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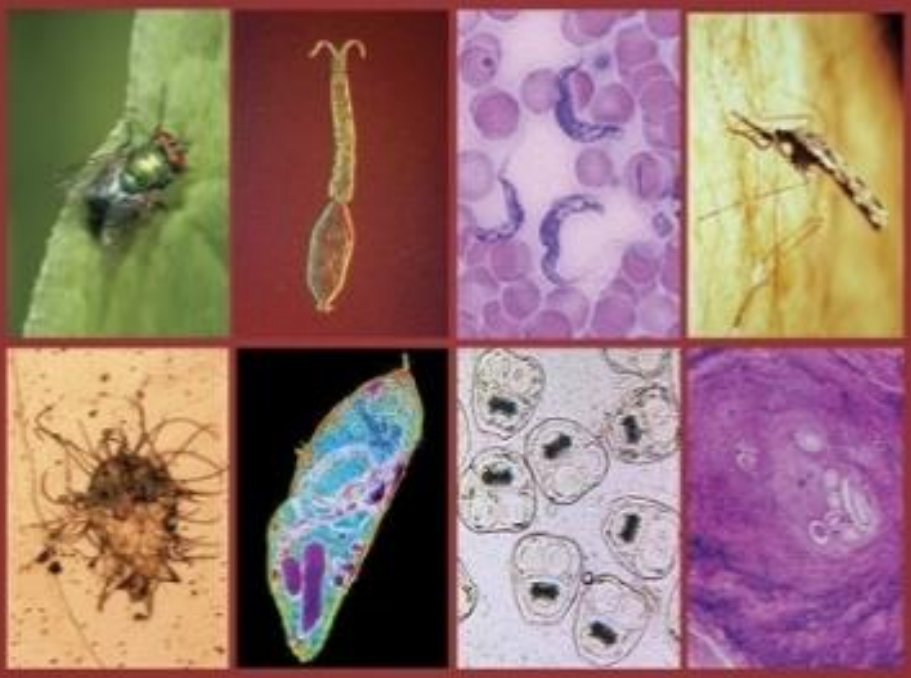

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Screening For the Burden of Enteric Parasitic Infections Among Egyptian Pre-Schoolers with Gastrointestinal Manifestations: A Cross-Sectional Study

Hebat-Allah S. A. Yousof[1*], Shaimaa A. Abdalgeleel [2] and Shaimaa H. El-sayed[3]

1- Medical Parasitology Department, Faculty of Medicine, Cairo University, Egypt.
2- Biostatistics and epidemiology Department, National Cancer Institute, Cairo University, Egypt
3- Medical Parasitology Department, Faculty of Medicine, Helwan University, Egypt.

*E-mail : drhebasalah@cu.edu.eg; drhebasalah@kasralainy.edu.eg

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ABSTRACT

Intestinal parasitic infections (IPI) continue to be a major public health problem that causes a variety of gastrointestinal symptoms in developing countries. Pre-schoolers are among the vulnerable population to getting such infections. Periodical research on the prevalence of IPI and associated manifestations is essential to improve different control measures in high-risk communities. A cross-sectional study on preschool children with gastrointestinal symptoms (n=200) was done to evaluate the burden of IPI. Demographic data, history taking, and clinical examination of the cases followed by macroscopic and microscopic examination of their stool samples by direct wet mount, concentration, and staining with acid-fast stain "cold method" was done. Among the study population, 49% of participants were positive for one or more IPI. Out of ten parasitic species detected, *G.intestinalis, E.histolytica/dispar* and *C.parvum* were the predominant IPI (35%, 16.5%, and 10.5% of participants) respectively. Diarrhoea, flatulence, and vomiting were significantly higher in positive cases for IPI (71.4%, 53.1%, and 36.7% respectively) than in cases without IPI whereas, Abdominal pain was significantly lower in positive cases for IPI (53.1%) than in cases without IPI (73.5%). The frequencies of loose/soft stool in *E.histolytica/dispar, C.parvum and E.coli*-infected cases (69.6%, 71.4%, and 81.8% respectively) were significantly higher, however, no statistically significant differences regarding stool consistency in *G. intestinalis*, *B. hominins*, *H. nana*-infected cases. Furthermore, a significantly higher proportion of bloody stool was among *E. histolytica/dispar*-infected children (27.3%). In a conclusion, there was a high prevalence of IPIs among preschoolers especially *G.intestinalis, E.histolytica/dispar,* and *C.parvum.* This necessitates enrolling preschoolers in the Egyptian deworming programs.

INTRODUCTION

It is well known that enteric parasitic infections are among the globally neglected tropical diseases, particularly in the developing world (Taghipour *et al*. 2021). Diarrhoeal is the second leading cause of death in children under the age of five in low-income and middle-income countries, with an approximate annual global burden of 1.7 billion cases.

Diarrhoeal diseases are responsible for one out of every ten deaths in children under the age of five. (LBD Diarrhoea Collaborators, 2020). Diarrhea is a common presentation of gastrointestinal infections caused by various pathogens such as parasites, bacteria, and viruses through contaminated food or drinking-water, or from person to person in poor hygienic communities. Enteric parasites and bacterial pathogens are more frequent causes of diarrhoea than viruses in the developing world (WHO, 2017). Transmission of intestinal parasitic infections (IPI) is closely related to geographic, and socioeconomic status, poor personal and community hygiene, low incomes, and a humid overcrowded environment (Amer *et al*. 2018).

Intestinal parasitic diseases produce various gastrointestinal symptoms such as diarrhoea, dysentery, constipation, flatulence, vomiting and abdominal pain. In addition, it can also cause malnutrition, anaemia, and growth retardation in vulnerable children (Mahmud *et al*. 2020). The most prevalent enteric parasitic protozoan infection causing diarrhoea in children are *Giardia intestinalis* (*G. intestinalis*), *Cryptosporidium parvum* (*C. parvum*) and *Entamoeba histolytica/dispar* (*E.histolytica/dispar*) (Farthing *et al*., 2013).

Despite significant developments in sanitation and hygiene in Egypt, IPI remains a public health problem that differs in their intensity among various Egyptian governorates. The prevalence of IPI among school children in Dakahlia governorate was 32.9% (Ahmed and Abu-Sheishaa, 2022). In Cairo, approximately 51% of patients with gastrointestinal symptoms were positive for IPI (Hussein *et al*. 2017). Also, In Sohag Governorate, there was a high infection rate reaching 63.5% of the children attending primary schools (El-Nadi *et al*., 2017). Additionally, in Sharkia Governorate, the prevalence of geo-helminths among school children was 21.07% (Farghly *et al*., 2016). The prevalence of IPI in primary school children in Aswan Governorate was 31% (Dyab *et al*., 2016). Moreover, the overall prevalence of IPI among schoolchildren in Damietta Governorate's rural and urban populations was 30.7% (Mohammad *et al*., 2012).

As the regional distribution of enteric parasites is variable, further periodical studies on the prevalence of IPI and associated manifestations among different age groups and different populations in different localities in Egypt are needed to identify high-risk communities and improve different control measures. A considerable proportion of diarrhoeal disease can be prevented through a clean water supply, improved sanitation and adequate public health and hygiene (WHO, 2017). Three approaches; protection, prevention, and treatment were named by WHO's integrated Global Action Plan for Pneumonia and Diarrhoea to lower the diarrhoeal disease burden (WHO, 2013).

The aim of the current study is to estimate the burden of enteric parasitic infections amongst Egyptian preschoolers with gastrointestinal manifestations who attended Helwan University Hospital's outpatient pediatrics clinics, in Cairo, Egypt.

MATERIALS AND METHODS

1. Study Design and Populations:

A cross-sectional parasitological study was conducted at the outpatient pediatrics clinics of Helwan University Hospital, Cairo, Egypt on 200 preschool children under 5 years of age who were attending the pediatric clinic of the hospital with various gastrointestinal manifestations such as diarrhoea, constipation, flatulence, vomiting, tenesmus, abdominal pain, pruritis ani, and fever within the previous 3 days from May to October 2022.

Inclusion Criteria: Preschool infants and children under 5 years of age of both genders who attended with various gastrointestinal manifestations and whose

parent/guardian give consent were included in the study.

Exclusion Criteria: Children with diarrhea associated with other identified diseases such as hepatitis, respiratory tract infection, lactose intolerance who needed emergency treatment and those with surgical problems such as appendicitis and children who were taking anti-helminth or anti-diarrhoeal treatment within the last 2 weeks.

The study population was considered diarrheic if complaining of an episode of stool passing characterized by three or more loose or liquid bowel movements per day or more frequent motions than normal for a person (WHO, 2009; WHO, 2017).

Cases were subjected to basic demographic information including age, sex, and residence, thorough history taking and clinical examination including general examination for the signs of dehydration, and abdominal examination. Evaluation of clinical symptoms was made by the pediatrician at the outpatient clinic according to the enrolment criteria.

2. Samples Collection and Copro-parasitological Examination:

Samples were collected in clean, leakproof, labeled, plastic stool cups, and transferred to the parasitology laboratory. The samples were examined macroscopically for color, consistency, and the presence of mucous or blood or adult worms and segments. The specimens were subjected to wet mount examination using a normal saline solution by mixing up a small amount of stool with a drop of 0.85% NaCl on a microscopic slide**.** Direct examination by iodine wet mounts was also done by adding a drop of Lugol's iodine to the pellet. Microscopic examination was done using low power (X10) and high power (X40) magnifications. The rest of each stool sample was preserved with 10% formalin and re-examined following Modified Ritchie's biphasic concentration technique. Part of each stool sample was stained with a modified Kinyoun acid-fast stain "cold method" (Eng Scientific Inc., N.J. 07012-1708, USA) and examined for detection of coccidian protozoa (Garcia, 2007).

3. Statistical Analysis of The Data:

Data were entered into Microsoft Excel and exported to the Statistical Package of Social Science (SPSS, version 26) software and analyzed. Data were described using numbers and percentages. Relation between qualitative data was done using the Chi-square test. P-value <0.05 was considered statistically significant.

2.4. Ethical Considerations:

The research ethics committee (REC) for human and animal research, Faculty of Medicine, Helwan University **(Serial: 145-2022)** granted ethical approval. Verbal consent was taken from the parent/guardian. All collected participant's data were confidential and anonymous. Participants with positive enteric parasitic infection results were told to be treated in the pediatric department of Helwan University.

RESULTS

1-Socio-Demographic Criteria of The Study Population:

A total of two hundred under 5 years children had joined this study. Equal distribution of males and females among the participants of the study was observed. Around 39% (n= 78) of the study population were newborns/infants (under 2 years of age) and 61% (n= 122) were toddler/preschool kids (2-5 years old). Amongst the studied group, 85.5% (n=171) were from urban/suburban areas within Cairo, provided with municipal tap water and sewage system. Only 14.5% (n= 29) of them were rural dwellers. None of them gave a recent history of travel**.**

2-Prevalent Symptoms Among the Study Population (Table 1):

Of the total study participants, the predominant symptom was abdominal pain in 127/200 (63.5%) participants followed by diarrhoea in 118/200 (59%) participants, tenesmus in 67/200 (33.5%) participants, flatulence in 63/200 (31.5%) participants, vomiting in 58/200 (29%) participants,

fever in 53/200 (26.5%) participants, constipation in 28/200 (14%) participants and only 7/200 (3.5%) participants complained of pruritis ani.

Diarrhoea and flatulence were significantly higher in positive cases for IPI (71.4% and 53.1%, respectively, p-value <0.001) than in cases without IPI. Also, vomiting was significantly higher in positive cases for IPI than in cases without IPI (36.7%, p-value =0.018). However, abdominal pain was significantly higher in cases without IPI (73.5%) than in positive cases for IPI (53.1%), p-value =0.003. No statistically significant difference between positive and negative cases for IPI regarding tenesmus, fever, constipation, and pruritis ani (p-value = 0.396, 0.515, 0.055, and 0.271 respectively).

Table 1: Frequency of symptoms among the study population**.**

Symptoms	Total (n= 200)		No IPI (n=102)		IPI (n=98)		P-value
	Number	%	Number	%	Number	%	
Abd. Pain	127	63.5%	75	73.5%	52	53.1%	0.003 *
Diarrhoea	118	59.0%	48	47.1%	70	71.4%	<0.001 **
Tenesmus	67	33.5%	37	36.3%	30	30.6%	0.396
Flatulence	63	31.5%	11	10.8%	52	53.1%	<0.001**
Vomiting	58	29.0%	22	21.6%	36	36.7%	0.018 *
Fever	53	26.5%	25	24.5%	28	28.6%	0.515
Constipation	28	14.0%	19	18.6%	9	9.2%	0.055
Pruritis ani	7	3.5%	5	4.9%	2	2.0%	0.271
*Significant difference at < P-value 0.05; **Significant at P-value <0.001							

3-Prevalent IPI Among the Study Population Their Frequent Symptoms (Table 2):

Of the total study participants, 49% (n= 98) were positive for one or more IPI. The predominant identified parasite was *G. intestinalis* which represents 70/200 (35%) of cases followed by *E. histolytica/dispar* 33/200 (16.5%) of cases, *C. parvum* 21/200 (10.5%) of cases, *Entamoeba coli* (*E. coli*) 11/200 (5.5%) of cases, *Blastocystis hominis* (*B. hominis*) 10/200 cases (5%), *Hymenolepis nana* (*H. nana*) 6/200 cases (3%), *Enterobius vermicularis* (*E. vermicularis*) 2/200 cases (1%) and only one case of *Trichuris trichiura* (*T. trichiura*), *Ascaris lumbricoides* (*A. lumbricoides*) and *Iodamoeba butschlii* (*I. butschlii*). Within the infected cases, 53.1% (n= 52) of them have single IPI, 34.7% (n= 34) of cases have double IPI and 12.2% (n= 12) of cases have triple IPI.

Diarrhoea was the most common presenting symptom in children infected with *G. intestinalis* (57.1%), *E. histolytica/dispar* (78.8%), *C. parvum* (90.5%), and *E. coli* (81.8%). However, the most frequent presenting symptoms in children infected with *B. hominis* were abdominal pain (60%), and flatulence (60%). Moreover, abdominal pain was the most frequent symptom in children infected with *H. nana* (83.3%).

Table 2: The associations between different diagnosed IPI and clinical symptoms of infected cases.

Symptoms	*G. intestinalis* (n=70)	*E. histolytica/dispar* (n=33)	*C. parvum* (n=21)	*E. coli* (n=11)	*B. hominis* (n=10)	*H. nana* (n=6)	Others (n=5)
Abd. Pain	40 (57.1%)	15 (45.5%)	9 (42.9%)	3 (27.3%)	6 (60%)	5 (83.3%)	2 (40%)
Diarrhoea	48 (68.6%)	26 (78.8%)	19 (90.5%)	9 (81.8%)	3 (30%)	4 (66.7%)	4 (80%)
Tenesmus	26 (37.1%)	12 (36.4%)	7 (33.3%)	2 (18.2%)	2 (20%)	2 (33.3%)	0 (0%)
Flatulence	43 (61.4%)	21 (63.6%)	10 (47.6%)	5 (45.5%)	6 (60%)	1 (16.7%)	1 (20%)
Vomiting	23 (32.9%)	13 (39.4%)	12 (57.1%)	3 (27.3%)	3 (30%)	2 (33.3%)	1 (20%)
Fever	16 (24.3%)	9 (27.3%)	6 (28.6%)	3 (27.3%)	2 (20%)	1 (16.7%)	1 (20%)
Constipation	9 (12.9%)	3 (9.1%)	0 (0%)	0 (0%)	3 (30.0%)	1 (16.7%)	0 (0%)
Pruritis ani	1 (1.4%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (20%)

4-The Relation Between Different IPI And Gross Macroscopic Pictures of The Samples of Positive Cases (Table 3):

There was a statistically significant higher frequency of loss/soft stool in *E. histolytica/dispar* (69.6%), *C. parvum* (71.4%), *E. coli* (81.8%), p-value= 0.025, 0.016, and 0.008, respectively. However, no statistically significant differences regarding stool consistency in *G. intestinalis*, *B. hominins*, *H. nana*-infected cases (p-value= 0.105, 0.401 and 0.585 respectively). Furthermore, there was a statistically significant high proportion of bloody stool among children infected with *E. histolytica/dispar* (27.3%, p-value= 0.001).

Table 3: The associations between different diagnosed IPI and the macroscopic picture of the stool samples of infected cases.

Macroscopic exam	*G. intestinalis* (n=70)	*E. histolytica/dispar* (n=33)	*C. parvum* (n=21)	*E. coli* (n=11)	*B. hominis* (n=10)	*H. nana* (n=6)	Others (n=5)
Formed	19 (27.1%)	5 (15.2%)	2 (9.5%)	0 (0%)	5 (50.0%)	3 (50.0%)	2 (40%)
Loose/ soft	42 (60%)	23 (69.6%)	15 (71.4%)	9 (81.8%)	5 (50.0%)	3 (50.0%)	2 (40%)
Watery	9 (12.9%)	5 (15.2%)	4 (19.1%)	2 (18.2%)	0 (0%)	0 (0%)	1 (20%)
P-value	0.105	0.025*	0.016*	0.008*	0.401	0.585	---
++Mucus	4 (5.7%)	3 (9.1%)	0 (0%)	1 (9.1%)	1 (10%)	0 (0%)	1 (16.7%)
++Blood	14 (20.0%)	9 (27.3%)	4 (19%)	1 (9.1%)	2 (20%)	0 (0%)	0 (0%)
P-value	0.056	0.001*	0.593	0.433	0.343	0.902	----
*Significant difference at P-value <0.05							

DISCUSSION

Epidemiological studies on the prevalence of IPIs in different regions are vital to recognize the high-risk regions and articulating satisfactory prevention and control measures (Teklemariam *et al*., 2014). Parasitosis represents a worldwide health problem in a lot of developing worlds including Egypt, with their morbidity prognosticators, not well-determined (El-Nadi *et al*., 2017).

The aim of the current study is to estimate the burden of intestinal parasitic infections among 200 Egyptian preschoolers under 5 years complaining of gastrointestinal manifestations. The choice of the under 5 years children in the present study is the reason that they are neglected compared to school children, who are currently the main focus of projects and health plans in Egypt.

Double concentration and permanent stains were used in the present study, this is not commonly implemented in epidemiological surveys, and this improves the sensitivity of the microscopic examination.Various Socio-demographic variables such as age, residence (rural/urban), the providence of municipal tap water and sewage system, history of

travel and others were recorded in this study. In the same context, these variables were previously recorded in many studies to predict IPIs among children worldwide (Forson *et al*., 2017; El-Nadi *et al*., 2017; Bakarman *et al*., 2019; Sitotaw and Shiferaw, 2020).

There was no significant association between children's gender and the occurrence of IPIs in this study. This agrees with previous reports (Weerakoon *et al*, 2018; Bakarman *et al*., 2019 and Elmonir *et al*., 2021), but conflicts with Nguhiu *et al*., 2009; Abdi *et al*., 2017; Khater and Elfar, 2019 who reported a higher IPIs rates among male children.

The current study revealed that 49% of children are infected with at least one parasite. This conclusion was consistent with an Egyptian study done in Assiut governorate, which observed that 55.7% of IPIs were among children aged 1-6 years (Yones *et al*., 2015) and similarly, a study done in Cairo governate, reported that the prevalence of IPIs was (51.8%) among children aged 1-5 years (Khater and Elfar, 2019). Yet, our finding was lower in another study done by Elmonir *et al*., 2021 in Tanta district of Gharbia governorate, the authors reported that the prevalence of IPIs among preschool children was 76.9% which was seven times more than among school-age children. The variation recorded in the prevalence rates between different areas might be attributed to various factors such as climatic features, environmental hygiene, socio-economic conditions, and the application of prophylactic control methods.

Out of ten parasitic species detected in this study, *Giardia intestinalis* was the predominant parasite (35%) among the infected children. This finding agreed with other studies which had demonstrated the widespread occurrence of *Giardia* among younger in comparison with older children (Keiser *et al*., 2002; Nguhiu *et al*., 2009). In the same context, Taha *et al.*, 2018 stated that *G. intestinalis* is the highly prevalent enteric parasite in children from 3 to 8 years with an infection rate of 20.68%. Also, Khater and Elfar, 2019 reported that *G intestinalis* was the most predominant parasite found in children (41%). The predominance of *G intestinalis* among preschoolers might be attributed to the various isolates/strains and inconsistent immune response which might play a vital role in the divergent patterns of Giardiasis (Majewska, 1994).

The prevalence rate of *E. histolytica/dispar* in this study reached 16.5%, this percentage changes in different nations and increases in the developing world. This parasite is thought to infect 10% of the world's population (Wadood *et al*., 2005).

Infection with *C. parvum* reached 10.5% of the cases in the current work. This percentage varies in different countries as reported by Kabayiza *et al*., 2014, Nasser 2016 and Tombang *et al*., 2019 where *Cryptosporidium* infection detected among children of ages 0–5 years was 9.4%, 10.4% and 8.93%, respectively. *Cryptosporidium* is commonly affecting children under five years old and was linked to malnutrition and death caused by diarrhea in developing countries (Kotloff *et al*., 2013).

G. intestinalis, E. histolytica/dispar, and *C. parvum* continue to be the common enteric parasitic infections transmitted through the faeco-oral route both directly from human to human and incidentally by ingesting polluted food or drinking polluted water (Mohammad *et al*., 2012). The differences in prevalence rates in different places might be attached to various sanitation levels, types of water sources, hygienic procedures, and nutrition manners (Bauomy *et al*., 2010).

The most predominant gastrointestinal symptom reported in the examined children was abdominal pain (63.5%) which was significantly lower in IPIs than in non-IPIs. Oh *et al*. 2004 stated that abdominal pain is one of the most common symptoms occurred in children. It

might be due to several gastrointestinal or extraintestinal reasons. These involve infection of the gastrointestinal tract, dietary indiscretion, infection of the urinary tract, and some surgical conditions such as acute appendicitis.

Diarrhea was the second symptom reported in the examined children (59%) and it was significantly higher in IPIs than in non-IPIs. Diarrhea was the most predominant symptom found in children infected with *G. intestinalis, E. histolytica/dispar, C. parvum and E. coli*. This result was aligned with the macroscopic picture of the stool samples of the cases infected with these parasites, in which the stool consistency was loose/soft. In the same context, Khurana *et al*., 2021 reported that the most prevalent infectious agents causing diarrhoea in children are parasites particularly *G. intestinalis, E. histolytica/dispar, Cryptosporidium.*

The present study showed that multiparasitism is a significant result recorded among Egyptian children; by which 34.7% of the children infected with parasites harbored 2 parasites and 12.2% harbored 3 parasites concurrently. In the same context, Khater and Elfar, 2019 reported that 47% of the infected children had two or more parasites. This finding also fits with other research that found polyparasitism to be a typical pattern for parasitic existence rather than single parasitic existence (Brooker *et al*., 2000; Keiser *et al*., 2002; Nguhiu *et al*., 2009).

CONCLUSION:

The present study highlighted the high prevalence rates of IPIs among children under five years. *G. intestinalis, E. histolytica/dispar,* and *C. parvum* were the most prevalent parasites and diarrhea was the most predominant symptom. Therefore, it is highly recommended to carry out regular inspections and treatment of IPIs in children. This study also highlighted the necessity of enrolling Egyptian preschool children simultaneously with school children in future deworming programs. Besides, poly-parasitism is considered a common finding among children less than five years in Egypt, so it should be assessed with more details regarding the intensity of infection, different age groups and impacts on the children's responses.

Contribution of Each Author:

All manuscript authors contributed to every aspect of it, including the paper's idea, study design, material collection, methodology, drafting the paper, and revising it.

Conflict of Interest:

The authors state that they do not have any competing interests.

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