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ORIGINAL ARTICLE

Helicobacter pylori and enteric parasites co-infection among diarrheic and non-diarrheic Egyptian children: seasonality, estimated risks, and predictive factors

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Abstract *Helicobacter pylori* (*H. pylori*) and intestinal parasites are known for their high prevalence in children. Both of them infect the gastrointestinal tract with overlapping clinical pictures. This study was conducted to determine *H. pylori* prevalence and its association with intestinal parasites in children, moreover to estimate risk and predictive factors for their detection in stool samples. Single fecal samples were collected from 226 Egyptian pediatric patients (125 diarrheic and 101 non-diarrheic) attending gastroenterology outpatients' clinics, from February 2016 to June 2017. All stool specimens were microscopically examined to search for ova and parasites. Copro-DNAs detection of *H. pylori* and *Cryptosporidium* were performed using nested-PCR assays. *H. pylori* was detected molecularly in 36.8% of the total study population, with a higher prevalence in diarrheic than in non-diarrheic children. Intestinal parasites were detected in 27.4% of the total study populations, of these, 43.9% had co-existence with *H. pylori* colonized patients and was significantly associated with *Cryptosporidium* spp. and *G. intestinalis*. Estimated risk of the presence of *H. pylori* was in January. Our data provide a better understanding of the epidemiology of *H. pylori* infection when associated with intestinal parasites. *H. pylori* co-existence with *G. intestinals* and *Cryptosporidium* may suggest the association of *H. pylori* infection with markers of fecal exposure. Whether *H. pylori* provides favorable conditions for intestinal parasitosis or vice versa, still further investigations are needed with an emphasis upon determining correlation with gut microbiomes.

Keywords *Helicobacter pylori* · Intestinal parasites · Risk factors · Diarrhea · Children · Egypt

Introduction

Helicobacter pylori (*H. pylori*) is a ubiquitous, helical shaped, motile, gram-negative bacillus bacterium, which colonizes the gastric mucosa (Rafeey et al. 2007). Colonization is generally acquired during the first 5 years of childhood (Rajindrajith et al. 2009). *H. pylori* prevalence in children ranges from 30 to 80%, with a predominance in developing countries and its prevalence differs from one region to the other in the same country (Suerbaum and Michetti 2002; Salih 2009). The mode of transmission of *H. pylori* is still unclear. Proposed *H. pylori* transmission modes include direct contact (fecal–oral increased among immunocompromised children and children suffering from diarrhea, vomiting, fever, and dehydration. *H. pylori* seasonality in our cohort of children showed a circannual pattern with peaking in winter, drinking contaminated water and ingestion of contaminated food (Frenck and

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Clemens 2003). *H. pylori* infection diagnosis is generally divided into invasive and non-invasive approaches. A combination of at least two tests is commonly used as a gold standard (Sethi et al. 2013). Parasitic infections, including intestinal parasites, are distributed worldwide and are endemic in tropical and subtropical countries. Globally about 3.5 billion individuals are infected with intestinal parasites, the majority of them being children. Diarrhea is the most commonly presented gastro- intestinal symptom and is mainly caused by intestinal parasites, bacterial pathogens, and viruses. Diarrheal diseases are globally estimated to be 1.7 billion annual cases (Brooker et al. 2009; Bhutta et al. 2013; WHO 2017). *Giardia intestinalis* (*G. intestinalis*), *Cryptosporidium* spp., and *Entamoeba histolytica* (*E. histolytica*) complex are the most common intestinal protozoan parasites which cause acute diarrheal diseases in children (Thompson and Ash 2016; WHO 2017).

PCR is considered a reliable test; it is performed rapidly and is cost-effective. Also, it can identify different types/strains of bacteria and protozoa for pathogenic and epidemiologic studies as well as for detection of antibiotic resistance (Mehmood et al. 2010). Both *H. pylori* and intestinal parasites share a common mode of transmission and may share the same risk and predictive factors, where one of them supports the colonization of the other. In addition, protozoa may transmit pathogenic bacteria and viruses (Yakoob et al. 2005).

There are few studies, which investigated co-infection between *H. pylori* and certain protozoa (*G. intestinalis*, *E. histolytica,* and *Blastocystis* spp.) (Torres et al. 2003; Moreira et al. 2005; Marini et al. 2007; Zeyrek et al. 2008; Escobar-Pardo et al. 2011; Sabah et al. 2015). The primary objective of the present study was to evaluate *H. pylori* prevalence and its co-existence with intestinal parasites among diarrheic and non-diarrheic Egyptian children. Additionally, we estimated risk and predictive factors, which are thought to influence the prevalence of this co-infection.

Subjects and methods

Study design and individuals

This cross-sectional study was carried on 226 Egyptian children (125 diarrheic which include both immunocompetent and immunocompromised and 101 non-diarrheic) attending gastrointestinal outpatients' clinics, Kasr Al-Ainy Pediatric hospitals, Cairo University, ranging from 0 to 16 years, from February 2016 to June 2017.

Stool specimen processing

Fresh single stool specimens were collected from each individual. The related socioeconomic, demographic, environmental and clinical data were collected with each sample. Each sample was examined microscopically and using PCR for detection of *H. pylori* and *Cryptosporidium* spp.

Copro-parasitological examination

All collected fecal samples were microscopically examined for detection of intestinal parasite and associated elements like pus, rbcs sand Charcot–Leyden crystals by direct wet mount before and after formal ether concentration technique (Chesbrough 2006). Fecal smears were stained by Kinyoun modified acid-fast stain for coccidian protozoa detection (Garcia et al. 1983).

Copro-PCR assay

Genomic DNA extraction

Thermal shocking was done for each fecal specimen to disrupt the oocyst wall, then genomic copro-DNA extraction from each sample was done with the Favor Stool DNA Spin Columns Isolation Kit (cat. no. *FAST1*; Favorgen Biotech Corporation, Taiwan) following the manufacturer's instructions.

Helicobacter pylori nested polymerase chain reaction (nPCR) assay

Helicobacter pylori extracted DNA amplification was performed by nPCR targeting the *H. pylori* UreA gene with two sequential PCR reactions. The first reaction amplified the 293 bp fragment by using the 81external primers set; 2F2 5′-ATATTATGGAAGAAGCGAGAGC-3′ and 2R2 5′-ATGGAAGTGTGAGCCGATTTG-3′. The second reaction amplified the 200 bp fragment by internal primers set; 2F3 5′-CATGAAGTGGGTATTGAAGC-3′ and 2R3 5′-AAGTGTTGAGCCGATTTGAACCG-3′. Amplification in each reaction was done following directions of Sasaki et al. (1999). The amplified nPCR products were stained with ethidium bromide and electrophoresed on agarose gel (1.5%) in TAE buffer and were visualized under a UV transilluminator.

Cryptosporidium spp nPCR assay

Cryptosporidium extracted DNA amplification was performed by nPCR that targeted the *COWP* gene, which included two sequential PCR reactions. The primary reaction amplified the 769- bp fragment by using

BCOWPF: 5′-ACCGCTTCTCAACAACCATCTTGTCCTC-3′; and BCOWPR: 5′-CGCACCTGTTCCCACTCAATGTAAACCC-3′. The secondary reaction amplified the 553-bp fragment by internal sets -Cry-15: 5′-GTAGATAATGGAAGAGATTGTG-3′ and Cry-9: 5′-GGACT-GAAATACAGGCATTATCTTG-3′. Amplification in each reaction was done according to steps carried out by Spano et al. (1997) and, Pedraza-Díaz et al. (2001). The amplified nPCR products were stained with ethidium bromide and electrophoresed on agarose gel (1.5%) in TAE buffer and were visualized under a UV transilluminator.

Analysis of Restriction-fragment length polymorphism (RFLP) was conducted following the manufacturer's instructions using *Rsa*I to fragment *Cryptosporidium* PCR products for genotyping (product no. ER1121; Thermo Scientific). Fragmented PCR products were electrophoresed in Metaphor agarose gel (3%) after staining with ethidium bromide, and gels were visualized using UV transillumination.

Statistical analysis

The statistical package SPSS 17 (Chicago, IL, USA) was used to statistically analyze the data with Fisher's exact test and multiple logistic regressions. Study variables, where associated with statistical significance with the prevalence of the bacterium *H. pylori* in the univariate analysis, were subjected to multivariate logistic regression. The *H. pylori* seasonality was performed by analysis of the number of positive cases of *H. pylori* per number of presenting patients per month, for duplicated months the mean was calculated.

Results

Helicobacter pylori DNA was detected in 36.8% (82/226) of total study population using PCR targeting *H. pylori* UreA gene (Fig. 1), with a higher occurrence in diarrheic (68.3% [56/82]) than in non-diarrheic patients (31.7% [26/82]) (Table 1).

Intestinal parasites were detected in 27.4% (62/226) of the study groups with *Cryptosporidium* being the predominant parasite (8.8%), followed by *G. intestinalis* (8.4%), *Blastocyst* spp. (4.4%) and *E. histolytica* complex (3.5%) (Table 4). Both *Cryptosporidium* genotypes, the anthroponotic *Cryptosporidium hominis* (*C. hominis*) and the zoonotic *Cryptosporidium parvum* (Fig. 2), were detected with a predominance of *C. hominis* genotype (80%) (Table 1; Fig. 3).

Intestinal parasites co-existed in 43.9% (36/82) of the *H. pylori* colonized patients, with a statistically significant association. *H. pylori* colonized half of the stool samples that were collected from diarrheic children (28/56) (Table 4). Polyparasitism (concurrent infection with multiple intestinal parasites species) occurred in six diarrheic cases (Table 5). They were significantly associated with the presence of *H. pylori in the* stool ($P < 0.05$).

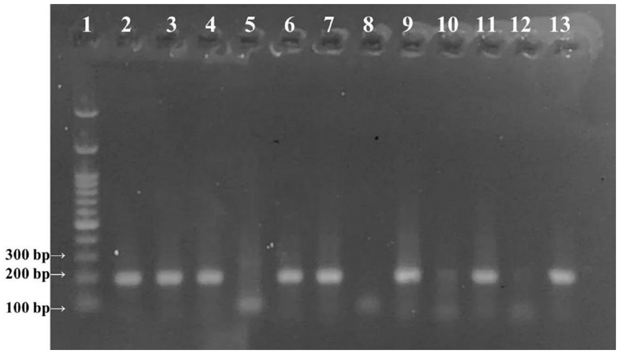

Fig. 1 Showing agarose gel electrophoresis for the products of the nPCR targeting *UreA* gene of *H. pylori* at 200 bp. Lane 1: 100 bp DNA molecular weight marker "ladder". Lanes 2–4, 6, 7, 9 and 11: Positive samples. Lanes 5, 8 and 10: Negative samples. Lane 11: Negative control. Lane 12: Positive control

Table 1 Results of molecular detection of *H. pylori* and *Cryptosporidium* spp and genotypes among study population

	H. pylori result using PCR		
	Positive	Negative	Total
Non-diarrheic			
Cryptosporidium			
Positive (Genotype)			
C. hominis	0	0	0
C. parvum	0	0	0
Total	0	0	0
Negative	26 (25.7%)	75 (74.3%)	101 (100%)
Total	26 (25.7%)	75 (74.3%)	101 (100%)
Diarrheic			
Cryptosporidium			
Positive (genotype)			
C. hominis	10 (8%)	6 (4.8%)	16 (12.8%)
C. parvum	2 (1.6%)	2 (1.6%)	4 (3.2%)
Total	12 (9.6%)	8 (6.4%)	20 (16%)
Negative	44 (35.2%)	61 (48.8)	105
Total	56 (44.8%)	69 (56.2%)	125 (100%)
Total			
Cryptosporidium			
Positive (genotype)			
C. hominis	10 (4.4%)	6 (2.6)	16 (7%)
C. parvum	2 (0.9)	2 (0.9%)	4 (1.8%)
Total	12 (5.3%)	8 (3.5%)	20 (8.8%)
Negative	70 (31%)	136 (60.2%)	206 (91.2%)
Total	82 (36.3%)	144 (63.7%)	226 (100%)

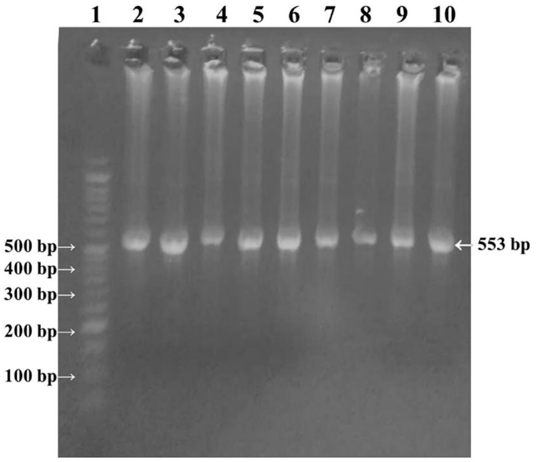

Fig. 2 Showing agarose gel electrophoresis for the products of the nPCR targeting *COWP* gene of *Cryptosporidium* spp. at 553 bp. Lane 1: 50 bp DNA molecular weight marker "ladder". Lane 2: positive control. Lanes 3–10: positive samples

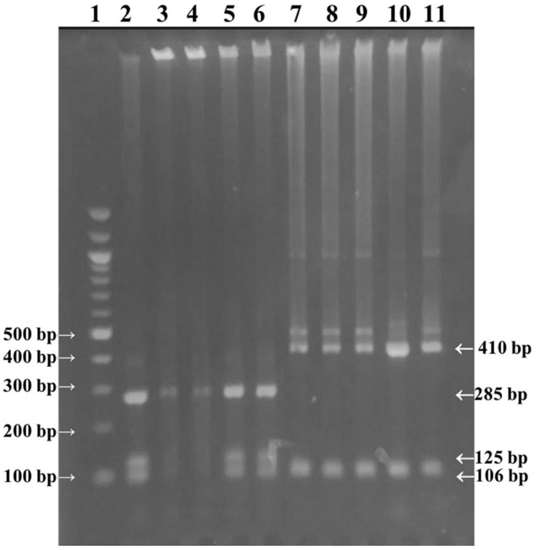

Fig. 3 Showing agarose gel electrophoresis for the products of the nPCR targeting *COWP* gene of *Cryptosporidium* spp. after digestion by *RsaI*. Lane 1: 100 bp DNA molecular weight marker "ladder", lanes 2–6: Positive *C. hominis* samples (285, 125, 106 and 34 bp). Lanes 7–11: positive *C. parvum* samples (410, 106 and 34 bp)

Helicobacter pylori was detected throughout the year, in both study groups, peaking in December only for non-diarrheic children (Fig. 4) with statistical significance ($P < 0.05$).

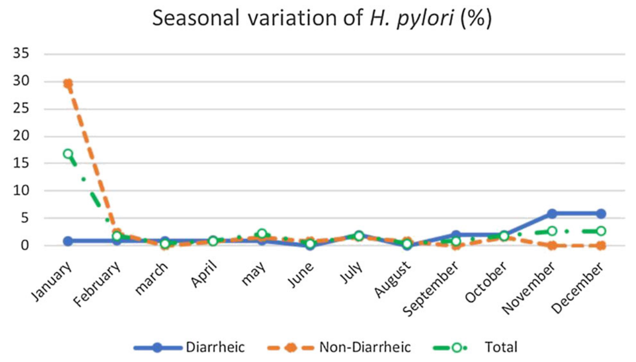

Fig. 4 Seasonal distribution of cases of *H. pylori* (%) among diarrheic and non-diarrheic children positive by PCR

In an effort to identify prospective shared risk factors that could elucidate the positive association between *H. pylori* and certain intestinal protozoan parasites, a number of the studied variables such as consumed milk, immune status (immunocompetent/immunocompromised) (Table 2), gastrointestinal symptoms (diarrhea, vomiting, fever, and dehydration) (Table 3), co- existence of *G. intestinalis* and *Cryptosporidium* parasites (Table 4) and polyparasitism were significantly associated (P <0.05) with detection of *H. pylori* in the stool (Table 5). These study variables were subjected to multivariate analysis by logistic regression and revealed an estimated increase in the risk of *H. pylori* within immunocompromised children, children presenting diarrhea, vomiting, fever, dehydration, and children who had *G. intestinalis*, *Cryptosporidium* spp. or multiple parasites in their stool (Table 6).

Discussion

Helicobacter pylori is the most prevalent human bacteria; its infection is a serious worldwide health problem, especially in developing countries. The infection is mainly acquired in early childhood, which can lead to gastritis in children and adults and may cause peptic ulcer (Whitney et al. 2000; Gallo et al. 2003; Mansour-Ghanaei et al. 2010). In our study, the overall *H. pylori* infection prevalence was 36.3%, rendering it the most prevalent pathogen detected in the stool of our study population. This finding is confirmed by a previous study from Egypt (33%) (Frenck et al. 2006) as well as the reported global average prevalence in children (32.6%) (Zamani et al. 2018).

There was a circannual seasonal variation of *H. pylori* for both diarrheic and non-diarrheic children, with peaking in mid-winter in non-diarrheic children. Though we reported seasonality of *H. pylori* in Egyptian children for the first time, this seasonal pattern with an increase in the rate of transmission in winter than in summer has been previously reported (Savarino et al. 1992; Raschka et al. 1999). Although we did not include peptic ulcer in our study variables, the seasonal variation in *H. pylori* was found to be parallel to peptic ulcer periodicity (Savarino et al. 1992; Raschka et al. 1999). Co-infections between *H. pylori* and protozoa namely, *G. intestinalis*, *E. histolytica,* and *Blastocystis* spp. have rarely been studied. The few

Table 2 Distribution of studied variables among study population in relation to diarrhea and *H. pylori* colonization

	Non-Diarrhoeic				Diarrhoeic				All study individuals			
	H. pylori negative	*H. pylori* positive	Total	*P*. value	*H. pylori* negative	*H. pylori* positive	Total	*P*. value	*H. pylori* negative	*H. pylori* positive	Total	*P*. value
Age group												
0–1 years	0	0	0		15 (12)	7 (5.6)	22 (17.6)		15 (6.6)	7 (3.1)	22 (9.7)	
> 2–5 years	46 (45.5)	16 (15.8)	62 (61.4)	0.72	33 (26.4)	23 (18.4)	56 (44.8)	0.24	79 (35)	39 (17.2)	118 (52.2)	0.34
> 5–12 years	28 (27.7)	9 (8.9)	37 (36.6)		18 (14.4)		24 (19.2)	42 (33.6)	46 (20.4)	33 (14.6)	79 (35)	
> 12–16 years	1 (0.9)	1 (0.9)	2 (1.8)		3 (2.4)	2 (1.6)	5 (4)		4 (1.7)	3 (1.3)	7 (3)	
Gender												
Female	41 (40.6)	14 (13.8)	55 (54.4)		31 (24.8)	23 (18.4)	54 (43.2)		72 (31.85)	37 (16.3)	109 (48.2)	
Male	34(33.6)	12 (11.9)	46 (45.5)	1.00	38(30.4)	33(26.4)	71 (56.8)	0.77	72(31.85)	45 (20)	117 (51.8)	0.49
Residence												
Urban	27(26.7)	12 (11.9)	39 (38.6)	0.36	35 (28)	22 (17.6)	57 (45.6)	0.21	62 (27.3)	34 (15)	96 (42.4)	0.89
Rural	48 (47.5)	14 (13.9)	62 (61.4)		34 (27.2)	34 (27.2)	68 (54.4)		82 (36.3)	48 (21.3)	130 (57.6)	
Water source												
No	71 (70.3)	25 (24.8)	96 (95)	1.0	67 (53.6)	52 (41.6)	119 (95.2)	0.41	138 (61.1)	77 (34.1)	215 (95.1)	0.53
Yes	4 (4%)	1 (1%)	5 (5)		2 (1.6)	4 (3.2)	6 (4.8)		6 (2.7)	5 (2.2)	11 (4.9)	
Animal at the house												
No	72 (71.3)	24 (23.8)	96 (95.1)		68 (54.4)	54 (43.2)	122 (97.6)		140 (62)	78 (34.6)	218 (96.5)	
Yes	3 (3%)	2 (2%)	5 (5%)	0.6	1 (0.8)	2 (1.6)	3 (2.4)	0.59	4 (1.7)	4 (1.7)	8 (3.5)	0.47
Feeding												
Milk												
Fresh	68 (67.3)	20 (19.8)	88 (87.1)		19 (15.2)	18 (14.4)	37 (29.6)		87 (38.5)	38 (16.8)	125 (55.3)	
Canned	3 (3%)	2 (2%)	5 (5%0		12 (9.6)	20 (16)	32 (25.6)		15 (6.6)	22(9.7)	37(16.3)	0.0001*
Breast	3 (3%)	1 (0.9)	4 (3.9)	0.110	26 (20.8)	6 (4.8)	32 (25.6)	0.002*	29 (12.8)	7 (3.1)	36 (15.9)	
Pasteurized	0	0	0		5 (4)	1 (0.8)	6 (4.8)		5 (2.2)	1(0.4)	6 (2.6)	
Not	1 (0.9)	3 (3%)	4 (3.9)		7 (5.6)	11 (8.8)	18 (14.4)		8 (3.5)	14 (6.2)	22 (9.7)	

Table 3 Associated clinical symptoms and immunity status among study population

	Non-diarrhoeic				Diarrhoeic				All study individuals			
	H. pylori negative	*H. pylori* positive	Total	*P*. value	*H. pylori* negative	*H. pylori* positive	Total	*P*. value	*H. pylori* negative	*H. pylori* positive	Total	*P*. value
Diarrhea												
Yes	0	0	0	No[a]	69 (55.2)	56 (44.8)	125 (100)	No[a]	69 (55.2)	56 (44.8)	125 (100)	0.003*
No	75(74.3)	26(25.7)	101(100)		0	0	0		75 (74.3)	26 (25.7)	101 (100)	
Vomiting												
Yes	4 (3.96)	5 (4.95)	9 (8.9)	0.047*	17 (13.6)	29 (23.2)	46 (36.8)	0.003*	21 (9.3)	34 (15)	55 (24.3)	0.0001*
No	71 (70.3)	21 (20.8)	92(91.1)		52(41.6)	27(21.6)	79 (63.2)		123 (54.4)	48 (21.2)	171 (65.6)	
Fever												
Yes	3 (3)	8 (7.9)	(10.9)		15 (12)	10 (8)	25 (20)		18 (8)	18 (8)	36 (15.9)	
No	72 (71.3)	18 (17.8)	90 (89.1)	0.001*	54 (43.2)	46 (36.8)	100 (69)	0.657	126 (55.8)	64 (28.3)	190 (84.1)	0.088
Abdominal pain												
Yes	64 (63.4)	23 (22.8)	87 (86.1)	1.000	60 (48)	54 (43.2)	114 (91.2)		124 (54.9)	77 (34.1)	201 (88.9)	0.081
No	11 (10.9)	3 (3%)	14 (13.9)		9 (7.2)	2 (1.6)	11 (8.8)	0.109	20 (8.8)	5 (2.2)	25 (11.1)	
Dehydration												
Yes	5 (5%)	8 (7.9)	13 (12.9)		10 (8)	1 (0.8)	11 (8.8)		15 (6.6)	9 (4)	24 (10.6)	1.000
No	70 (69.3)	18 (17.8)	88 (87.1)	0.004*	59 (47.2)	55 (44)	114 (91.2)	0.022	129 (57.1)	73 (32.3)	202 (89.4)	
Alternating constipation												
Yes	3 (3)	2 (2%)	5 (5%)		3 (2.4)	3 (2.4)	6 (4.8)		6 (2.7)	5 (2.2)	11 (4.9)	0.533
No	72 (71.3)	24 (23.8)	96 (95)	0.601	66 (52.8)	53 (42.4)	119 (95.2)	1.000	138 (61.1)	77 (34.1)	215 (95.1)	
StatusImmuno												
Immuno-competent	75 (74.3)	26 (25.7)	101 (100)	No[a]	46 (36.8)	31 (24.8)	77 (61.6)	0.20	121 (53.6)	57 (25.2)	178 (78.8)	0.02*
Immuno-compromised	0	0	0		23 (18.4)	25 (20)	48 (38.4)		23 (10.2)	25 (11)	48 (21.2)	
Total	75 (74.3)	26 (25.7)	101 (100)		69 (55.2)	56 (44.8)	125 (100)		144 (63.7)	82 (36.3)	226 (100)	

Table 4 Associated parasites with *H. pylori* colonization among study individuals

	Non-Diarrhoeic				Diarrhoeic				All study individuals			
	H. pylori negative	*H. pylori* positive	Total	*P*. value	*H. pylori* negative	*H. pylori* positive	Total	*P*. value	*H. pylori* negative	*H. pylori* positive	Total	*P*. value
Microscopy												
G. intestinalis	5 (5.0)	5 (5.0)	10 (9.9)	0.12	3 (2.4)	6 (4.8)	9 (7.2)	0.297	8 (3.5)	11 (4.9)	19 (8.4)	0.05*
Hymenolepis nana	0	0	0	No^a	0	3 (2.4)	3 (2.4)	0.252	0	3 (1.3)	3 (1.3)	0.56
Entrobius vermicularis	0	0	0	No^a	0	2 (1.6)	2 (1.6)	0.119	0	2 (0.9)	2 (0.9)	0.13
E. histolytica complex	1 (1.0)	1 (1.0)	2 (2.0)	0.45	3 (2.4)	3 (2.4)	6 (4.8)	1.000	4 (1.8)	4 (1.8)	8 (3.5)	0.46
Blastocystis spp.	4 (4.0)	2 (2.0)	6 (6.0)	0.65	2 (1.6)	2 (1.6)	4 (3.2)	1.000	6 (2.7)	4 (1.8)	10 (4.4)	1.00
Cryptosporidium spp. by PCR	0	0	0	No^a	8 (6.4)	12 (9.6)	20 (16)	0.107	8 (3.5)	12 (5.3)	20 (8.8)	0.02*
Total	10 (9.9)	8 (7.9)	18 (17.8)	–	16 (12.8)	28 (22.8)	44 (35.2)	–	26 (11.5)	36 (15.9)	62 (27.4)	–
No parasite	65 (64.4)	18 (17.8)	83 (82.2)	0.07	53 (42.4)	28 (22.8)	81 (64.8)	–	118 (52.2)	46 (20.4)	164 (72.6)	–
Total	75 (74.3)c	26 (25.7)	101 (100)	–	69 (55.2)	56 (44.8)	125 (100)	–	144 (63.7)	82 (36.3)	226 (100)	–

existent studies had different objectives and non-conclusive outcomes.

Both *H. pylori* and intestinal parasites colonize the human gastrointestinal tract and are the most common childhood infections (Torres et al. 2003; Moreira et al. 2005; Marini et al. 2007; Zeyrek et al. 2008; Escobar-Pardo et al. 2011; Sabah et al. 2015). There was a 28.6% prevalence of intestinal parasitic infections in our study populations, predominantly anthroponotic *Cryptosporidium* and *G. intestinalis*, of which 43.9% co-existed with *H. pylori* with statistical significance (*p* value, 0.02 and 0.05, respectively).

Our study revealed that more than half of cryptosporidiosis (60%) and/or giardiasis (58%) cases coexisted and showed a duplicated risk for *H. pylori* (O.R 2.9 and 2.6, respectively) with statistical significance. *Escobar-Pardo* et al. (2011) and Moreira et al. (2005) reported an association between detection of *G. intestinalis* microscopically and *H. pylori* with two different method Elisa to determine anti-*H. pylori* IgG antibodies and using the ^{13}C urea breath test among children. To our knowledge, the present study is the first study to include *Cryptosporidium* protozoa in association with *H. pylori* using molecular assays.

Co-existence of *H. pylori* and intestinal parasites mostly occur in low income developing countries and may be linked mechanically or pathologically. *H. pylori* shares the associated gastrointestinal symptoms of intestinal parasites and shares the same mode of transmission. This may suggest the association of *H. pylori* infection with markers of fecal exposure.

This hypothesis may be supported by our findings of a statistically significant association between presence of *H. pylori* and polyparasitism of intestinal parasites in diarrheic children. Polyparasitism may increase human susceptibility to *H. pylori* and other intestinal microbial infections. Both *H. pylori* and gastrointestinal parasites share the same estimated risk factors, including poor sanitation and hygiene, low socioeconomic conditions and overcrowded populations (Cheng et al. 2009). These factors affect the dynamics of pathogen transmission and are the main drivers of the seasonal distribution of infectious enteric diseases (Lal et al. 2012).

In addition, *H. pylori* may support *Cryptosporidium* spp. and *G. intestinalis* colonization in human gastrointestinal tract by producing urease enzyme to overcome gastric acidity (Suerbaum and Michetti 2002; David and William 2006; Rodriguez et al. 2011). On the other hand, gastrointestinal parasitic infection may affect inflammatory response to *H. pylori* (Whary et al. 2011). This significant co-existence may suggest that *H. pylori* could be a risk factor for intestinal parasitic infection or vice versa, which still needs further investigations. Co-existence of *H. pylori* and intestinal parasites might interact synergistically leading to serious health consequences which could be

Table 5 Cases showed polyparasitism

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
H. pylori	+	+	+	+	+	−
Cryptosporidium spp.	+	+	+	+	+	−
G. intestinalis	+	+	−	−	−	−
E. histolytica complex	−	−	−	−	+	+
Blastocystis spp.	−	−	−	−	+	+
Entrobius vermicularis	−	−	+	+	−	−

Table 6 Multivariate analysis for nPCR *H. pylori* positive cases

	OR	95% CI	*P* value*
Immunity			
Immunocompetent/immunocompromised			
All study group	2.3	(1.2–4.4)	0.017*
Diarrhoea			
Yes/no			
All study group	2.3	(1.3–4.1)	0.003*
Non-diarrhoeic group	4.2	(1.0–17.2)	0.047*
Associated symptoms			
Vomiting			
Yes/no			
Diarrhoeic group	3.3	(1.5–7.0)	0.003*
All study group	4.1	(2.2–7.9)	0.0001*
Fever			
Yes/no			
Non-diarrhoeic group	10.7	(2.6–44.3)	0.001*
Dehydration			
Yes/no			
Non-diarrhoeic group	6.2	(1.8–21.3)	0.004*
Associated parasitic infection			
G. intestinalis			
Yes/no			
All study group	2.6	(1.0–6.8)	0.048*
Cryptosporidium spp			
Yes/no			
All study group	2.9	(1.1–7.5)	0.02*
Polyparasitism			
Yes/no			
Diarrhoeic group	2.1	(1.3–4.2)	0.01*

Data presented as n, with (*) *P* value for OR < 0.05 is significant

influenced by hosts and environmental factors (Torres et al. 2003; Marini et al. 2007).

Intestinal parasites and *H. pylori* colonized more than half of the stool samples collected from diarrheic children with statistical significance. Though many pathogens such as bacteria, viruses, and intestinal parasites can cause diarrhea, a large proportion of cases is caused by parasitic protozoan (Kotloff et al. 2013). Diarrhea is currently considered the second cause of death in children during the first 5 years of life; rotavirus being the most deadly infectious agent, followed by *Cryptosporidium* (Striepen 2013; Vos et al. 2016). We classified our study population into diarrheic and non-diarrheic groups of children. Diarrhea represented 55.3% of the total study population. *G. intestinalis*, *Cryptosporidium* spp and *E. histolytica* are known to be the most prevalent protozoan parasites that cause acute diarrhoeal disease in children (WGO 2012), they were also the most prevailing parasites in our study populations (Table 4).

Although a previous study reported that infection with *H. pylori* had a protective role in reducing frequency of diarrhoeal illness in children (Chen et al. 2003), in our study, there was a higher *H. pylori* prevalence in diarrheic children (44.8%) than non-diarrheic children (25.7%). This may be due to co-infection with intestinal protozoa (Bhan et al. 2000).

Cryptosporidium spp. was one of the top diarrhea associated pathogens in children (Kotloff et al. 2013). *Cryptosporidium* is the second most common organism causing diarrhea and death in children, with a higher death rate in immunocompromised than immunocompetent patients (Sow et al. 2016). In our study, *Cryptosporidium* was the most prevailing parasite with a predominance of *C. hominis* species, which agrees with the result of other studies in Egypt (Abd El Kader et al. 2012; Helmy et al. 2013; El-Badry et al. 2015).

Similarly, *G. intestinalis* is a common protozoan parasite causing diarrhea worldwide (Einarsson et al. 2016). Based upon the microscopic examination, *G. intestinalis* was the second most common parasite in the present study; however, if the molecular method had been used, it might have revealed a higher prevalence.

Helicobacter pylori was associated with vomiting with statistical significance in both diarrheic and non-diarrheic children. Fever and dehydration were statistically significant symptoms in non- diarrheic children and could be predictors for suspecting *H. pylori* in these patients. This finding agrees with Jacoby and Porter (1999)and *Shahinian* et al. (2000).

Many socio-behavioral, demographic and environmental variables in association with *H. pylori* were previously studied with controversial results (Moayyedi et al. 2002; Rodrigues et al. 2004; Tanih and Ndip 2013). Our study results showed no significant association between age, gender, residency, and source of drinking water, however consumption of raw animal (cow, goat, and sheep) milk was linked as one of the major sources of *H. pylori* infection (Vale and Vitor 2010). Drinking milk in our study was significantly associated with the presence of *H. pylori* in the stool; however, after being subjected to multivariate analysis by the logistic regression test, consuming milk was not estimated for the presence of *H. pylori* in children's stool.

Conclusion

Our results documented significant association *of H. pylori* with *G. intestinals* and *Cryptosporidium* species. This co-existence may suggest the association of *H. pylori* infection with markers of fecal exposure. Furthermore, our study documented the circannual pattern of *H. pylori* seasonality in Egyptian children. Our findings would indicate that in addition to searching for *H. pylori* in gastrointestinal symptomatic children, screening for cryptosporidiosis and giardiasis in diarrheic children is recommended.

Helicobacter pylori may support the colonization by intestinal parasites or vice versa. The interaction between *H. pylori* and intestinal parasites may have serious health consequences. This point needs further investigations with an emphasis upon determining correlation with gut microbiomes. The findings of the present study provide a better understanding of the epidemiology and the estimated risks of *H. pylori* infection when associated with intestinal parasites. Further research is needed to provide better insight into their co-infection and ensure future improvements in clinical practice, testing, and development of therapies to these pathogens.

Authors contribution AI: corresponding author, participate in all stages from study design to manuscript writing and revision, YBMA: participate in study design and manuscript revision); AA-A provide technical help; AAE-B: participated in Study design, supervised the lab work, analysis and interpretation of data and involved in drafting the manuscript.

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Compliance with ethical standards

Conflict of interest The authors have declared that no competing interest exists.

Ethical approval Ethical board of University of Sadat City, Genetic Engineering, and Biotechnology Research Institute, Egypt approved the study. Parents of all the children included in the study were verbally informed about the study's aims, and collection of the specimens was done after their consent was obtained.

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