Prevalence of intestinal parasitic infections and *Campylobacter* spp. among children with gastrointestinal disorders in Tehran, Iran

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ABSTRACT

Co-infection of intestinal parasitic infections (IPIs) and *Campylobacter* spp. are public health problem in both developing and developed countries. This study was conducted to determine prevalence of IPIs and *Campylobacter* spp. among children with gastrointestinal disorders in Tehran. In this descriptive cross-sectional survey, 283 fresh stool samples were collected from all individuals and examined by standard parasitological methods including direct slide smear, formalin-ether concentration, trichrome staining, modified Ziehl-Neelsen staining, and chromotrope 2R staining techniques were used for detection of intestinal protozoa and helminths. Furthermore, culture and multiplex-PCR were also used to identify the species of *Campylobacter*. Data analysis was performed using SPSS version 16. IPIs were detected microscopically in 22.26% of the total study population, with a higher prevalence in *Giardia duodenalis* (7.06%) and *Blastocystis hominis* (7.06%). *Campylobacter* were detected molecularly in 14.8% (95.2% of *C. jejuni* vs. 4.8% of *C. coli*) of the total study populations; of these, 3.5% had co-existence with IPIs colonized patients. Our results showed a relatively high prevalence of IPIs and *Campylobacter* in children with diarrhea. Further research is needed to better understand their co-infection and ensure future advances in clinical trials, testing, and development of therapeutic approaches for these pathogens.

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1. Introduction

Intestinal parasitic infections (IPIs) are one of the most important public health concerns worldwide (Ngui et al., 2011; Taghipour et al., 2019a). According to the previous literatures, it is estimated that approximately 3.5 billion people are affected of which 450 million peoples suffer from a broad spectrum of intestinal parasites (Bethony et al., 2006; Watkins, 2003). IPIs can cause iron deficiency anemia, growth retardation, and gastrointestinal complications including diarrhea, nausea, vomiting and abdominal pain in children and immunocompromised patients (Mehraj et al., 2008; Omrani et al., 2015; Taghipour et al., 2019c; Taghipour et al., 2019d). As shown in previous studies, various co-infections such as *Mycobacterium tuberculosis* (Taghipour et al., 2018; Taghipour et al., 2019b), *Helicobacter pylori* (Ibrahim et al., 2019; Taghipour et al., 2020), human immu-

nodeficiency viruses (HIV) (Obateru et al., 2017) with intestinal parasites lead to the more severity of gastrointestinal disorders and even death. In recent researches, there are some evidence showing a probable linkage between the presence of IPIs and *Campylobacter* infection on development and exacerbation of gastrointestinal complications (Ekdahl and Giesecke, 2004; Goodman et al., 1980).

Campylobacter, an enteric gram-negative bacterium, is the cause of bloody diarrhea, abdominal pain, fever, headache, nausea, and vomiting, that considered as one of the four main causes of diarrheal diseases, called campylobacteriosis (Endtz, 2020; Sadiq et al., 2019). There are various methods for transmission of this bacterium including food-borne, waterborne, contact with animals (Bronowski et al., 2014; Luangtongkum et al., 2009). In developing countries, the isolation rate of *Campylobacter* for foodborne illness ranges from 5% to 20% (Epps et al., 2013). In this regard, campylobacteriosis can occur in all age groups, although it mainly affects children (Jeffs et al., 2019).

Co-infection of *Campylobacter* and IPIs occurs more frequently in developing countries with lower income levels; this co-infection may be due to the common pathway of transmission of these infections (Bronowski et al., 2014; Taghipour et al., 2019d). On the other hand, since both infections affect the gastrointestinal tract, these infections share similar clinical pictures that may have led to misdiagnosis (Epps et al., 2013; Taghipour et al., 2018). Children are at higher risk for *Campylobacter* infection and IPIs, especially those living in communities with low levels of health. Hence, the primary objective of the present study was to evaluate the prevalence of IPIs, *Campylobacter* and their co-existence among children with gastrointestinal disorders. Moreover, we estimated symptoms associated to IPIs.

2. Materials and methods

2.1. Study population and criteria

The sample collection was conducted during a cross-sectional study from April 2015 to June 2017 at *Children's Medical Center* Hospital in Tehran. For this purpose, confirmed gastrointestinal disorders, verified age less than 15 years and parental consent for children participating were considered as inclusion, and consumption of anti-parasitic and bacterial drugs during the 3 months' period prior to sampling were exclusion criteria. Stool samples were taken from 283 confirmed children with gastrointestinal disorders, as confirmed by a physician. Along with sample collection, a standardized questionnaire including the sociodemographic features and clinical symptoms related to IPIs was completed by a trained nurse for patients.

2.2. Copro-parasitological analysis

Briefly, all collected stool samples were microscopically examined for IPIs using formalin-ether concentration and Lugol's iodine staining (Taghipour et al., 2018). In this regard, we investigated associated elements like WBC, RBC and mucus by direct wet mount before and after the formalin-ether concentration technique. Accordingly, trichrome staining for the detection of intestinal protozoa such as *Giardia duodenalis* and *Entamoeba* complex, *modified Ziehl-Neelsen* technique for *Cryptosporidium* spp. oocysts, and chromotrope-2R staining for the identification of microsporidia spores, were performed (Taghipour et al., 2019d).

2.3. Examinations for the detection of Campylobacter

2.3.1. Culture and diagnostic tests

In order to isolate *Campylobacter*, stool samples were cultured immediately on modified Charcoal-Cefoperazone-Deoxycholate Agar (mCCDA) (Merck, Germany) and Brucella agar (Merck, Germany) supplemented with 5% sheep blood and vancomycin, polymyxin B, and trimethoprim. Then, the media were incubated at 42 °C for 48 h under microaerobic conditions in a sealed jar using gas packs (Merck, Germany). In the following, suspected colonies to *Campylobacter* spp. were confirmed using Gram staining and specific morphology, catalase and oxidase positive, nitrate reduction positive, and indoxyl acetate hydrolysis positive. To differentiate between *Campylobacter* species, hippurate hydrolysis test positive (*C. jejuni*) and negative (*C. coli*) as well as susceptibility to nalidixic acid (*C. coli*) were done (Shams et al., 2017).

2.3.2. DNA extraction and multiplex PCR assay

Molecular examinations were also performed to accurately evaluate and identify *Campylobacter* species. Briefly, DNAs were extracted from 200 mg of stool samples for the detection of *Campylobacter* using a direct multiplex PCR as previously described (Shams et al., 2016a; Shams et al., 2017). In each run of PCR for either *Campylobacter* spp., positive and negative controls were included. Next, PCR products were visualized by electrophoresis on 1% agarose gel stained with ethidium bromide. Finally, PCR products of positive samples were sequenced using Applied Biosystems 3730/3730xl DNA Analyzers (Bioneer, Korea) and the results were compared using BLAST software in the GenBank database.

2.4. Statistical analysis

Data analysis was performed using SPSS software version 16 (SPSS, Chicago, IL, USA). The frequency and percentages of intestinal parasites, *Campylobacter* spp. and other descriptive were calculated by a binomial distribution. The Chi-square and Fisher's

exact tests were used to compare the prevalence of IPIs with potential risk factors and related clinical symptoms. *P* values <0.05 were considered statistically significant.

3. Results

3.1. Participants

In total, 283 samples include 142 (50.2%) male and 141 (49.8%) female were collected. The participants were aged between 1 month and 14 years old, with a mean age of 3.48 years. In this study, all patients were suffering from diarrhea and other gastro-intestinal disorders.

3.2. Prevalence and risk factors of IPIs

Of the 283 samples, 63 children (22.26%) were infected with at least one intestinal parasites, although multi-parasitism infection was not found in stool samples. In this regard, six species of IPIs were found in the study population. The prevalence of intestinal protozoa was 21.55% (61/283) and helminths parasites were found in 0.70% (2/283) of participants. The most common protozoan parasite was *Giardia duodenalis* (20 participants, 7.06%) and *Blastocystis hominis* (20 participants, 7.06%) followed by microsporidia (12 participants, 4.2%), *Cryptosporidium* spp. (5 participants, 1.7%) and *Entamoeba* complex (4 participants, 1.4%) (Table 1 and Fig. 1). There were also only two positive microscopic examinations for helminth infection, both of which were related to *Hymenolepis nana*.

The infection rate in females (24.64%, 35/142) was slightly higher than males (19.71%, 28/142), although, there were no statistically significant differences regarding two sexes. Moreover, risk factors and clinical symptoms related to IPIs in the children are presented in Table 2.

3.3. Prevalence of Campylobacter spp. infection

According to the culture results, the number of *Campylobacter* positive cases was 19 (6.71%), in which 17 cases (89.5%) were infected by *C. jejuni,* and 2 cases (10.5%) were infected by *C. coli*.

With regard to molecular examination by PCR and using specific primers showed that 42 positive cases for *Campylobacter* spp., of which forty were *C. jejuni* and two were *C. coli*. Molecular investigations showed expected fragments for *Campylobacter* spp. (Fig. 2).

3.4. Co-infection of IPIs and Campylobacter spp.

In the present study, stool from 3.5% (10/283) of children were positive for co-infection of IPIs and *Campylobacter* spp.

4. Discussion

Every year about 2–4 billion cases of infectious diarrhea caused by bacteria, viruses, and parasites occur in the world that is especially prevalent in children under the age of 5 years (Farthing et al., 2013; Harb et al., 2019; Thapar and Sanderson, 2004). Several factors such as weather conditions, lack or absence of health facilities, poor hygiene practices, and unfavorable economic condition are issues affecting the spread of parasitic and bacterial diseases in different regions of the world, especially in under-developed or developing countries (Fletcher et al., 2013; Patz et al., 2000). Our findings revealed that 22.26%, 6.71%, and 3.5% of patients were infected with intestinal parasites, *Campylobacter*, and their co-infection, respectively.

The most important intestinal parasites cause of the diarrheal disease are *Giardia*, *Cryptosporidium*, and microsporidia (Liu et al., 2014). All three parasites mainly infect the small intestine and affect the lumen and epithelial surface, but do not invade deeper mucosal layers (Certad et al., 2017; Liu et al., 2014). Findings of the current study showed that 7.06%, 4.2%, and 1.7% of children were infected with *Giardia*, microsporidia and *Cryptosporidium*. In this line, *Giardia* and *Cryptosporidium* prevalence rates is almost consistent with reports from previous comprehensive studies in Iranian preschool and school children (5.6% vs. 1.6%) (Daryani et al., 2017). In this study, *Giardia* and *Blastocystis* were reported as the most common intestinal parasites. Giardiasis as a pathogenic protozoan commonly causes malnutrition, vitamin B12, and folate deficiency (Olivares et al., 2002) and *Blastocystis* as a non-pathogenic protozoan causes gastrointestinal disorders by altering the normal intestinal flora (Audebert et al., 2016; El-Sayed and Ramadan, 2017; Mirjalali et al., 2020). The high prevalence of these parasites occurs mainly in deprived and rural areas as drinking water supply and sewage networks are less developed compared to advanced and urban areas (Fletcher et al., 2012). But about *Cryptosporidium* and microsporidia, both of these protozoa are usually associated with persistent diarrhea, which causes gastrointestinal disorders and even death in children and people with immune deficiencies, and against which there is no effective therapy (La Hoz et al., 2019; Maggi et al., 2000). One of the most important reasons for the relatively low prevalence of these two protozoa in many studies is that it is difficult to diagnose these pathogens, but considerable progress has been made in the past decade in the production of molecular diagnostic reagents for these organisms (Collins and Wright, 1997).

Table 1

Frequency of the intestinal parasites among children with gastrointestinal disorders ($n = 283$).

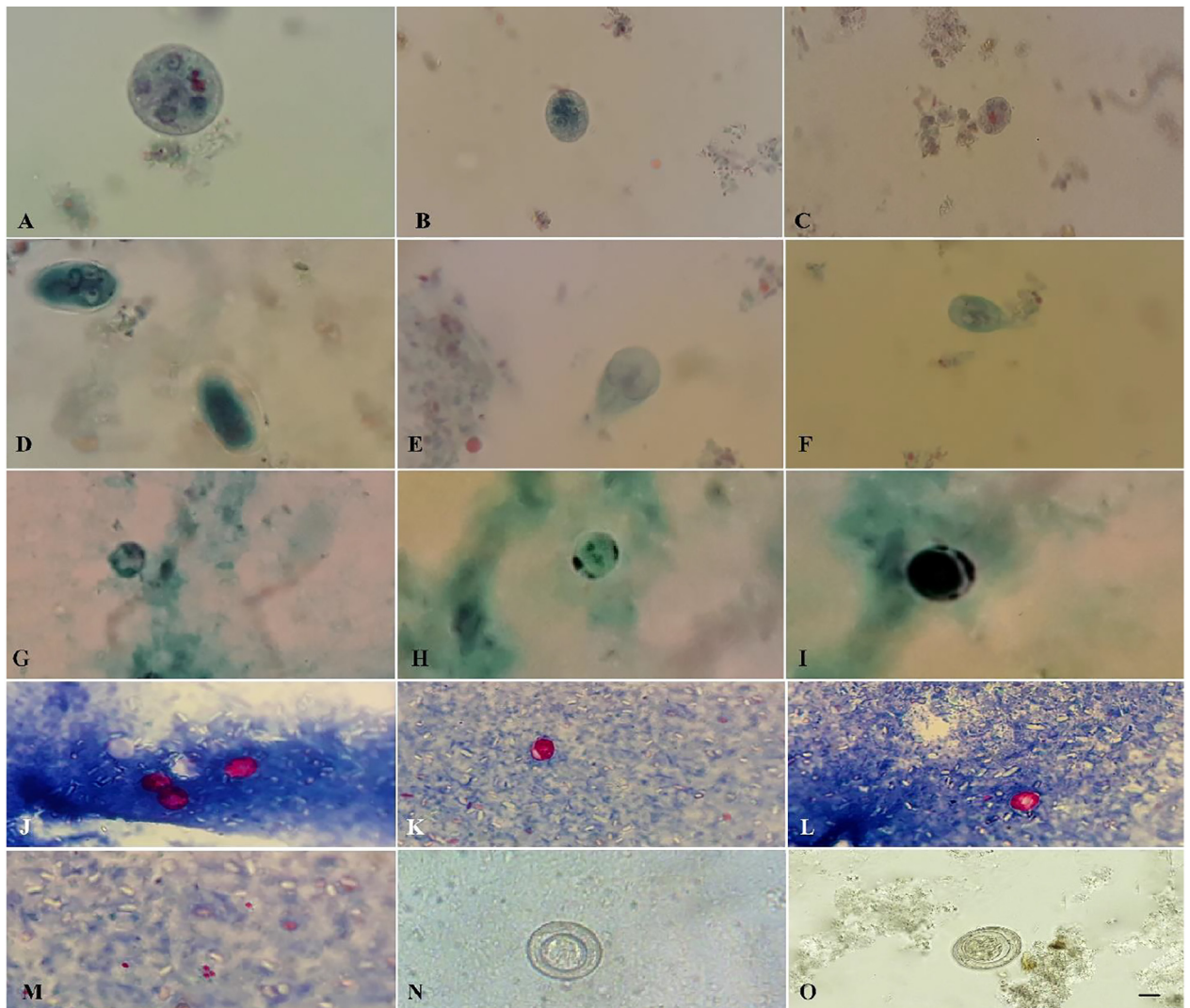| Type of parasites	Children with gastrointestinal disorders ($n = 283$)
	Positive n (%)
Blastocystis	20 (7.06)
Giardia duodenalis	20 (7.06)
Microsporidia	12 (4.2)
Cryptosporidium spp.	5 (1.7)
Entamoeba complex	4 (1.4)
Total protozoa	61 (21.55)
Hymenolepis nana	2 (0.70)

Fig. 1. Images of intestinal parasites isolated in the present study. –An index of 5 μm was used for all images of intestinal parasites. A, B and C: *Entamoeba* complex. D, E and F: *Giardia duodenalis*. G, H and I: *Blastocystis hominis*. J, K and L: *Cryptosporidium* spp. M: Microsporidia. N and O: *Hymenolepis nana*.

Campylobacteriosis is also one of the major gastrointestinal transmitted diseases worldwide, especially in children younger than 5 years of age. According to the latest report from the European Center for Disease Control and Prevention, campylobacteriosis was the most reported case of zoonosis (with 214,268 confirmed human cases) in 2012 (Authority et al., 2014). Theses can infect the gastrointestinal tract and cause abdominal pain, fever, and cramps (Endtz, 2020). Post-infection with some *Campylobacter* strains may occur including Guillain-Barré Syndrome (GBS), the Reactive Arthritis (REA), and Irritable

Table 2
Demographic characteristics and clinical symptoms of children with gastrointestinal disorders, according to the presence or absence of intestinal parasites.

| Variables	Parasitized ($n = 63$)	Non-parasitized ($n = 220$)	P-value
Gender			0.30
Male	28	114	
Female	35	106	
Age			0.68
1 month–7 year	51	183	
8–14	12	37	
Underlying disorders			0.72
Yes	10	12	
No	53	75	
WBC			0.02*
High	37	94	
Normal	18	106	
Low	8	20	
RBC			0.00*
High	23	60	
Normal	31	155	
Low	9	5	
Clinical symptoms			
Fever			0.78
Yes	26	95	
No	37	125	
Stomach pain			0.14
Yes	26	69	
No	37	151	
Headache			0.56
Yes	4	10	
No	59	210	
Nausea and/or vomiting			0.008*
Yes	24	48	
No	39	172	

* Statistical significantly.
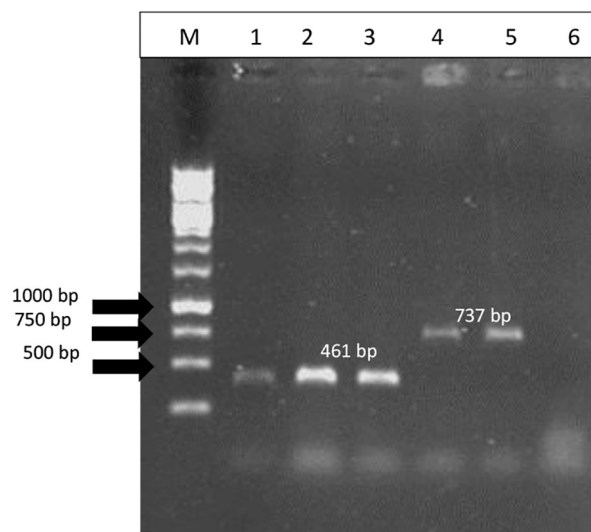
Fig. 2. Identification of *C. coli* and *C. jejuni* using PCR. M: 1000 bp marker, 1 and 2: samples of *C. coli*, 3: positive control of *C. coli*, 4: *C. jejuni*, 5: positive control of *C. jejuni*, 6: negative control.

Bowel Syndrome (IBS) (Facciolà et al., 2017). Accurate and correct diagnosis of *Campylobacter* spp. is crucial clinically for the control and prevention of the disease and the avoidance of antimicrobial resistance whenever possible.

In this study, 19 Campylobacters (6.71%) were isolated by culture, while 42 positive cases were detected by PCR assay. In both methods, the isolation rate of *C. jejuni* was higher than *C. coli*. Culture is a standard gold method for the detection of *Campylobacter* spp., but unfortunately in most clinical laboratories the results are reported as false-negative due to lacking the required standards and fastidious nature of the bacteria (Shams et al., 2016b). As the results of this study show, the detection ability of

multiplex PCR is approximately twice that of the culture diagnostic method. Therefore, the molecular assay can be considered as a highly sensitive method (Buss et al., 2019). In a study by Ghorbanalizadgan et al. (2014), Tehran, Iran, reported 6% *Campylobacter* (4.5% *C. jejuni* and 1.5% *C. coli*).

The common transmission route of *Campylobacter* and IPIs is one of the most important reasons that these two infections occur simultaneously (Devane et al., 2005; Dorny et al., 2009). On the other hand, due to the same anatomical location of both infections, they have common clinical symptoms that make it difficult to accurately and timely diagnose the infection (Endtz, 2020; Marques et al., 2020). Therefore, using accurate and efficient diagnostic tools will help the physician to prevent worsening or prolonging the course of treatment by administering the drug correctly.

In conclusion, the interaction between *Campylobacter* and IPIs may have serious health consequences. However, 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 co-existence of *Campylobacter* infection when associated with IPIs. Thus, appropriate personal hygiene is recommended as well as regular hygiene screening for these microbial pathogens.

Authors' contributions

All authors contributed to study design. MB, SS and MP contributed to all parts of the study. SS and MP contributed to study implementation. AT and BB collaborated in the analysis and interpretation of data. AT and MB collaborated in the manuscript writing and revision. All the authors commented on the drafts of the manuscript and approved the final version of the article.

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Availability of data and materials

Not applicable.

Ethics approval and consent to participate

This study received ethical approval from the Ethics Committee of Tarbiat Modares University, Tehran, Iran (Code: IR. MODARES.REC).

Consent for publication

Not applicable.

Declaration of Competing Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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