Original Article

Prevalence of Intestinal Protozoan Parasites among Children Attending the Hevi Pediatric Hospital in Duhok Province, Kurdistan Region, Iraq

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ABSTRACT

The present study aimed to determine the prevalence rate of intestinal protozoan infection among children in Duhok Province, North of Iraq, from October 2022 to May 2023. This cross-sectional study was conducted on 740 children attending Hevi Pediatric Hospital, Duhok Province, Iraq. Firstly, all collected stool samples were examined directly by preparing a wet stool smear for the detection of the vegetative stage (Trophozoite) of both *Entamoeba histolytica* and *Giardia lamblia*. Thereafter, all stool samples were preserved at -22°C until further use for identifying cysts of protozoa and oocysts of *Cryptosporidium parvum* by the Telmman concentration stool method. The children were within the age range of 1-14 years. Out of 740 stool samples collected, 205 (27.7%) cases were positive for intestinal protozoan infections as follows: 160 (78.0%) children tested positive for *E. histolytica*, 35 (17.1%) were positive for *C. parvum*, and only 10 (4.9%) were positive for *G. lamblia*. Nonetheless, the results indicated a statistically significant difference between the prevalence of *E. histolytica*, *C. parvum*, and *G. lamblia* infections across different age groups ($P<0.05$). The highest infection rates of intestinal protozoa, namely *E. histolytica*, *C. parvum*, and *G. lamblia*, were reported in the age ranges of 5-9 years (53.75%, 25.0%, and 60.0%), 1-4 years (28.75%, 20.0%, and 30.0%), and 10-14 years (17.55%, 8.6%, and 10.0%), respectively. In this study, the prevalence of amoebiasis and cryptosporidiosis was reported to be higher than in other studies performed in Iraq, while the prevalence of giardiasis was reported at a lower rate than the rate reported previously in Iraq, especially in Duhok. These infections continue to pose a challenging public health issue, necessitating the implementation of stronger and more effective preventative measures.

Keywords: Children, Duhok Province, Intestinal Protozoa, Prevalence

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1. Introduction

Gastrointestinal infections and diarrhea are widespread in developing countries, including various tropical regions. Infections of the gastrointestinal tract can be caused by viruses, bacteria, protozoa, helminths, and occasionally fungi (1). The World Health Organization reported that diarrhea affects more individuals than any other disease, even in high-income countries (2). Poor quality drinking water is one of the factors that have been linked to an increased prevalence of diarrheal diseases (3). Intestinal protozoan infections are generally common across the globe. They represent a major cause of diarrheal diseases, especially among children in developing countries (1). *Entamoeba histolytica*, *G. lamblia*, and *C. parvum* are the most frequently identified protozoan parasites in stool samples. *Cryptosporidium parvum*, first described in humans in 1976, is now recognized as an important cause of diarrhea in young children and immunocompromised adults (4, 5). The transmission dynamics of enteric protozoa vary considerably among geographic areas (1). Understanding the significance of different sources of infection is needed to set priorities for controlling measures. The present study aimed to investigate protozoan infections in children with diarrhea and its relationship with protozoan contamination of drinking water and other possible risk factors in Duhok Province.

2. Materials and Methods

This cross-sectional study was conducted on 740 children referred to Hevi Pediatrics Hospital, Duhok Province, Iraq, from October 2022 to May 2023.

2.1. Sample Collection and Processing

About 4 g of fresh stool samples were collected from all children and put in a plastic cube. Each stool cup was labeled with the main information of the child, including name, age, and gender. All diarrheic samples were examined directly under the microscope for the detection of the trophozoite stage of *E. histolytica* and *G. lamblia*. Following that, all stool samples were preserved at -22°C until used for the identification of cysts of *E. histolytica* and *G. lamlia*, and oocysts of *C. parvaum*, by direct wet stool smear and concentration stool methods.

2.1.1. Direct stool smear

A direct stool smear examination was performed using the methods previously mentioned (6). A small amount of stool sample was mixed with a few drops of normal saline, put on a cover slide, and examined directly under the microscope first at low power and then moved to other powers. From each sample, at least three slides were prepared and examined.

2.1.2. Telmman stool concentration technique

Firstly, all frozen stool samples were left at room temperature to be thawed; thereafter, about 1 g of stool sample was mixed well with 5 ml of acetic acid and set for a few minutes. It was then filtered through a fine sieve, and the filtered fluid was pureed into a clean test tube. Following that, the same volume of ether was added to the test tube, covered with a cover, and centrifuged at 2000 rpm for 3 min. After centrifugation, the supernatant was discarded and a drop of sediment was put on the slide and mixed with a few drops of normal saline, covered, and examined under the microscope for the detection of cysts. For the detection of the oocyst of *C. parvum*, a thin stool smear was prepared from the sediment and dried at room temperature. Subsequently, the slide was put in Methyl alcohol for 4 min for fixation, and the smear was then stained with Modified Ziehl- Neelsen Stain.

2.3. Statistical analysis

GraphPad Prism software (version 8.0) was used to evaluate the data gathered for the current investigation. The Chi-square test was used to examine the relationship of infections with age and gender.

3. Results

The children were in the age range of 1-14 years. Out of the 740 stool samples collected, 205 (27.7%) were positive for intestinal protozoan infections as follows: 160 (78.0%) children tested positive for *E. histolytica*, 35 (17.1%) were positive for *C. parvum*, and only 10 (4.9%) were positive for *G. lamblia*, as presented in table 1 and displayed in figures 1, 2, and 3. Nonetheless, the results showed no statistically significant difference between the prevalence of amoebiasis, cryptosporidiosis, and giardiasis across different age groups ($P<0.05$). As tabulated in table 2, the rate of amoebiasis was higher in females than in males (60.6% vs 39.4%), respectively. However, the rate of cryptosporidiosis was higher in males than in females (65.7% vs 34.4%), while the rate of giardiasis was equal in both genders (5%). These results were significant at $P < 0.05$. Table 3 gives information about the infection rate of intestinal protozoa among children according to the age group. The highest infection rates of intestinal protozoa, namely *E. histolytica*, *C. parvum*, and *G. lamblia*, were reported in the age range of 5-9 years (53.75%, 25.0%, and 60.0%), 1-4 years (28.75%, 20.0%, and 30.0%), and 10-14 years (17.55, 8.6%, and 10.0%), respectively. These differences according to the age groups were statistically significant at $P < 0.05$.

4. Discussion

The results of the present study demonstrated a relatively high prevalence rate of intestinal protozoan parasites among children in Duhok Province, Iraq. The overall frequency rate of protozoal infections was estimated at 27.7%, consistently reported by several studies performed in other countries (7).

Table 1: Prevalence of *E. histolytica*, *C.parvum*, and *G. lamblia* in Duhok City, Iraq (n=740)

Intestinal Protozoa	Frequency	Percent (%)
E.histolytica	160	78.0
C.parvum	35	17.1
G. lamblia	10	4.9
Total	205	100

The chi-square statistic is 0.0023. The *p*-value is 0.998869.
The result is *not* significant at a $P < 0.05$.

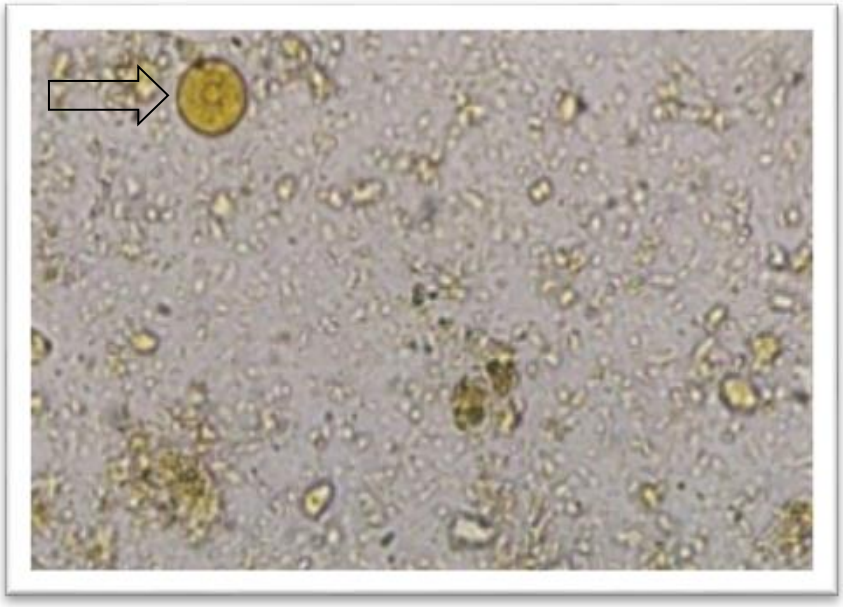

Fig 1: Cyst stage of *Entamoeba histolytica*

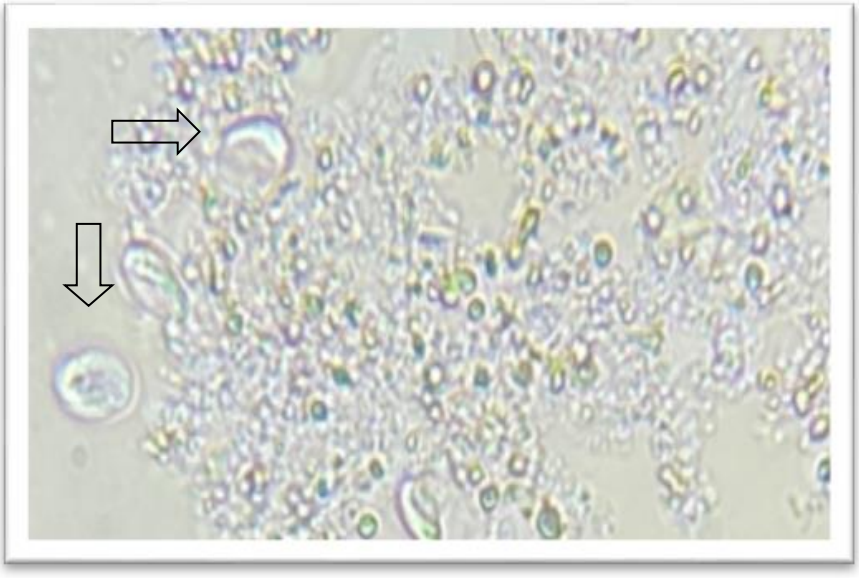

Fig 2: Cyst stage of *Girdia lamblia*

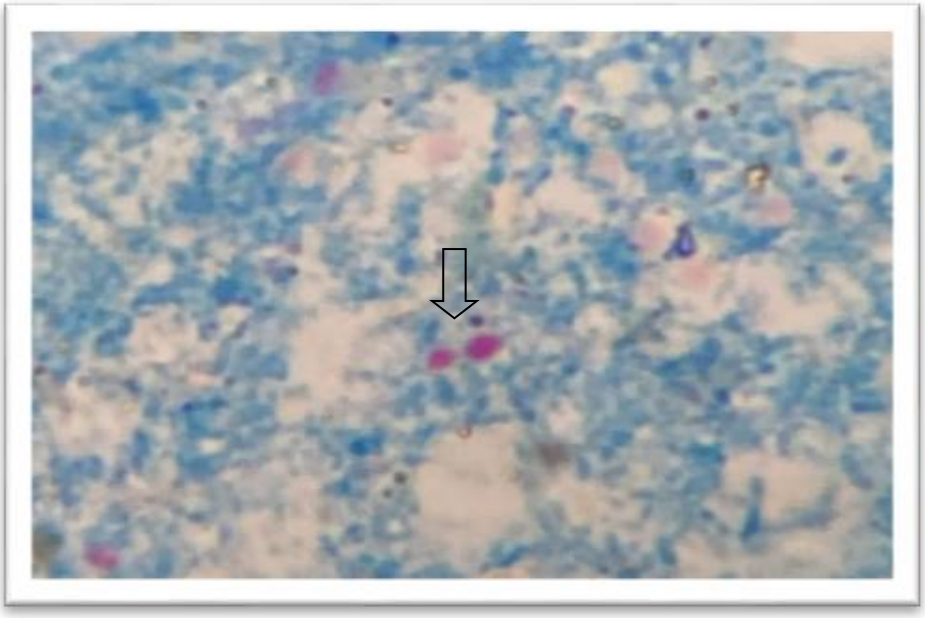

Fig 3: Oocyst stage of *Cryptosporidum parvum* with acid fast stain

Table 2: Frequency of *E. histolytica*, *C.parvum, and G. lamblia* protozoan regarded to the gender

Intestinal Protozoa	Total No. (%)	Gender No. (%)	
		Males	Females
E. histolytica	160 (78)	63(39.4)	97(60.6)
C.parvum	35(17.1)	23(65.7)	12(34.3)
G.lamblia	10 (4.9)	5(50)	5(50)
Total No. (%)	205 (100)	91(44.4)	114(55.6)

The chi-square statistic is 21.3248. The *p*-value is 0.000023. The result is significant at a $P < 0.05$.

Table 3: Prevalence of intestinal parasites among children according to the age groups

Intestinal Protozoa	Total No. (%)	Age group (year) No. (%)		
		1-4	5-9	10-14
E.histolytica	160(78.0)	46(28.75)	86(53.75)	28(17.5)
C.parvum	35(17.1)	7(20.0)	25(71.4)	3(8.6)
G. lamblia	10(5.88)	3 (30.0)	6(60.0)	1(10.0)
Total No. (%)	205 (100)	56(27.3)	117(57.1)	32(15.6)

The results are significant at a $P < 0.05$

In the present study, the most prominent intestinal protozoa infections were *E. histolytica* (78.0%) and *C.parvum* (17.1%), while the lowest infection rate was caused by *G. lamblia* (4.9%). Earlier studies in Iraq have revealed that the most prevalent intestinal protozoan infections among patients who visited Azadi Teaching Hospital in Duhok City Kurdistan Region were *E. histolytica* and *G. lamblia*. The prevalence of intestinal protozoan infections and the reported species vary in different studies conducted in Iraq. Nevertheless, most of these studies have reported that *E. histolytica* caused the highest prevalence of infection. The highest prevalence rate of *E. histolytica* recorded in the present research was in agreement with the results of a study performed by Salman et al. (2019) (8). They reported that the highest rate of Amoebiasis was 27.0% among diarrheic children attending a hospital in Al-Karkh region, Baghdad Province. The second-highest prevalence rate of *C. parvum* in the current study was in line with the results of a study conducted by Kanabe and Darogha (9) in Erbil City, Iraq, where the prevalence of *C. parvum* was 18.94%. Finally, the lowest infection rate of *G. lamblia* was calculated at 3.0% in the present study, which was consistent with that reported by Bazzaz et al. (10). The findings of the current research was in agreement with a previous study conducted in Erbil City in term of reporting similar species of intestinal parasites; however, prevalence rates were slightly lower than those found in our study (11). This research reported that 61.24% and 26.60% of subjects were infected with *E. histolytica* and *G. lamblia*, respectively. A further study conducted in Mosul, Iraq, on 2,296 subjects found that the rates of *E. histolytica* and *G. lamblia* in children associated with diarrhea were 14.5% and 0.96%, respectively (12). Another study performed in Duhok, Northern Iraq, revealed that the frequency rates of *E. histolytica* and *G. lamblia* among patients attending Azadi Teaching Hospital were 28.3% and 1.1%, respectively (13). The results of a study conducted in Mazandaran Province, Iran, demonstrated that the prevalence of *G. lamblia* infection among the general population was 4.6% (14). This variation in the rate of parasitic infections can be attributed to differences in the number of samples, design of the study, laboratory tests, sanitary conditions, type of water consumed, economic standards, climate and environmental factors, hygiene levels, behavior style, and type of drinking water. The present study pointed out that the prevalence of *E. histolytica* was higher than that of *G. lamblia* infection across different age groups. In a similar study performed by Omar and Ismael (15) in Duhok, it was found that the infection rate of amoebiasis was higher than that of giardiasis in children. These results were inconsistent with those of the two studies carried out in Iran by Kia et al. (16) and Zaki (11), who recorded that the infection rate of giardiasis was higher than amoebiasis in children. The prevalence rate of *E. histolytica* was higher among females than males in this study. In a similar vein, Haji and Bamarni (17) reported that the infection rate was higher among female children. Inconsistent with the results of the present research, a related study reported that the rate of *E. histolytic* (61.7%) was higher in males than in females (18). The differences observed between genders in terms of susceptibility to infections can be attributed to variations in endocrine immune interactions, specifically in the levels of sex steroid hormones, such as androgens and estrogens. These hormones have been shown to modulate different aspects of the host immune response by regulating the expression of Toll-like receptors and the production of humoral immunity (19). In contrast to the current research findings, a study has reported a higher prevalence of giardiasis among males compared to females. In disagreement with the results of the present research, one study reported that the prevalence rates of giardiasis were 4.7% and 4.5% in males and females, respectively (13). The higher rate of infection with *C. parvum* in males in this study was in agreement with a study by Ahmed et al. performed in Zakho District, Duhok, Iraq (20). The results of our study found that the infection rate of amoebiasis, cryptosporidiosis, and giardiasis was higher in the age range of 5-9 years. The results of the present study agreed with a study conducted in Zakho City on primary school children. The referred study found that the highest infection rate occurred in 9-year-old children (21, 22). The higher prevalence of infection among 5-9-year-old students could be attributed to less attention paid by their mothers to personal hygiene as children in this age group are more active and tend to play outdoors, exposing them to more dirt and potential sources of infection. On the other hand, older individuals tend to be more conscious of personal hygiene and take better care of themselves. It is suggested that timely measures be taken by carrying out long-term control strategies, enhancing personal cleanliness standards, and improving living environments and sanitary conditions.

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Authors' Contribution

Study concept and design: Ismael, SS. Abdullah, BH. & Sadiq, AJ.

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Acquisition of data: Ajaj, JS. Ali, NS. Omer, DM. Nori, NY.

Analysis and interpretation of data: Sadiq, AJ.
Drafting of the manuscript: Ismael, SS. & Abdullah, BH
Critical revision of the manuscript for important intellectual content: Ismael, SS. & Abdullah, BH
Statistical analysis: Sadiq, AJ.
Administrative, technical, and material support: Ismael, SS.

Ethics
The study proposal were approved by the ethics committee of the College of Health Sciences, University of Duhok, Duhok, Iraq

Conflict of Interest
The authors declare that they have no conflict of interest.

Data Availability
The data that support the findings of this study are available on request from the corresponding author.

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