

Isolation and diagnosis of *Entamoeba histolytica* and *Giardia lamblia* from children infected with diarrheal at Mohammed AL-Moussawi Hospital in Dhi-Qar Province

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Abstract: - *Entamoeba histolytica* causes amebiasis and is responsible for 100,000 deaths each year in humans, making it the third leading cause of death due to protozoan parasite. Giardiasis is a major globally transmitted diarrhoeal disease caused by *Giardia lamblia*. The present research has been conducted in the province of Dhi Qar to explore the spread of *Entamoeba histolytica* and *Giardia lamblia* in children with diarrhea and to investigate some factors that may affect the spread of infection rates, such as age and sex. From the beginning of November 2017 to the end of March 2018, 500 stool samples were collected from Muhammad Al-Mousawi Hospital. Results indicated number of positive samples for *E. histolytica* was 63 (12.6%) higher than the *G. lamblia* infection which reached 3 (0.6%) samples. On the other hand, the study showed that the rate of infection according to age groups was highest in the age group of 1-3 years, reaching (42.4%), while the infection rate in advanced age groups 10-12 years was (9.1%). In males, 37 (56.1%) was higher than the females who had 29 samples (43.9%). The results of the statistical analysis showed that there were significant differences between the infections according to the type of parasite, age and sex. Finally, we conclude that *E. histolytica* is the main causative agent of childhood diarrhea than *G. lamblia* in young age groups in males.

Key words—*Entamoeba histolytica* , *Giardia lamblia*, Diarrhea, Amebiasis, Giardiasis

INTRODUCTION

Parasitic infections are a major global public health issue, particularly in developing countries. Intestinal parasite infections are most widespread and highly prevalent in Iraq [1] Various protozoan species parasitize humans' intestines, some of which are *G. lamblia* and *E. histolytica* , causing a remarkable quantified burden of disease, particularly in humid tropics [2]. However, epidemiological research and public health initiatives are more often based on helminths (e.g., *Schistosoma mansoni*, *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworm) than on intestinal protozoa [3]. Partly because microscopic diagnosis of intestinal protozoa is harder. It requires qualified technicians to better recognize and distinguish intestinal protozoa cysts. *E. histolytica* infections leads to 40,000 – 100,000 deaths per year due to amoebiasis [4]. *Giardia intestinalis* could affect 200 million people annually [5]. Due to the lack of up-to - date epidemiological data, the burden of pathogenic bowel protozoa infection continues to be established [6]. Human may become infected with Parasites through various means, such as direct contact with an infected organism, animal

vectors, or indirectly by human microbial transmission, while most parasites reach to the body through the skin or mouth. Infection may also be acquired by handling very many parasites in chickens, dogs , and cats. Food pollution also causes germs to spread from one source to other people [7]. There are many parasitic diseases, but amebiasis and giardiasis are the most common ones, spreading all over the world including Iraq and contributing to severe diarrhea. Although diarrhoea means the pathogenic condition of an excreted stool, it can also mean that it can easily be transmitted through the gut for a few reasons: large intestinal and rectal infections caused by intestinal parasites, food poisoning, infected milk, or certain diseases such as tuberculosis, cholera , typhoid, or dysentery. Fever, gastrointestinal colic, coughing, vomiting and abdominal pain are frequently followed by bacterial diarrhea [8]. In developing countries diarrheal diseases are common and considered to be key causes of morbidity and mortality [9]. Diarrhea etiology involves viruses , bacteria and parasites [10], the major intestinal parasite being *G. lamblia* and *E. histolytica* . [11]. These species are major pathogens widespread in developing countries that cause significant

public health issues [12]. The purpose of this study is to detect *E. histolytica* and *G. lamblia* intestinal parasites in patients with diarrhea as well as their relationship with sex and age.

MATERIALS AND METHODS

a. Collection data:

The current study was conducted on children with diarrhea to investigate intestinal parasites and different age groups to both sexes. since start of November 2017 to the end of March 2018, samples have been collected at Mohammed Al Mousawy Hospital. The data were collected in a questionnaire (patient age , gender) format.

b. Stool specimen collection:

Stool samples were collected from each patient in a clean , dry, tight-fit cover, and examined in the parasitology lab within half an hour. The samples were analyzed in hospital for the presence of the parasites *E. histolytica* and *G. lamblia*.

c. Stool examination:

Macroscopic examination : The stool samples were examined with naked eyes before microscopically examination for color, consistency, blood mucous.

Microscopic examination : The Olympus light microscope was used to detect trophozoite and cyst stage of *Entamoeba histolytica* and *Giardia lamblia*, after mixing a double blind check mixed with physiological saline (Normal saline solution) prepared from dissolving 8.5 gm of pure sodium chloride NaCl in one liter of distilled water^[2].

2.4. Statistical analyses:

Using SPSS soft wear kit version 20, the data was analyzed to discover the variability in the results of the study. As a (P-value < 0.05) statistical significance was established. For data processing, statistical tests such as (percentage and Chi-square) were used.

RESULTS AND DISCUSSION

The current study recorded *E. histolytica* as being the highest infection risk. Of the 500 samples, the number of positive samples was 66 (13.2%) for the parasites under study, of which 63 (12.6%) were *E. histolytica* and only 3 (0.06%) were infected with *G. lamblia*. The statistical analysis demonstrated a significant difference in P < 0.05 as seen in **Table 1**.

Table 1. Total number and percent of meningitis infection according to sex

Type of parasites	N. Positive	N. Negative	Sig=
<i>E. histolytica</i>	63 (12.6%)	434 (86.8%)	0.0001
<i>G. lamblia</i>	3 (0.6%)	(13.2%)	Df=1
Total	500(100%)		

The age-specific statistical study revealed that there is a significant association between the age group and the percentage of infection with parasites at (P≤0.05). Figure (1) indicate the high prevalence of infection in the age group (1-3) years, reaching 28 (42.4%), and the lowest infection rate in the age group (10-12) years, which was 6 (9.1%).

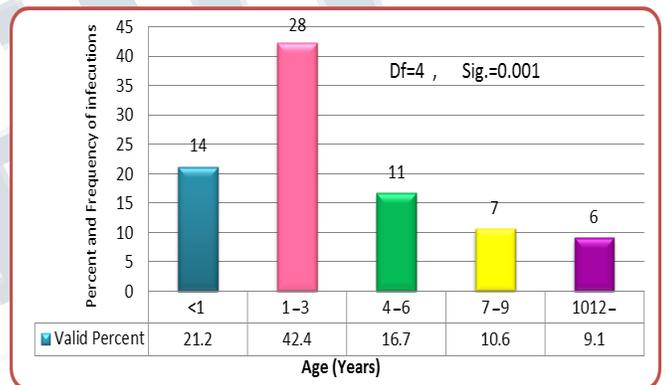


Figure1. Percentage and Frequency of *E. histolytica* and *G. lamblia* according to the age groups

The results in Table (2) showed that the incidence between the sexes and the parasites was distinct. The high prevalence of infection among males was 37 (56.1%) higher than among females, which was 29 (43.9%).

Table 2: Distribution of *E.histolytica* and *G. lamblia* according to gender

Gender	Frequency	Sig=
Male	37 (43.9%)	0.001 Df=1
Female	29 (56.1%)	
Total	66	

DISCUSSION

Current study conducted on 500 stool samples collected from children with diarrhea in Mohammed al-Musawi Hospital in the province of Dhi Qar and for different age

groups. The incidence of gastrointestinal infections of *E. histolytica* and *G. lamblia* was (12.6%) and (0.06%), respectively. This study was in accordance with [13] and differed with [14,15]. The reason of higher incidence of *E. histolytica* because it is endemic, has a simple life cycle, rapid reproduction, and ease of transmission through contaminated foods and drinks as well as its resistance to acidity, chlorine, and rotting, and its ability to survive for several weeks in humid environments [16]. The low incidence of Giardia parasites may be due to the lack of contamination of drinking water, which is the main source of transmission [17]. The findings also indicated a higher percentage of male infection than females (56.1 %) and (43.6 %), respectively. The study agreed with [18, 19, 20] but it does not agree with [21], which showed that the intestinal parasite infection was higher in females compared to males. The cause of increase in male infected rate belong to whose visits out clinic & inpatient hospitalized in contrast with number of female & may be due to social habit role in this, such as more care to males than females or due to the males effective more and contact with without environment than females [22]. The current study found that the rate of infection in the lower age groups highest compared to the advanced age groups, the rate of infection in the (1-3) years was (42.4 %) higher than the (10-12) age group the infection rate was (9.1 %). The study agreed with [23], which was conducted in Baghdad, the age range (1-2) years higher rate of infectious - disease to age group (10-12) years with (91.83 %). The study differ with [24] where infection in small age groups from (0-1) years is high by (69.1 percent) In the age group (6-10), parasite infection was highest (75.5 %). The low immunity to various pathogens may be due to this high prevalence as those age groups are relatively less disease-resistant as stated in the previous study [25]. The other cause might be a variety of factors, such as poor health and toileting, overpopulation, low socio - economic status, and weather [26]. The children often try and play anywhere, regardless of suppleness or quality, since there are no different playgrounds. The playgrounds are key sources of disease, because household and industry waste material is thrown there [27].

2) Conclusions

We conclude, eventually, that *E. histolytica* is more of a childhood cause of diarrhea than *G. lamblia*, and that the rate of infection in males was greater than that in females, and that the young age groups were more affected than the advanced age. It is recommended that extra steps should be taken by the Ministry of Health in Iraq to control the complications caused by these two active parasites.

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REFERENCES

- [1] Al-Saeed1, AT., & Issa, SH. Frequency of *Giardia lamblia* among children in Dohuk, Northern Iraq. *Eastern Mediterranean Health Journal*,2006, 12(5): 555-561.
- [2] Hamad, N. R., & Ramzy, I. A.. Epidemiology of *Entamoeba histolytica* among children in Erbil province, Kurdistan Region-Iraq. *J Res Biol*, 2012.2(1), 57-62.
- [3] AL-Shaheen, Z., et al. A study on prevalence of *Entamoeba histolytica* & *Giardia lamblia* infection among patient attending qurna hospital in Basrah. *Bas.J.Vet.Res.*, 2007.6(2):30-36.
- [4] Ouattara, M., et al. Prevalence and Spatial Distribution of *Entamoeba histolytica* /dispar and *Giardia lamblia* among Schoolchildren in Agboville Area (Co^{te} d'Ivoire). *PLoS Negl Trop Dis.*, 2010.4(1):e574.
- [5] Okhuysen,PC. Traveler's diarrhea due to intestinal protozoa. *Clin.Infect. Dis.*,2001.33:110-114.
- [6] Stanley, SL. Jr. Amoebiasis. *Lancet.* ,Minenoa, T., & Avery MA. Giardiasis: recent progress in chemotherapy and drug development. *Curr Pharm Des.* (2003). 361:1025–1034.
- [7] Hotez PJ., et al. Rescuing the bottom billion through control of neglected tropical diseases. *Lancet.* 2009. 373:1570–1575.
- [8] Ravdin, J.I. and Stauffer, W.M. *Entamoeba histolytica* (amoebiasis). In: Mandell, G.L., Bennett, J.E. and Dolin, R., Eds., Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases, 6th Edition, *Churchill Livingstone*, Philadelphia, PA, 2005. 3097-3111.
- [9] Guerrant, J.M. Hughes, N.L. Lima, J. Crane,. Diarrhea in developed and developing countries magnitude, special settings, and etiologies, *Rev. Infect. Dis.*1990.12 ,S41eS50.
- [10] Tinuade, O. John, O. Saheed, O. Oyeku, N. Fidelis, D. Olabisi,. Parasitic etiology of childhood diarrhea, *Indian J. Pediatr.* 2006.73 ,1081e1084.
- [11] Blessmann J, Van Linh P, Nu PA, et al. Epidemiology of amebiasis in a region of high incidence of amebic liver abscess in central Vietnam. *Am J Trop Med Hyg.*; 2002.66(5):578-583.
- [12] R. Haque,. Human intestinal parasites, J. Health Popul. Nutr. Kerkuk City for Years 2009-2014. *European Journal of Pharmaceutical and Medical Research*, 3, 13-19. 25 (2007) 387e391.
- [13] Baban, A. A. Epidemiology of diarrheal diseases in Ramadi area (Iraq) among infants admitted to pediatric hospital in six months. Dip. Comm (Doctoral dissertation, Thesis, Coll. Med. AL-Nahrain Univ. (1992).

- [14] Al-Warid HS. Some factors influencing the prevalence of *Giardia lamblia* and *Entamoeba histolytica* in a sample of patients in North of Baghdad. J Al Nahrain Univ Sci; 2012.15:121-5.
- [15] Yassin MM, Shubair ME, al-Hindi AI, Jadallah SY.: Prevalence of intestinal parasites among school children in Gaza city, Gaza strip. J Egypt Soc Parasitol; 1999.29:365-73
- [16] Hashem, W. H.; Ali, J.K. & Hiessen, A. M. : Prevalence of intestinal parasites among primary schools pupils at Hilla city. J. Techn./Med.Rech. , 1999 .50:17-23. (In Arabic)
- [17] Lechevatiliar, M. W.; W; W.D. Norton and R.G. Lee. Occurrence of *Giardia* and *Cryptosporidium* spp., in surface water supplies, App. Environ .Microbiol., 1991. 57: 2610-2616 .
- [18] AL-Killaby, K.K.A. Epidemiological study for common intestinal pathogens & related with acute diarrhea in children in Najaf governorate. M. Sc. Thesis., Coll. Al- qaid of education for women, Univ. Kufa.: 1999. 126pp.
- [19] AL-Mashhadani, W.S.H. Isolation & diagnosis for some Microbial causes to diarrhea & resistant bacterial isolations to antibiotic & product it of betalactamase enzyme. M. Sc. Thesis., Coll. Sci., Univ. Al-mustansiriya: 2000. 91pp
- [20] Salman, A.O. Epidemiology study to intestinal parasites in infective children with diarrhea & intended two children hospital in Baghdad city. M. Sc. Thesis., Coll. Education (Ibn al-Haytham), Univ. Baghdad.: 2002. 119pp.
- [21] Kadir, M. A.; Kadir, A. A. and Faraj, K. K. Survey study of intestinal parasites among different population of Arbil city. J. Fac. Med. Baghdad 1987; 29: 455-458.
- [22] Mahdi, N.K., AL-Sadoon, I. and Mohamed, A.J. First report of cryptosporidiosis among Iraqi children Eastern Medit Hlth .J. , 1996. 2(1) :115-120.
- [23] Ibrahim, Amjed Q. Prevalence of *Entamoeba histolytica* and *Giardia lamblia* in Children in Kadhmiyah Hospital The Iraqi J. Vet. Med. 2012. 36 (1):32– 36; 2012
- [24] Al-Moussawi, Ahmed M.. Prevalence of Intestinal Parasites Among Rural Population in Babylon Province, College of Dentistry, University of Babylon. 2006.2(4): 491-498.
- [25] Haq, R.; Mondal, D.; Duggal, P.; Kabir, M.; Roy, S.; Farr, BM.; Sack, RB.; and Petri, WA. *Entamoeba histolytica* Infection in Children and Protection from Subsequent Amebiasis. Infect. Immunity, 2006. 74: 904–909.
- [26] Ulukanligil, M. and Seyrek, A. Demographic and socio-economic factors affecting the physical development, haemoglobin and parasitic infection status of school children in Sanliurfa province, Tur. Pub. Health, 2004. 118: 151-158.
- [27] Munazza, E.; Ghulam, M.; Mahmood, A.; Shujaat, AK.; Qazi, NS.; Muhammad, HH. Amir, W.; Kalsoom, F. and Izhar, H. Determination of the prevalence of *Entamoeba histolytica* in human at a private fertilizer company hospital in Pakistan using microscopic technique. African J. of Microbio. Res., 2011. 5(2) 149-152.