotyping:** Restriction fragment length polymorphisms (RFLP) technique was used for *Cryptosporidium* genotyping. Amplified *Cryptosporidium* nPCR products were digested by the restrictive enzyme RasI (Thermo Scientific) 2 µl green buffer, and 17 µl nuclease-free water to reach a volume of 30 µl. The mix was mixed gently followed by spinning down for a few sec and then incubated at 37°C for 5 min and the resulting restriction fragments were then separated by electrophoresis in 3% metaphor agarose followed by ethidium bromide staining to detect *Cryptosporidium* genotypes[19,20].

Statistical analysis: Data were coded and entered using the statistical package of social science (IMB SPSS) version 20 (Chicago, IL, USA) for statistical analysis. The qualitative and quantitative data were presented, and the chi-square test and Fisher's exact test were used to compare groups when applicable. Diagnostic yield (specificity and sensitivity), accuracy, and Kappa agreement of the diagnostic tests were conducted. All variables significantly associated with *H. pylori*, *Cryptosporidium* spp. prevalence and co-infection in the univariate model were included in multivariate logistic regression. Significance was statistically considered when *P* value was <0.05.

Ethical considerations: This study was approved by the Genetic Engineering and Biotechnology Research Institute, Sadat City University. All samples were collected after obtaining parents' or guardians' consent who were informed about the study objectives. The treating physicians were informed with the study results to prescribe the appropriate treatment to the infected children.

RESULTS

Our study included 102 immunocompromised diarrheic children, 50 with primary immunodeficiency, and 52 with secondary immunodeficiency. The mean age was 3.8±2.4; 39.2% were girls and 60.8% were boys; 56.9% were from urban areas; 90.2% used tap water, and 33.3% consumed raw milk daily. The majority of children were in the pre-school age group (41.2%).

Cryptosporidium spp. and *H. pylori* prevalence and diagnostic performance of used assays: *Cryptosporidium* oocysts were detected in 6 (5.9%) patients, with modified AF stain, while ICT detected *Cryptosporidium* copro-antigen in 12 (11.8%) patients, and nPCR detected *Cryptosporidium* DNA in 22 (21.6%) patients. Upon analyzing *cowp* gene and RFLP for *Cryptosporidium* spp., *C. hominis* was the predominant species (81.8%), followed by *C. parvum* (18.2%). The diagnostic performance and accuracy of the used *Cryptosporidium* diagnostic methods used

were calculated (Table 2). *H. pylori* copro-antigen was detected in 18 (17.6%) patients using ICT, and *H. pylori* copro-DNA was detected in 56 (54.9%) patients using nPCR. The diagnostic performance and accuracy of *H. pylori* diagnostic methods were calculated (Table 2). Furthermore, more than half of the immunocompromised children had *H. pylori* (54.9%). One-fifth of the immunocompromised children had *Cryptosporidium* (22 patients), 72.2% (16 cases) of which were coinfected with *H. pylori* without significant association.

The association of sociodemographic and clinical variables with cryptosporidiosis and *H. pylori* infection: The association of sociodemographic and clinical variables with each pathogen and their co-infection rate was statistically analyzed to identify shared risk factors (Tables 3-5). None of the studied

Table 2. Diagnostic performance of conventional methods for detection of *Cryptosporidium* spp. and *H. pylori* using nPCR as a reference standard.

	***Cryptosporidium* spp.**		***H. pylori***
	Microscopy (AF stain)	**ICT**	**ICT**
Sensitivity	27.3%	54.5%	34.6%
Specificity	100%	95%	100%
Positive predictive value (PPV)	100%	75%	100%
Negative predictive value (NPV)	83.3%	88.4%	59.5%
Accuracy	84.3%	86.3%	66.7%
Kappa*	0.37	0.55	0.34

***Key for Kappa:** <0 poor agreement; 0.01-0.20 slight agreement; 0.21-0.40 fair agreement; 0.41-0.60 moderate agreement; 0.61-0.80 substantial agreement; >0.80 almost perfect agreement.

Table 3. Socio-demographic and clinical data of nPCR positive cases for *Cryptosporidium* spp.

Variables			**Samples**	**Positive**	**Statistical analysis**		
			N (%)	**N (%)**	**OR**	**95% CL**	***P* value**
Age group (Year)	**<1**		18 (17.6)	2 (9.1)	1.13	0.30-4.32	**0.73**
	>1-5		42 (41.2)	12 (54.5)			
	>5-12		20 (19.6)	4 (18.2)			
	>12-16		22 (21.6)	4 (18.2)			
Sex	**Male**		62 (60.8)	16 (72.7)	1.97	0.45-8.55	**0.49**
	Female		40 (39.20)	6 (27.3)			
Immunodeficiency	**Primary**		50 (49.0)	8 (36.36)	1.13	0.30-4.32	**0.86**
	Secondary		52 (51.0)	14 (63.63)			
Residence	**Urban**		58 (56.9)	12 (54.5)	1.93	0.49-7.66	**0.34**
	Rural		44 (43.1)	10 (45.5)			
Water source	**Tap**		92 (90.2)	18 (81.8)	2.74	0.40-18.92	**0.29**
	Filtered		10 (9.8)	4 (18.2)			
Milk source	**No milk**		16 (15.7)	0 (0.0)	N/A	N/A	**0.16**
	Raw (cow milk)		34 (33.3)	8 (36.4)			
	Pasteurized milk		30 (29.4)	12 (54.5)			
	Breastfeeding		14 (13.7)	2 (9.1)			
	Industrial milk		8 (7.8)	0 (0.0)			
Animal contact	**No**		100 (98.04)	20 (90.9)	0.2	0.12-0.35	**0.22**
	Yes		2 (1.96)	2 (9.1)			
Clinical symptoms	**Vomiting**	**No**	62 (60.8)	12 (54.5)	1.39	0.36-5.35	**0.63**
		Yes	40 (39.2)	10 (45.5)			
	Fever	**No**	68 (66.7)	14 (63.63)	1.18	0.29-4.79	**0.81**
		Yes	34 (33.3)	8 (36.36)			
	Dehydration	**No**	92 (90.2)	18 (81.8)	2.74	0.4-18.92	**0.29**
		Yes	10 (9.8)	4 (18.2)			
	Constipation	**No**	98 (96.1)	20 (90.9)	3.9	0.22-67.93	**0.32**
		Yes	4 (3.9)	2 (9.1)			
	Abdominal pain	**No**	12 (11.8)	2 (9.1)	1.43	0.15-13.68	**0.76**
		Yes	90 (88.2)	20 (90.9)			
	Growth	**Normal**	28 (27.5)	2 (4.1)	N/A	N/A	**0.3**
		Weight loss	66 (64.7)	18 (81.8)			
		Retard	8 (7.8)	2 (4.1)			
	Total		**102 (100)**	**22 (100)**			

Table 4. Socio-demographic and clinical data of nPCR positive cases for *H. pylori*.

Variables			Samples	Positive	Statistical analysis		
			N (%)	N (%)	OR	95% CL	*P* value
Age group (Year)	**<1**		18 (17.6)	8 (14.3)	N/A	N/A	**0.12**
	>1-5		42 (41.2)	28 (50.0))			
	>5-12		20 (19.6)	14 (25.0)			
	>12-16		22 (21.6)	6 (10.7)			
Sex	**Male**		62 (60.8)	16 (28.6)	2.72	0.86- 8.69	**0.15**
	Female		40 (39.20)	40 (71.4)			
Immunodeficiency	**Primary**		50 (49.0)	28 (50.0)	0.91	0.31-2.76	**0.88**
	Secondary		52 (51.0)	28 (50.0)			
Residence	**Urban**		58 (56.9)	30 (53.6)	1.35	0.44-4.13	**0.6**
	Rural		44 (43.1)	26 (46.4)			
Water source	**Tap**		92 (90.2)	50 (89.3)	1.26	0.19- 8.27	**0.81**
	Filtered		10 (9.8)	6 (10.7)			
Milk source	**No milk**		16 (15.7)	10 (17.8)	N/A	N/A	**0.1**
	Raw (cow milk)		34 (33.3)	22 (39.3)			
	Pasteurized milk		30 (29.4)	20 (35.7)			
	Breastfeeding		14 (13.7)	2 (3.6)			
	Industrial milk		8 (7.8)	2 (3.6)			
Animal contact	**No**		100 (98.04)	56(100.0)	0.44	0.32- 0.60	**0.45**
	Yes		2 (1.96)	0 (0.0)			
Clinical symptoms	**Vomiting**	**No**	62 (60.8)	30 (53.6)	1.89	0.62-6.31	**0.24**
		Yes	40 (39.2)	26 (46.4)			
	Fever	**No**	68 (66.7)	38 (67.8)	0.89	0.28- 2.86	**0.84**
		Yes	34 (33.3)	18 (32.2)			
	Dehydration	**No**	92 (90.2)	52 (92.8)	0.52	0.08-3.37	**0.48**
		Yes	10 (9.8)	4 (7.8)			
	Constipation	**No**	98 (96.1)	54 (96.4)	0.82	0.05– 13.76	**0.89**
		Yes	4 (3.9)	2 (3.6)			
	Abdominal pain	**No**	12 (11.8)	8 (14.3)	0.57	0.95 –3.44	**0.54**
		Yes	90 (88.2)	48 (85.7)			
	Growth	**Normal**	28 (27.5)	16 (28.6)	N/A	N/A	**0.55**
		Weight loss	66 (64.7)	34 (60.7)			
		Retard	8 (7.8)	6 (10.7)			
	Total		**102 (100)**	**22 (100)**			

variables had a significant association with any of the tested pathogens (*H. pylori* and *Cryptosporidium* spp.), neither separately nor combined.

The seasonal distribution of each pathogen and their co-infection were detected throughout the 4 seasons, with a clear peak in summer for *Cryptosporidium* spp., *H. pylori*, and co-infection (Figure 1), without statically significant association (*P* value = 0.932, 0.911, 0.438 respectively).

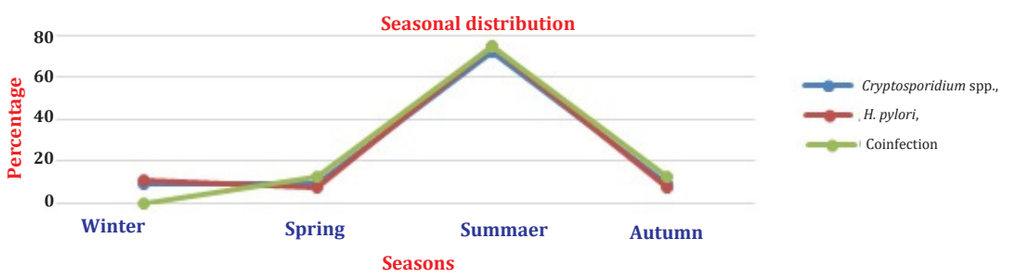

Fig. 1. Seasonal distribution of the percentage of cases of *Cryptosporidium* spp., *H. pylori*, and co-infection among diarrheic children positive by PCR.

Table 5. Socio-demographic and clinical data of nPCR positive cases for *H. pylori* and *Cryptosporidium* spp. co-infected cases.

Variables			Samples	Positive	Statistical analysis		
			N (%)	N (%)	OR	95% CL	*P* value
Age group (Year)	**<1** **>1-5** **>5-12** **>12-16**		18 (17.6) 42 (41.2) 20 (19.6) 22 (21.6)	2 (12.5) 8 (50.0) 4 (25.0) 2 (12.5)	N/A	N/A	**0.48**
Sex	**Male** **Female**		62 (60.8) 40 (39.20)	12 (75.0) 4 (25.0)	2.16	0.40-11.96	**0.46**
Immunodeficiency	**Primary** **Secondary**		50 (49.0) 52 (51.0)	13 (81.2) 3 (18.8)	1.75	0.37–8.24	**0.48**
Residence	**Urban** **Rural**		58 (56.9) 44 (43.1)	8 (50.0) 8 (50.0)	1.39	0.31–6.30	**0.67**
Water source	**Tap** **Filtered**		92 (90.2) 10 (9.8)	14 (87.5) 2 (12.5)	1.39	0.14–14.38	**0.78**
Milk source	**No milk** **Raw (cow milk)** **Pasteurized milk** **Breastfeeding** **Industrial milk**		16 (15.7) 34 (33.3) 30 (29.4) 14 (13.7) 8 (7.8)	0 (0.0) 6 (37.5) 10 (62.5) 0 (0.0) 0 (0.0)	N/A	N/A	**0.13**
Animal contact	**No** **Yes**		100 (98.04) 2 (1.96)	16 (100.0) 0 (0.0)	0.84	0.74-0.95	**0.66**
Clinical symptoms	**Vomiting**	**No** **Yes**	62 (60.8) 40 (39.2)	8 (50.0) 8 (50.0)	1.69	0.37-7.97	**0.49**
	Fever	**No** **Yes**	68 (66.7) 34 (33.3)	10 (62.5) 6 (37.5)	1.24	0.25-5.96	**0.76**
	Dehydration	**No** **Yes**	92 (90.2) 10 (9.8)	14 (87.5) 2 (12.5)	1.78	0.14-14.38	**0.78**
	Constipation	**No** **Yes**	98 (96.1) 4 (3.9)	14 (87.5) 2 (12.5)	6.0	0.36-107.42	**0.17**
	Abdominal pain	**No** **Yes**	12 (11.8) 90 (88.2)	2 (12.5) 14 (87.5)	0.92	0.09-9.13	**0.94**
	Growth	**Normal** **Weight loss** **Retard**	28 (27.5) 66 (64.7) 8 (7.8)	2 (4.1) 18 (81.8) 2 (4.1)	N/A	N/A	**0.55**
	Total		**102 (100)**	**22 (100)**			

DISCUSSION

Diarrhea is one of the most common gastrointestinal symptoms among immunocompromised children, especially those receiving chemotherapy[5]. Recently, diarrhea was recognized as the second leading cause of death for children 0-5 years of age[21]. In the current study, enteric pathogens, *H. pylori* and *Cryptosporidium*, had a high prevalence rate among studied diarrhoeic immunocompromised children and 16 (15.7%) of them had *H. pylori* and *Cryptosporidium* spp co-infection. *H. pylori* is the most prevalent enteric bacteria, which is commonly acquired in early childhood[22].

In the present study, the enteric pathogens revealed that 72.7% of children infected by *Cryptosporidium* were co-infected with *H. pylori* without a statistically significant association. Both *H. pylori* and *Cryptosporidium* infections are localized in the gastrointestinal tract (stomach and small intestine respectively); infections are acquired fecorally, mainly during early childhood[13]. Ibrahim *et al*.[13] reported the presence of an association between *Cryptosporidium* and *H. pylori* in diarrheic immunocompetent Egyptian children. *H. pylori* is the most common enteric bacterium in previous studies, it has a supportive role to other pathogens, including intestinal parasites, especially *Giardia intestinalis*, by depending on its urease enzyme production, which overcomes gastric acidity[13]. In contrast, parasitic infections lead to inflammatory responses that may reduce *H. pylori* infection rate[23]. While *H. pylori* supports colonization of enteric parasites, in contrast parasites do not support *H. pylori* colonization.

In our study, AF stain detected *Cryptosporidium* oocysts only in 6 (5.9%) of 22 children with poor sensitivity (27.3%), and with high false-negative results (16 cases) and perfect specificity (100%). Several studies reported similar findings; AF stain may give false-negative results due to low parasitic load, irregular excretion and small size of oocysts,

thus increasing the potential for misdiagnosis[24-26]. *Cryptosporidium* ICT test showed good specificity (95%), however, it had a low sensitivity (54.5%). Acceptable sensitivities (75–88%) with high false positives of ICT were reported due to the presence of *Cryptosporidium* oocysts in scanty numbers[8,26]. These findings indicate that neither one of AF stain or ICT can be used as a single diagnostic test for laboratory diagnosis of *Cryptosporidium* infection. In our study, *H. pylori* ICT showed poor sensitivity (34.6%) with perfect specificity (100%) for the diagnosis of *H. pylori* infection. Many studies with different categories of patients reported false negative (poor sensitivity) and false positive (low specificity) results of ICT when diagnosing *H. pylori* in the stool. This may be explained by the use of polyclonal and monoclonal antibodies for antigen capture[27-30].

In our study, *C. hominis* was the predominant genotype (81.8%), while 18.2% of the children were infected by *C. parvum*. This result agrees with other results from Egypt[18,26], reporting *C. hominis* predominance in more than 80% of tested patients. In contrast, Eida *et al*.[31] reported a predominance of *C. parvum* (66.7%).

Association between enteric pathogens (*Cryptosporidium* spp., *H. pylori*, or their co-existence) and socio-behavioral and environmental factors have been previously studied, with controversial results[13]. Our study showed no significant association between detection of enteric pathogens (*Cryptosporidium* spp., *H. pylori*, or both) and age group, sex, residency, immunodeficiency type, source of drinking water, source of milk, and animal contact.

The seasonal pattern of infectious diseases could be influenced by long-term climate changes that may extend the transmission cycle and change their seasonal peaking[32]. Our findings detected both *Cryptosporidium* spp. and *H. pylori* in all 4 seasons, with seasonal variations and a peak in summer. El-Badry *et al*.[17] reported a bimodal pattern of *Cryptosporidium* in Egyptian children, with a major peak in the summer preceded by high weather temperature. Many studies have reported peaking of enteric pathogens, including *Cryptosporidium* spp. and *H. pylori*, in the wet seasons, while others reported their peak in summer[13,17]. The difference in the seasonality of infection may be due to the difference in the contaminating conditions of water. Winter peaking is affected by rainfall/floods and agricultural practices, while summer peaking could be a consequence of contamination of water sources due to human activities[33,34].

Exposure to microbes and febrile infectious diseases may cause diarrhea, resulting in inadequate nutrition, in turn leading to growth retardation[35]. The enteric pathogens, *H. pylori* and *Cryptosporidium* were reported to significantly slow growth in children. The true burden of *Cryptosporidium* is underestimated[36]. In our study, 66.7% of cases with *Cryptosporidium* spp., *H. pylori*, or their co-existence suffered from weight loss, and 7.8% showed growth retardation without statistical significance.

The association between *H. pylori* infection and upper gastrointestinal symptoms is debatable in childhood[37]. The enteric pathogen *Cryptosporidium* and *H. pylori* are commonly associated with gastrointestinal symptoms, mainly abdominal pain and diarrhea that may be considered as predictors for the presence of enteric pathogens[38]. Among our hospital-based study of diarrheic children, abdominal pain was the most prevalent GIT symptom, followed by vomiting. However, none of the GIT symptoms showed a significant association between either *Cryptosporidium* spp., *H. pylori*, or both of them.

It was concluded that enteric infection by *Cryptosporidium* spp. and *H. pylori* is common in our chosen study group of immunocompromised diarrheic Egyptian children, with co-existence in most patients, and a clear predominance of *C. hominis*. Microscopy and ICT tests were limited by their low sensitivity, which may lead to misdiagnosis. Based on these findings, using molecular assays to search for *Cryptosporidium* spp. and *H. pylori* in the diarrheic stool of immunocompromised children is recommended. Due to disturbance in gastric acidity associated with *H. pylori* its co-existence with *Cryptosporidium* may provide a good understanding of the correlation between different gut microbiota. The actual underlying mechanisms are still unknown. Further studies are still required to know more about the mechanisms of the pathogenesis of this co-existence.

Author contribution: Ibrahim A and El-Badry AA proposed the study topic and planned the study design. Ibrahim A did the practical work and wrote the original draft. Ibrahim A and El-Badry AA evaluated the data. Abdel-Aziz A, Ali YBM and El-Badry AA did the conceptualization, validation, and supervision. All authors reviewed the final draft.

Conflict of interest: The authors declare no competing interests.

Funding statement: None.

REFERENCES

1. Chinen J, Shearer WT. Secondary immunodeficiencies, including HIV infection. J Allergy Clin Immunol 2010; 125(Suppl 2): S195–S203.
2. Frenkel, L. Infectious diseases as a cause of global childhood mortality and morbidity: Progress in recognition, prevention, and treatment. Adv Pediatr Res 2018; 5:14.
3. Khan A, Shams S, Khan S, Khan MI, Khan S, Ali A. Evaluation of prevalence and risk factors associated with

Cryptosporidium infection in rural population of district Buner, Pakistan. PLoS One 2019; 2; 14(1):e0209188.
4. Checkley W, White AC, Jr., Jaganath D, Arrowood MJ, Chalmers RM, Chen XM, *et al*. A review of the global burden, novel diagnostics, therapeutics, and vaccine targets for *Cryptosporidium*. Lancet Infect Dis 2015; 15:85-94.
5. Boschi-Pinto C, Velebit L, Shibuya K. Estimating child mortality due to diarrhoea in developing countries. Bull World Health Organ 2016; 86(9):710-717.
6. Caccio SM, Chalmers RM. Human cryptosporidiosis in Europe. Clin Microbiol Infect 2016; 22:471–480.
7. Chalmers RM, Campbell BM, Crouch N, Charlett A, Davies AP. Comparison of diagnostic sensitivity and specificity of seven *Cryptosporidium* assays used in the UK. J Med Microbiol 2011; 60:1598-1604.
8. Ghoshal U, Jain V, Dey A, Ranjan P. Evaluation of enzyme-linked immunosorbent assay for stool antigen detection for the diagnosis of cryptosporidiosis among HIV negative immunocompromised patients in a tertiary care hospital of northern India. J Infect Public Health 2018; 11:115–119.
9. Laude A, Valot S, Desoubeaux G, Argy N, Nourrisson C, Pomares C, *et al*. Is real-time PCR based diagnosis similar in performance to routine parasitological examination for the identification of *Giardia intestinalis*, *Cryptosporidium* parvum/*Cryptosporidium hominis* and *Entamoeba histolytica* from stool samples? Evaluation of a new commercial multiplex PCR assay and literature review. Clin Microbiol Infect 2016; 22(2):190.e1-190.e8.
10. Dore MP, Marras G, Rocchi C, Soro S, Loria MF, Bassotti G, *et al*. Changing prevalence of *Helicobacter pylori* infection and peptic ulcer among dyspeptic Sardinian patients. Intern Emerg Med 2015; 10:787–94.
11. Sabbi T, de Angelis P, Colistro F, Dall'Oglio L, di Abriola GF, Castro M. Efficacy of noninvasive tests in the diagnosis of *Helicobacter pylori* infection in pediatric patients. Arch Pediatr Adolesc Med 2005; 159:238-241.
12. Sabbagh P, Mohammadnia-Afrouzi M, Javanian M, Babazadeh A, Koppolu V, Vasigala VR, *et al*. Diagnostic methods for *Helicobacter pylori* infection: Ideals, options, and limitations. Eur J Clin Microbiol Infect Dis 2019; 38(1):55-66.
13. Ibrahim A, Ali YBM, Abdel-Aziz A, El-Badry A. *Helicobacter pylori* and enteric parasites co-infection among diarrheic and non-diarrheic Egyptian children: seasonality, estimated risks, and predictive factors. J Parasit Dis 2019; 43(2):198-208.
14. Smolka AJ, Schubert ML. *Helicobacter pylori*-induced changes in gastric acid secretion and upper gastrointestinal disease. Curr Top Microbiol Immunol 2017; 400:227-252.
15. Chakarova, B. Comparative evaluation of the diagnostic methods for detection of *Giardia intestinalis* in human fecal samples. Trakia J Sci 2010; 8(2):174-179.
16. Garcia LS, Shimizu RY, Novak S, Carroll M, Chan F. Commercial assay for detection of *Giardia lamblia* and *Cryptosporidium* parvum antigens in human fecal specimens by rapid solid-phase qualitative immunochromatography. J Clin Microbiol 2003; 41:209–212.
17. El-Badry AA, Al-Antably ASA, Hassan MA, Hanafy NA, Abu-Sarea EY. Molecular seasonal, age and gender distributions of *Cryptosporidium* in diarrhoeic Egyptians: Distinct endemicity. Eur J Clin Microbiol Infect Dis 2015; 34(12):2447-2453.
18. El-Badry AA, Ghieth MA, Ahmed DA, Ismail MA. *Giardia intestinalis* and *Helicobacter pylori* co-infection: estimated risks and predictive factors in Egypt. J Egypt Soc Parasitol 2017; 47(1):19–24.
19. Spano F, Putignani L, McLauchlin J, Casemore DP, Crisanti A. PCR-RFLP analysis of the *Cryptosporidium* oocyst wall protein (COWP) gene discriminates between C. wrairi and *C. parvum*, and between *C. parvum* isolates of human and animal origin. FEMS Microbiol Lett 1997; 150:209-217.
20. Pedraza-Díaz S, Amar C, Nichols GL, McLauchlin J. Nested polymerase chain reaction for amplification of the *Cryptosporidium* oocyst wall protein gene. Emerg Infect Dis 2001; 7:49-56.
21. Vos T, Allen C, Arora M, Barber RM, Bhutta ZA, Brown A, *et al*. Global, regional, and national incidence, prevalence and years lived with disability for 310 diseases and injuries1990-2015: A systematic analysis for the Global Burden of Disease Study 2015. Lancet 2016; 388(10053):1545–1602.
22. Mansour-Ghanaei F, Taefeh N, Joukar F, Besharati S, Naghipour M, Nassiri R. Recurrence of *Helicobacter pylori* infection 1 year after successful eradication: A prospective study in Northern Iran. Med Sci Monit, 2010; 16(3):144–148.
23. Whary MT, Taylor NS, Feng Y, Ge Z, Muthupalani S, Versalovic J, *et al*. *Lactobacillus reuteri* promotes *Helicobacter hepaticus*-associated typhlocolitis in gnotobiotic B6.129P2- IL10(tm1Cgn) (IL-10(-/-)) mice. Immunology 2011; 133:165–178.
24. Shimelis T, Tadesse E. Performance evaluation of a point-of-care test for detection of *Cryptosporidium* stool antigen in children and HIV infected adults. Parasit Vect 2014;7:1–5.
25. Omoruyi BE, Nwodo UU, Udem CS, Okonkwo FO. Comparative diagnostic techniques for *Cryptosporidium* infection. Molecules 2014; 19:2674-2683.
26. Ghallab MMI, Aziz IZA, Shoeib EY, El-Badry AA. Laboratory utility of coproscopy, copro immunoassays and copro nPCR assay targeting Hsp90 gene for detection of *Cryptosporidium* in children, Cairo, Egypt. J Parasit Dis 2016; 40:901–905.
27. Osman HA, Hasan H, Suppian R, Bahar N, Hussin NS, Rahim AA, *et al*. Evaluation of the atlas *Helicobacter pylori* stool antigen test for diagnosis of infection in adult patients. Asian Pac J Cancer Prev. 2014; 15(13):5245-5247.
28. Korkmaz H, Findik D, Ugurluoglu C, Terzi Y. Reliability of stool antigen tests: investigation of the diagnostic value of a new immunochromatographic *Helicobacter pylori* approach in dyspeptic patients. Asian Pac J Cancer Prev 2015; 16(2):657-660.

29. Calik Z, Karamese M, Acar O, Aksak Karamese S, Dicle Y, Albayrak F, *et al*. Investigation of *Helicobacter pylori* antigen in stool samples of patients with upper gastrointestinal complaints. Braz J Microbiol 2016; 47(1):167-171.
30. Hasosah M. Accuracy of invasive and noninvasive methods of *Helicobacter pylori* infection diagnosis in Saudi children. SJ. 2019; 25(2):126-131.
31. Eida, AM, Eida MM, El-Desoky A. Pathological studies of different genotypes of human *Cryptosporidium* Egyptian isolates in experimentally mice. J Egypt Soc Parasitol 2009; 39(3):975-990.
32. Martinez PP, King AA, Yunus M, Faruque AS, Pascual M. Differential and enhanced response to climate forcing in diarrheal disease due to rotavirus across a megacity of the developing world. PNAS 2016; 113(15):4092-4097.
33. Lal A, Hales S, French N, Baker MG. Seasonality in human zoonotic enteric diseases: A systematic review. PLoS ONE 2012; 7(4):e31883.
34. Ismail MAM, El-Akkad DMH, Rizk EMA, El-Askary, HM, El-Badry AA. Molecular seasonality of *Giardia lamblia* in a cohort of Egyptian children: A circannual pattern. Para Res 2016; 115(11):4221–4227.
35. Black RE. Patterns of growth in early childhood and infectious disease and nutritional determinants. Nestle Nutr Inst Workshop Ser 2017; 87:63-72.
36. Khalil IA, Troeger C, Rao PC, Blacker BF, Brown A, Brewer TG, *et al*. Morbidity, mortality, and long-term consequences associated with diarrhea from *Cryptosporidium* infection in children younger than 5 years: A meta-analyses study. Lancet Glob Health 2018; 6(7):e758–e768.
37. Spee LA, Madderom MB, Pijpers M, Medjahed N, Azzouz MM. Association between *Helicobacter pylori* and gastrointestinal symptoms in children. Pediatrics 2010; 125:e651–e669.
38. Barati M, Taghipour A, Bakhshi B, Shams S, Pirestani M. Prevalence of intestinal parasitic infections and *Campylobacter* spp. among children with gastrointestinal disorders in Tehran, Iran. Parasite Epidemiology and Control 2021; e00207.