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INVESTIGATION OF INTESTINAL PROTOZOAN INFECTIONS AMONG FOOD-HANDLERS IN ERBIL CITY, IRAQ

Ahmed A. Khudhair Al-Daody^{1*}, Fattma A. Ali², Lana Barzan Sadiq³, Asma Samer Mamand⁴, Rawezh Salah Ismail⁵ and Hana Fatah Muhammed⁶

^{1,2,3,4,5,6}Department of Medical Microbiology, College of Health Sciences, Hawler Medical University, Erbil, Kurdistan Region, Iraq,

¹Email: ahmed.akil@hmu.edu.krd, ²Email: Fattima.ali@hmu.edu.krd, ³Email lanabarzan98@gmail.com, ⁴Email: asmashekhany.21@gmail.com, ⁵Email: rawezhsalah200@gmail.com, ⁶Email: hana.fatah43@gmail.com

ABSTRACT

Intestinal protozoa parasitic infection are one of the most common problems in developing countries, the spread of parasitic disease via food handlers is of great importance for maintaining hygienic quality of food prepared and served by them, so the objective of this study was to determine the magnitude of intestinal protozoa parasitic infection and associated factors among food handlers serving in Erbil City food handling establishment.

A total of (10561) samples were examined for detection of intestinal protozoa parasitic infection among food handlers in Erbil City from July (2019) to February (2020) using direct wet mount preparation in normal saline and iodine solution, microscopically examined. The data collected were coded, using specially designed coding system and entered into Microsoft Excel sheets, and then imported to a data management solution, statistically package for social sciences (SPSS).

Out of (10561) stool samples examined of food handlers revealed that 769 (7.3%) were positive for intestinal protozoa parasitic infection by direct microscopic examination. The predominant had one intestinal parasite 766 (99.61%) and 3 (0.39%) of food handlers have been diagnosed with double intestinal parasites. *Entamoeba histolytica* was the most prevalence parasite 619 (80.1%), followed by *Giardia lamblia* 152 (19.8%) and *Hymenolepis nana* 1 (0.1%). The rate of intestinal protozoa infection among females 102 (7.9%) was higher than males 667 (7.2%), in spite of statistically there was non-significant difference. The highest rate of infection was observed among 10-19 year age groups and the lowest rate was among ≥ 60 age groups. According to months the prevalence of intestinal protozoa parasitic infection, showed significant differences and the highest rate of infection was recorded in September (10.3%) and the lowest rate was observed in October and November (6.2%).

The high prevalence of intestinal protozoa parasitic infections in this study highlights the importance of food handlers are probable sources of parasitic infections. We also concluded that the rate of infection was affected by age, months and the diagnostic technique used in hospitals and laboratories.

Keywords: Protozoa, Food handler, Prevalence, Food-borne disease, Erbil, Iraq.

Introduction

Intestinal protozoan parasitic infections are of global public health concern Thomas *et al.* (2015). It continues to be an important cause of morbidity and mortality in the developing countries Babiker *et al.* (2009). Each year millions of people suffer or die due to food-borne disease. However, the harmful outcomes of food-borne outbreaks are more severe in developing regions than anywhere else this due to the missing of proper intervention strategies and poor levels of nutrition in these regions Habiballah *et al.* (2017). Most of the intestinal parasites of medical importance are known to be transmitted by ingestion of food or water contaminated with infective stages of these parasites Damen *et al.* (2015). Often parasitic infections are asymptomatic or have mild and non-specific symptoms, which makes difficult the diagnosis and treatment Colli *et al.*, (2013).

Some jobs may facilitate the transmission of parasitic infection such as food handler worker which deals with food Hengami *et al.* (2018). Infection of food handlers with

intestinal pathogens, including parasites could be a potential cause for the spreading of these pathogens to the people (the customers) or the surrounding community Wakid *et al.* (2009). The health of food handlers is of great importance for maintained hygienic and inadequate knowledge on food safety could be potential source of infection of many intestinal protozoa Wadilo *et al.* (2016). The role of food handlers, as the final agent of product provider, is very important because they can make unsafe and hazardous food for consumption Balarak *et al.* (2016). The food handling personnel play a vital role in the transmission of food-borne disease Wadilo *et al.* (2016).

A food-borne disease has been affecting large number of people and is an improper public health problem Solanki *et al.* (2014). Many outbreaks of diarrhea and vomiting also occur by eating raw foods, raw material composition of foods, or foods from unsafe sources Schlipkoter and Flahault *et al.* (2010), Newell *et al.* (2010). Compared with the rest part of the hand, fingernail, due to the surviving of

microorganism, is filthier than other parts which are common more difficult to keep them clean Hengami *et al.* (2018). In this group asymptomatic carrier act as continuous sources of infection within public leading to problems in control of parasitic intestinal infection Motazedian *et al.* (2015). The causative agent of gastrointestinal parasitic infection includes protozoa, protozoa are microscopic, one-celled eukaryotic organisms that can be found in nature as parasitic or free living, and classification of protozoa according to their morphology and movement include flagellates, ciliates and amoeba Al-khayat *et al.* (2017). Numerous protozoa inhabit gastrointestinal tract of human. This list includes representative from many divers' protozoa groups. The majority of these protozoa are non-pathogenic commensals or only result in mild disease under certain circumstance Egbuobi *et al.* (2014).

However, the disease causing parasites may produce serious infections and occasionally can harm their hosts through injury or illness or may cause death (Kassani *et al.*, 2015; Asires *et al.*, 2019). It has been indicated that high intensity infections with intestinal parasites and other food-borne diseases would negatively affect the health status of the individuals. Among those negative sequels, it may worth to mention malnutrition, anemia, stunting and cognitive impairment altogether interfering with the overall productivity (Girma *et al.*, 2017). The most common protozoan reported to lead to digestive disorders include *Giardia lamblia* and *Entamoeba histolytica* (Babiker *et al.*, 2009). intestinal protozoa tend to exhibit similar life cycle consisting cystic and trophozoite stage (Egbuobi *et al.*, 2014).

According to national food safety standard all food handlers should be referred to a health center medical diagnostic laboratory in order to check for intestinal parasitic infection prior to receiving their health certificate (Hengami *et al.*, 2018). Fecal sample was collected from each food handler in a sterile, dry container and transported to the laboratory within one hour of collection Solanki *et al.* (2014). Each stool specimen was examined macroscopically for detecting the color, consistency, the presence of the mucus and blood and any trophozoite and cyst Amer *et al.* (2018). Microscopically several methods used for detection of protozoa infection including saline wet mount and staining test for trophozoites and cysts observation in stool sample of patient Al-Khayat *et al.* (2017). Saline direct smear is used mainly for detection of motility of intestinal protozoan trophozoites, which are seen in liquid or semi liquid specimens. Iodine direct smear shows the characteristic feature of the diagnostic stage in more detail Wakid *et al.* (2009). In the case of light infection which is the number of parasites in the stool specimen is low, examination of direct wet mount may not reveal them and hence the stool should be concentrated, cysts can be recovered after the concentration procedure, whereas trophozoites can get destroyed during this procedure, this makes a direct wet mount examination obligatory as the initial phase of microscopic examination Santosh, (2012).

Numerous Studies in the world have been carried on the prevalence of intestinal parasite among food handlers Motazedian *et al.* (2015). Globally, 3.5 billion people are affected with intestinal parasitic infections, in which 450 million are symptomatic and more than 1.2 million deaths occurred annually Damen *et al.* (2015). The aims of the study

were to assess the prevalence of intestinal protozoal infections among food handlers, distribution of protozoal infection according to months, distribution of protozoal infection in both males and females, and to detected single and mix infection.

Materials and Methods

Study area

The study was conducted among food handlers in the various categories of sources of food within Erbil city and it was collected from July 2019 to February 2020.

Study Population

The study was carried out on food handlers working in restaurants, cafeterias, hotels, bakers and food factories. A total sample were taken from (10561) food handlers, (9270) males and (1291) females from different age groups were randomly chosen to be included in this study.

Sample collection

Each of the recruited subjects was given a clean, dry, leak-proof, wide mouthed, and well labeled by identification code, container with screwed cup. They were instructed on how to collect their stool samples into the containers Egbuobi *et al.* (2014) Damen *et al.* (2015).

Macroscopic Examination

Generally, macroscopic reports of the stool samples were given in terms of color, consistency, odor, texture, presence of blood and mucus Solanki *et al.* (2014).

Microscopic Examination

In order to detect the intestinal parasites, different methods were used: routine direct fecal examination, staining by lugol's iodine solution and direct smear with saline Motazedian *et al.* (2015), were used to detect the protozoan parasite Amer *et al.* (2018). Generally, direct smear is cheap, easy, and the best simple way for detection of microscopy cellular exudates including RBC's and WBC's and mucus Wakid *et al.* (2009). The sample was processed immediately without preservation for saline direct smear, using (0.85%) normal saline for detecting the actively motile intestinal protozoan trophozoites, which are seen in liquid or semi-liquid specimens Al-Saeed and Issa (2010). Which performed by emulsifying about 1mg of stool by clean, wood stick, the fecal specimen was touched in various sites especially were streaks of blood or pus were noticed, the mixed thoroughly with each drop of normal saline on microscopic slide, then covered with a cover slip Hussein *et al.* (2016). The same procedure used for iodine, which is used for staining the nucleus of protozoan parasite which gives the ability to diagnosis by their nuclear character. The smear was scanned microscopically under low and high (x10 and x40) objective lenses Wakid *et al.* (2009).

Statistical Analysis

The Statistical Package for the Social Sciences (SPSS) program will use to analysis data in this Statistical analysis was done using the statistical package for the social sciences (SPSS v.23) program, with statistical significance (P) value < 0.05 and also with non-significance (P) value > 0.05 the date was analyzing using "Independent sample T-test" and "Chi-square".

Results

Total prevalence of intestinal protozoa among food handlers

A total of (10561) fecal samples were examined for intestinal protozoa in this study by direct microscopic examination. The overall percentage of infection was (7.3%), while the remaining (92.7%) were negative as seen in Figure [1].

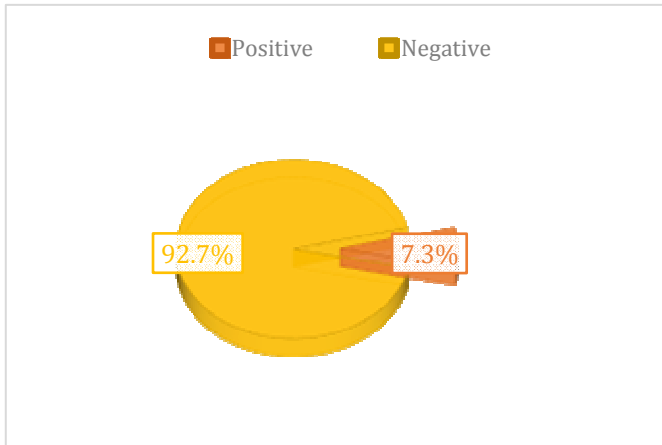


Fig. 1 : Total prevalence of Intestinal protozoa among food handlers

Prevalence of intestinal protozoa in relation to gender

As shown in Table [1], in total (10561) samples, (9270) samples were taken from males and (1291) samples were from females. The higher prevalence rate of intestinal protozoa infection was observed among females (7.9%) than males (7.2%). The difference was statistically not significant (p -value= 0.361).

Table 1: Prevalence of intestinal protozoa infection in relation to gender

Gender	No. +ve	%	No. -ve	%	Total
Male	667	7.2	8603	92.8%	9270
Female	102	7.9	1189	92.1%	1291
Total	769	7.3	9792	92.7%	10561
P. value	0.361				

Prevalence of Intestinal Protozoa according to age group

As shown in Table [2], the highest proportion (8.1%) of intestinal protozoal infection were found in the age group of 10-19 years followed by (7.9) in the age group 30-39 years (7.1) in the age group 20-29, (6.8) in the age group 50-59, (6.2) in the age group 40-49, and the lowest proportion (3.4%) of infection were found in the age group of ≥ 60 . The difference was statistically not significant (p -value= 0.145).

Prevalence of intestinal protozoa infection according to month

As shown in Table [3]. Highest rate of infection was observed in September (10.3%) while the lowest rate was observed in October and November (6.2%). These differences were shown to be statistically highly significant with a p -value of 0.001.

Table 2: Prevalence of Intestinal protozoa according to age group

Age	No. -ve	%	No. +ve	%	Total
10-19	1254	91.9	111	8.1	1365
20-29	4792	92.9	368	7.1	5160
30-39	2,319	92.1	198	7.9	2517
40-49	911	93.8	60	6.2	971
50-59	372	93.2	27	6.8	399
≥ 60	144	96.6	5	3.4	149
Total	9792	92.7	769	7.3	10561
P. value	0.145				

Table 3: Prevalence of Intestinal protozoa infection according to the months of study

Month	No. +ve	%	No. -ve	%	Months	No. +ve	%
July	101	7.5	1246	92.5	Hot	310	8.7
August	93	8.5	1000	91.5			
September	116	10.3	1009	89.7	Cold	459	6.6
October	122	6.2	1845	93.8			
November	115	6.2	1729	93.8			
December	101	6.9	1368	93.1			
January	121	7.1	1595	92.9			
Total	769	7.3	9792	92.7		769	7.3
P. value	0.001						

Types of intestinal protozoa parasitic infection in this study

The most predominant species of the protozoan parasites were *Entamoeba histolytica* 80.1% (619/769) and *Giardia lamblia* 19.8% (152/769) as shown in Figure [2]. Meanwhile, only one egg of *Hymenolepis nana* 0.1% (1/769) was detected in food-handlers in this study.

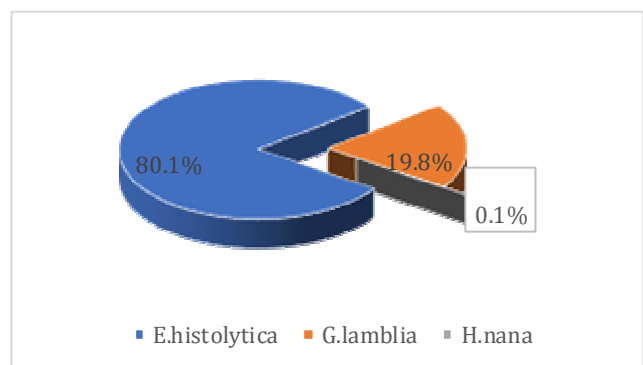


Fig. 2: Types of intestinal protozoa parasitic infection

Prevalence of Single and Double parasitic infection

Stool examination of food handlers revealed that 766 (99.61%) had one and 3 (0.39%) food handlers have been diagnosed with mixed intestinal parasites as shown in Table [4]. Statistically these differences were significant with a p -value of 0.0001

Table 4: Prevalence of Single and Double parasitic infection

Type of infection	No. +ve	%
Single infection	766	99.61
Mixed infection	3	0.39
Total	769	100
P. value	0.0001	

Types of Single and Double parasitic infection

As shown in Table 5 the highest prevalence of intestinal parasites was recorded in single infection. The most common pathogenic parasites associated in people harboring double infections were between *Entamoeba histolytica* and *Giardia lamblia* followed by *Giardia lamblia* and *Hymenolepis nana*.

Table 5: Types of Single and Double parasitic infection

Single infection	No. +ve	%
<i>Entamoeba histolytica</i>	617	80.23
<i>Giardia lamblia</i>	149	19.37
Double infection		
<i>Entamoeba histolytica</i> + <i>Giardia lamblia</i>	2	0.26
<i>Giardia lamblia</i> + <i>Hymenolepis nana</i>	1	0.13
Total	769	100

Discussion

Parasitic infections must receive increasing attention for their responsibility to cause parasitic diseases. The world Health Organization regards illness due to contaminated food as one of the most widespread health problems in the contemporary world Sheth and Dwivedi, (2006). Infection of asymptomatic person of intestinal protozoa are a particular public health hazard, especially workers dealing with food (food handlers), could become a potential cause of dissemination of variety of pathogens including intestinal protozoan parasites. Intestinal parasitic infections are one of the problems that affect human health, especially in developing countries Wadilo *et al.* (2016).

In this study, the overall prevalence of intestinal protozoa infection among food handlers in Erbil city. During the period of our study that we did in this city in Central Lab, we collected a total of (10561) samples among males and females during seven months from July 2019 to January 2020 (769) samples were positive (7.3%). Several studies agree and disagree with our study as following: Similar result was observed by previous studies, which were done in United Arab Emirates (7.7%) by Dash *et al.* (2010), and in Libya (8.28%) by (Kubti *et al.*, 2011), Whereas higher results were recorded in Erbil (26.58%) by Faraj and Koyee, (2012), and in Ethiopia (14.8%) by Alemu, *et al.*, (2019). and lowest rates were recorded in Iran (3.73%) by Balarak *et al.* (2016), in Baghdad (1.41%) by Al-khayat *et al.* (2017), and in Saudi Arabia (0.5%) by Amer *et al.* (2018). The existence of such variations may be explained by the differences in practices of personal hygiene, environmental sanitation, health promotion practices, geographical location and type of diagnostic sensitivity Marami *et al.* (2018).

Higher rate of infection was recorded among females (7.9%) than male (7.2%) (Table 1) and this difference was statistically non-significant (P. value =0.361). Similar results were obtained by other studies carried out in Saudi Arabian by (Amer *et al.*, 2018), while other studies like that of Negeria by (Damen *et al.*, 2015) and in Brazil by (Brauer *et al.*, 2017) have shown significant difference between female and male positive rates of intestinal protozoa parasitic infection. These finding may be due to that male and female did not have the same chance to be exposed to intestinal protozoa, some high risk habits such as male do not look for medical assistance, therefore the males have less chance for the detection of positive result among them. Also may be due to the fact woman are much more involved in kitchen work than men. Most of the males participate in the selling of the prepared food, while women are those who do the washings of vegetables and fruits Egbuobi *et al.* (2014).

In the present study we observed that the highest prevalence of intestinal protozoa was found among 10-19 years (8.1%), while the age group of ≥ 60 years had the less prevalence of (3.4%) (Table 2). similar results were observed by other studies carried out in Iran by Motazedian *et al.* (2015), also in Nigeria by (Damen *et al.*, 2015) and Ethiopia by Alemu *et al.* (2019). Our study was disagreed with results reported by a study done in Sudan by Babiker *et al.* (2009). These differences may be due to this age group having dirty and untrimmed nails, or having the habit of nail biting, also wearing jewelry and ignore washing their hands or keep it clean; in addition, these age groups widely work in crowded areas mostly such as cafeterias and restaurants.

The epidemiology of intestinal protozoa parasitic infections is associated with climate, our study revealed the higher prevalence 10.3% (116/1125) was found in hot season than in cold season 6.6% (459/6996), so there is a significant difference between intestinal protozoa parasitic infection and season (P. value =0.0001) (Table 3). Which might be due to the fact that Iraq is one of the countries that have long hot dry summer and short cold rainy winter. Hot and dry weather encourages parasitic infection; also the population characteristics play a role, such as swimming in rivers and lakes in hot weather increases the susceptibility for infections Stuart *et al.* (2003). Consumption of fresh vegetables which are an important part of Iraqi diet such as Lettuce, Leek and Celery, without good washing can play a major role in infections Al-khayat *et al.* (2017).

The current study results showed that the highest prevalence was by *Entamoeba histolytica* (80.1%), followed by *Giardia lamblia* (19.8%) and the lowest (0.1%) by *Hymenolepis nana*. Similar results were obtained in Ninevah Governorate by AL-Daoudy and Rahemo (2002), in Yemen by (Baswaid and Al-Haddad, 2008), also in United Arab Emirates by Dash *et al.* (2010) and Southern Iran by Hengami *et al.* (2018). While other studies disagree with our results, like that of Tunis by Siala *et al.* (2011), also in Jordan by Abdel-Dayem *et al.* (2014) and in Baghdad province/Iraq by Al-khayat, *et al.* (2017). The differences in prevalence of these intestinal protozoal parasite infections between these studies, maybe due to several factors, including environmental, nutrition, socio-economic and geographical conditions, as well as demographic and health-related behavior.

In our study we observed the prevalence of intestinal protozoa parasitic infection was higher among single infection than double infection. In previous studies also shown the similar results done in Ninevah Governorate by AL-Daoudy and Rahemo, (2002), in Erbil City by Faraj and Koyee, (2012), in South Ethiopia by Wadilo *et al.* (2016), and in East and West Ethiopia by Asires *et al.* (2019). These differences maybe, due to poor personnel hygiene and low diagnostic sensitivity. Among double infection the prevalence by two protozoa was higher than the protozoa with helminths, this outcome may result because the life cycle of protozoa is simpler than the helminths so it is easier to introduce infection.

Conclusions

In our study we concluded that: The incidence of intestinal protozoa infection among food handler was high in Erbil City, the highest incidences were among 10-19 years. Higher incidences were recorded in hot weather than in cold weather.

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