

Epidemiological Study of *Entamoeba histolytica* among Children in Kirkuk Province, Iraq

Jingeez A.Hameed , Omaima I. Mahmood , Adnan F.Al-azawy

Microbiology Department, College of Veterinary Medicine, University of Tikrit, Tikrit, Iraq.

Microbiology Department, College of Veterinary Medicine, University of Tikrit, Tikrit, Iraq.

Biology Department, College of Science, University of Tikrit, Tikrit, Iraq.

jingeezpasha@gmail.com.

Abstract

The current study included the microscopic diagnosis of *Entamoeba histolytica* parasite, where (140) stool samples were collected from children ranging in age from one to fourteen years with diarrhea from the Children's Hospital in Kirkuk from September 2020 to August 2021 using direct examination methods. The percentage of total infection was (23.57%), and the highest percentage of infection was recorded in the category (5-10) years with a percentage of (36.58%), and there were no significant differences between male and female infection, while there was a clear difference in the percentage of infection according to the different seasons of the year. Where the highest rate of injuries was recorded in the summer months (June, July, August, September) at 33.33%, 45%, 33.34%, 35.7%, respectively.

Keywords: Diarrhea

Introduction

Entamoeba histolytica was discovered by Feder Losch for the first time in 1875 when treating a person with bloody diarrhea, where he noticed the vegetative phase of the parasite in the patient's stool and because it is present in the colon, it was called Amoeba coli, but its relationship to bloody diarrhea was not confirmed until 1887 by Kartulis (Kockwell, 2002).

Entamoeba histolytica parasite exists in the human body in two forms, the trophozoite and the cyst, both of which cause infection. The life cycle of the parasite includes the following stages: the vegetative stage, the pre-cysts stage, the cysts stage, and the post-cysts stage. The transition between the stages is affected by two factors, the availability of food and the surrounding environment (Arredondo *et al.*, 2014), Excitation phase is release of the vegetative phase from the cystic phase, which has the ability to invade tissues. And cause infection to new host (Junaidi *et al.*, 2020)

Besides humans, some mammals can be infected with the parasite, such as dogs and cats, as well as mice and pigs, but it is not possible to develop infection with the parasite from humans to animals, and this is due to the characteristics of the parasite, which is rarely found in the lumen of the intestines of animals (Regan *et al.*, 2014; Schuster and Visvesvara, 2004).

the pathogenesis can be described into three steps, firstly, host cell death, secondly inflammation, and thirdly the parasite invasion. Adherence is carried out by the parasite's adhesion molecule Gal/GalNAc lectin. The vegetative phase is able to kill host cells using several mechanisms, including stimulating programmed cell death, and phagocytosis (Ralston and Petri Jr, 2011). Most tissue amoeba infections are asymptomatic, only about 10-20% of cases develop into a symptomatic infection. There is no explanation for this case, but it is believed that there are several factors related to the parasite, the host and the environment (Marenga *et al.*, 2019). The incubation period of the disease ranges from 7 to 28 days after exposure, so the symptoms may be mild in the form of abdominal cramps, diarrhea that progresses to bloody diarrhea (Petri *et al.*, 2012).

The most common clinical forms of dysentery are:

Intestinal amoebiasis

The infection is only in the intestine and includes:

Acute amoebic dysentery: Acute intestinal amoebiasis, the disease begins when the vegetative phase of the parasite attaches to the epithelial cells of the colon and then produces its lytic enzymes that cause inflammation in the intestines. The presence of the vegetative phase stimulates the intestinal cells to produce the enzyme Cyclooxygenase-2 (COX-2), which in turn leads to Increased production of Prostaglandin, which leads to stimulating the inflammatory process. The vegetative phase also secretes certain peptides called Amoebapores, which cause lysis of cells in the lining of the intestine. Also, lysis of cells occurs as a result of apoptosis by the vegetative phase, and thus ulcers that develop abnormally and may rupture, causing massive bleeding that threatens the patient's life (López *et al.*, 2015). The most important pathological symptoms accompanying this case are diarrhea with a lot of mucus and blood in the stool, abdominal pain, nausea, vomiting, weakness, bloating, and high body temperature. Electrolyte solutions balance, and in extreme cases, cardiovascular instability and fainting. Persistent diarrhea is considered a serious danger, especially for children and infants, because it leads to dehydration and an acid-base imbalance in the body (Bansal *et al.*, 2015; Nowak *et al.*, 2015).

Chronic intestinal amoebiasis: The acute phase (if not treated) can develop into a chronic state, which is characterized non-bloody diarrhea and constipation with symptoms of varying severity, chronic (Nowak *et al.*, 2015)

Material and Methods

Microsocial examination:

140 stool samples were collected from children attending the Children's Hospital in Kirkuk city who suffer from severe diarrhea and diarrhea accompanied by blood for the period from the beginning of October 2020 until the end of October 2021.

The direct smear method was used by taking a quantity of stool (especially mucous from it to increase the possibility of the parasite's appearance) by means of a wooden stick and placing it

on the glass slide (microscopically slide) and adding a drop of glass physiological solution and a drop of iodine dye, and then Examine the slide under the microscope.

Statistical Analysis

All samples in this study were subjected to statistical analysis to find out the significant differences for the studied variables using (chi-squer) and the significant differences were determined at the probability level ($P \leq 0.05$ (Schiefer, 1980).

Results & Discussion

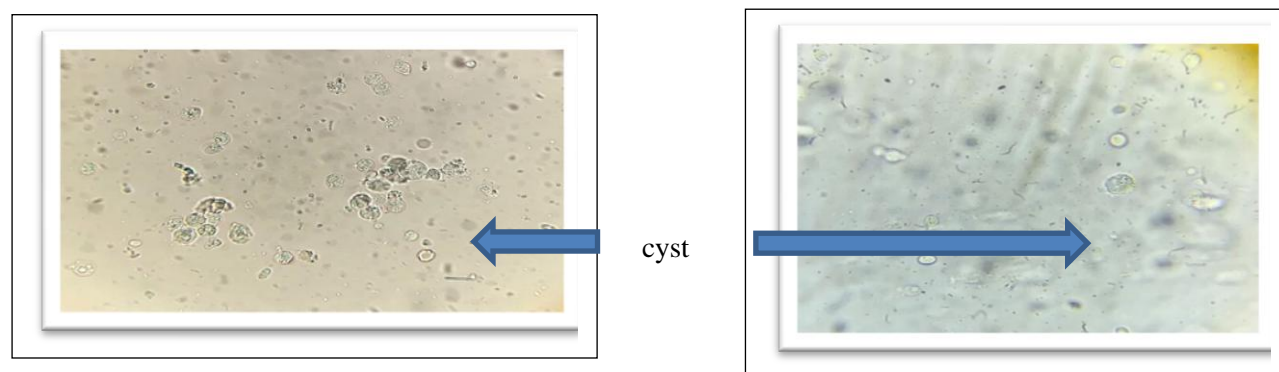
The percentage of infection with the *Entamoeba histolytica* parasite of the samples examined by direct swab

140 fecal samples were examined for children attending the Children's Hospital / Kirkuk using the direct swab method, and the number of positive infections was (33) (27.57%) as shown in the tab.1

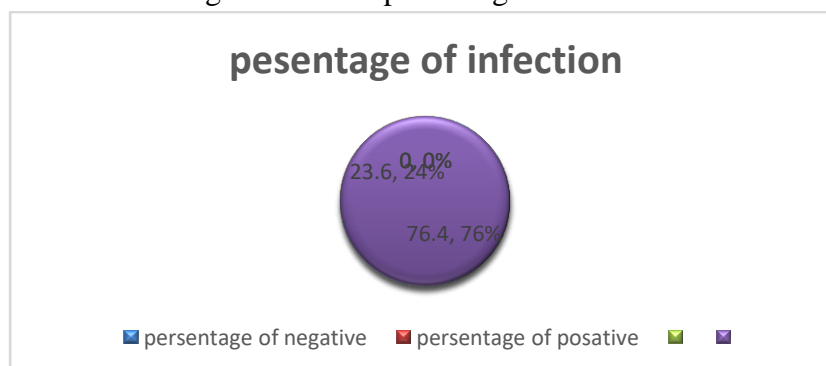
Tab.1. percentage of samples positive for *Entamoeba histolytica* infection

No. of samples	No. of positive	No. of negative
140	33 (%23.57)	56107 (%76.4)

Fig.1. Direct swab of faces shows *Entamoeba histolytica* cysts under a force of 40 X



Feg.2. show the percentage of infection



The percentage of the current study was close to what was reached by Jaleel (2021) in Basra city (21.19%), Hussein (2016) (26.41%) in Dhi Qar city and Al-Emam (2015) (23.37%) in Babil city. While the percentage was lower than what was reached by jaffar (2021) in Wasit city with percentage (47.7%), Al-zubadi (2021) 34.3% in the city Najaf and Al-Tufaili (2020) (43.29%) in Kufa and Surra (2018) (76.82%) in Maysan City, Alabdullah (2017) (46.6%) in Erbil and dhok city and Al-saqur (2017) in all of Iraq (45.40%) and (2012) Hamad with a percentage (30%) in Erbil city.

As for the infection rate in neighboring and Arab countries, the percentage was lower than what was found by Mezeid (2021) by 28.5% in Palestine, Qureshi (2018) by (23.1%) in Pakistan, Dincer (2017) (31%) in Jordan and Amir Saeed (2015) with a percentage of 32% in Sudan and Al Laham (2015) with a percentage of (43.8%) in Palestine. The percentage was higher than what was recorded by kadir (2018) with a rate of 9.7% in the city of Tikrit.

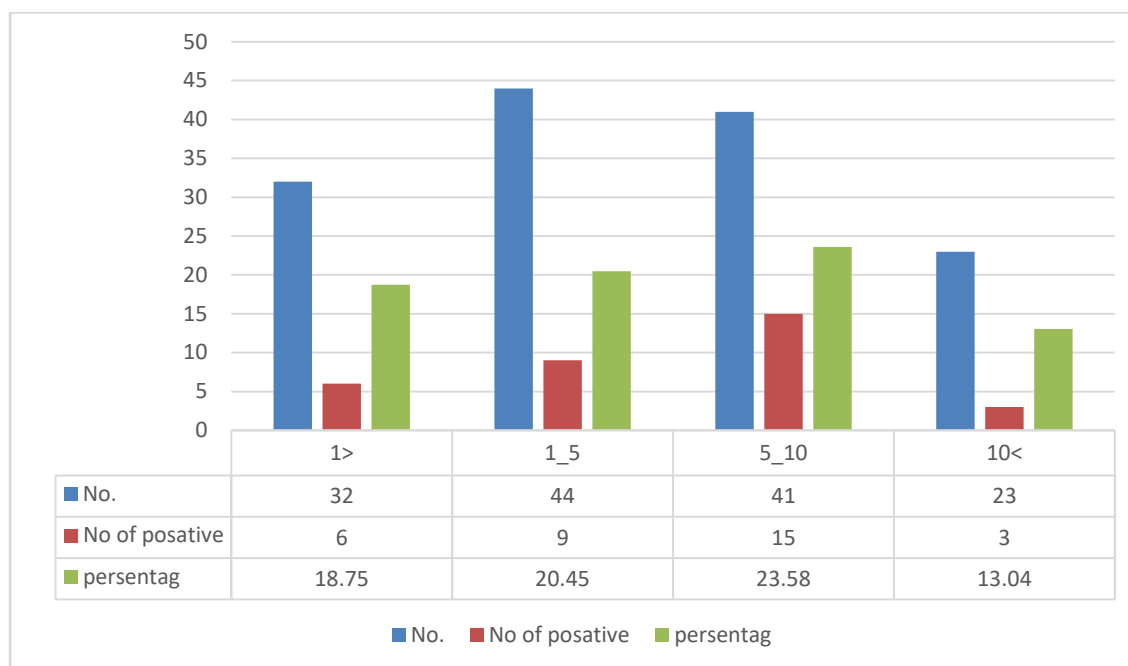
The difference in the rates of infection recorded in the current study and previous studies may be due to the difference in the level of personal hygiene, sanitation, geographical location, climatic conditions, the level of poverty, style of living conditions and the availability of clean water supplies (Nath *et al.*, 2015; Zeb *et al.*, 2018). Where it was observed that people in crowded places with inappropriate health and environmental system are more susceptible to infection than others (Al-Taei, 2019) and different methods of diagnosis (Mezeid *et al.*, 2021), sample size and study duration (Dincer *et al.*, 2017). Climatic conditions and large population numbers all play important roles in infection (Dhubyan Mohammed Zaki, 2021).

Age relationship

Tab.2. percentage of infection with *Entamoeba histolytica* according to age

Age	No.	No.of posative	percentage %
> 1	32	6	18.75%
5 - 1	44	9	20.45%
5 - 10	41	15	36.58%
10 <	23	3	% 13.04
المجموع	140	33	%23.57
Chi-Square = 5.920		P-Value = 0.046	

Fig.3. percentage of infection with *Entamoeba histolytica* according to age



The results of Table.2 showed that there were no significant differences, as the highest rate of infection was recorded for the age group (1-5) years, at a rate of 46.5%, and this percentage was close to what was recorded by Taei (2019) in the province of Babylon, the highest percentage for the age group (1-10).) with percentage 44.35%, and Hamad (2012) in Erbil city, where the infection rate was higher in the age group (4-6 years)

In the Arab and neighboring countries, Qureshi (2018) in Pakistan recorded the highest infection rate for the age group (4-6) years, while Al Laham (2015) in Gaza recorded the highest infection rate for four-year-olds with (43.6%).

While this study contradicted what was recorded by Mohammed (2021) in Mosul city, where he recorded the highest infection rate in the ages of less than a year (49.9%) and Jaffar (2021), where he recorded the highest infection rate for the age group (1-5) years 23.9%. Majeed (2019) in Wasit City, the highest rate of infection in the age group (21-40) years

In the Arab countries , Al-Jawabreh (2019) in Palestine recorded the highest incidence of infection in children under four years of age, and Al-Areeqi (2017) in Yemen mentioned the highest incidence of infection for the age group (21-30) years.

The results indicate that the parasite is more prevalent in younger age groups because children have less resistance compared to adults. Parasitic infections are mostly found in younger people who are exposed to crowded environment such as school, playgrounds, etc., lack of awareness and lack of personal hygiene (Zeb *et al.*, 2018). In addition, these groups of children are completely independent in toilet use and participate more in both outdoor activities and nutrition. In addition to their low level of education (Hamad and Ramzy, 2012) .

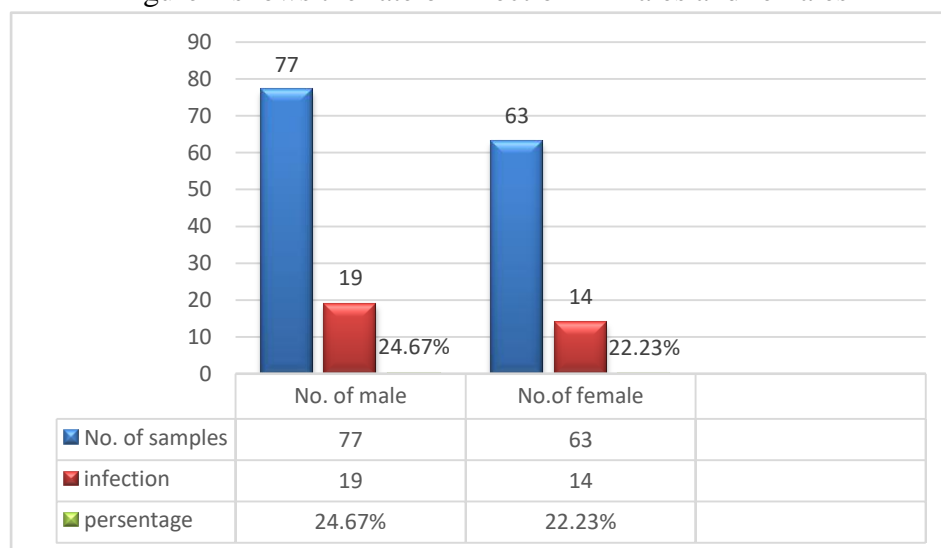
Relationship to infection with sex

Tab. 3. percentage of infection with *Entamoeba histolytica* according to age sex

sex	No.	No of positive	percentage
male	77	19	%24.67
female	63	14	%22.23
total	140	33	%23.57
ns		Chi-Square = 0.116	P-Value = 0.734

The results of Table 3 showed the variation in the infection rates between female and male, and there was no significant differences ($P \geq 0.05$) in the percentage of infection between the sexes, according to the statistical analysis, and the percentage of male infection is 19 (24.67%) of the total number of males 77, and in females the infection rate was 14 (22.23%) of the total number of females 63.

Figure 4 shows the rate of infection in males and females



This result was close to what reported by , Al-Tufaili (2020) in Kufa, Ahmed (2020) in Mosul, and Majeed (2019) in Wasit.

In the Arab and neighboring countries, the results were similar to those recorded by Alanazi (2017) in Saudi Arabia, Al-Areeqi (2017) in Yemen, and Qureshi (2018) in Pakistan. While this study differed from Jaffar (2021) in Wasit city, hamad (2012) in Erbil city, and Mezeid (2021) in Palestine that females are infected more than males.

Males are usually more susceptible to infection with parasites than females because they have a lower immune response compared to females. (Zeb *et al.*, 2018) and slight differences between the sexes may be attributed to the methodology and some difference in the personal and public health service as well as environmental factors, demographic, climatic (Bazzaz *et al.*, 2017).

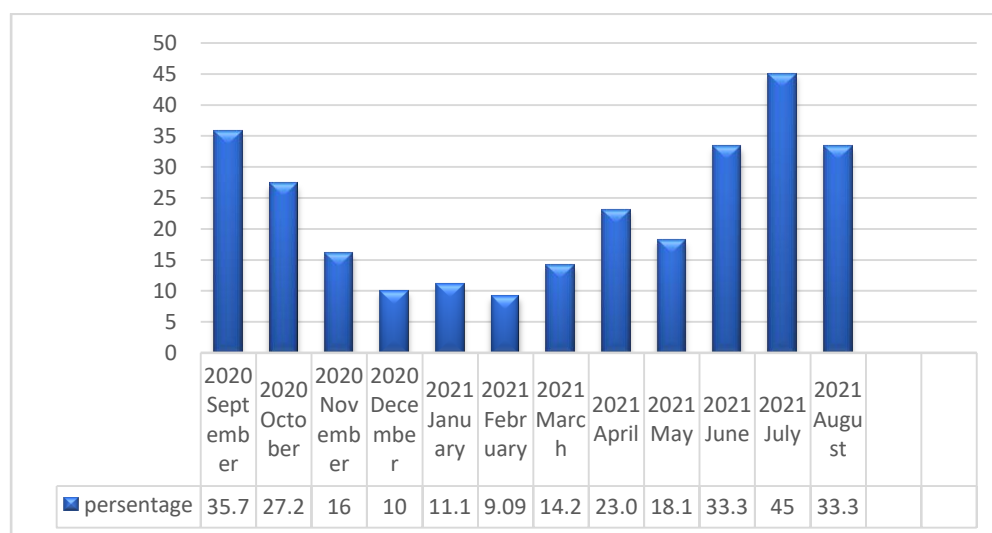
The relationship of infection to the months of year

Tab.4 shows the percentage of *Entamoeba histolytica* according to months of year

Month	العدد	العينات الموجبة	% النسبة المئوية
2020 September	14	5	35.7%
2020 October	11	3	27.27%
2020 November	12	2	16%
2020 December	10	1	10%
2021 January	9	1	11.11%
2021 February	11	1	9.09%
2021 March	14	2	14.2%
2021 April	13	3	23.07%
2021 May	11	2	18.18%
2021 June	12	4	33.33%
2021 July	11	5	45%
2021 August	12	4	33.34%
ns	Chi-Square = 9.668		P-Value = 0.560

The results of Tab.4. showed that there were no significant differences in infection between the months of the year according to the statistical analysis, where the highest rate of infection was recorded in the summer in months (July, August, September, October) at rates (45%, 33.34%, 35.7%, 27.27%).

Fig.5. shows the percentage of infection for each month



This result was similar to Mohamed (2020) in Erbil city and Nassar (2019) in Karbala and Tikrit cities, and a (2018) in Maysan city recorded the highest infection rate in July and June with a rate of (92.73%, 90.87%) respectively. Bazzaz (2018)) in Samarra city, Hussein (2016), in Dhi Qar city and Al-Emam (2015), where the highest infection rate was recorded in September with 41.93% in Babil city.

In the Arab and neighboring countries, Alanazi (2017) in Saudi Arabia, Dincer (2017) in Jordan, Abd-Aldayem (2014) in Jordan and Qureshi (2018) in Pakistan.

The increase in the infection rate was during the summer months due to the availability of suitable environmental conditions for the growth and spread of *Entamoeba histolytica* (Bazzaz et al., 2017), in addition to the fact that the cystic stage of the parasite does not tolerate low temperatures (Hamad and Ramzy, 2012) and the spread of flies that help contaminate food with bags (Flaih et al. ., 2021).

Conclusions:

E.histolytica is one of the parasites that causes diarrhea in children in Kirkuk city , Climate change and seasons have a severe impact on the spread of *E. histolytica* ,Young ages are more infected with *E.histolytica* . Infection with *E.histolytica* is more prevalent compared to other Entamoeba spp.

Reference

1. Abdel-Dayem, M., Al Zou'bi, R., Hani, R. B., & Amr, Z. S. (2014). Microbiological and parasitological investigation among food handlers in hotels in the Dead Sea area, Jordan. *Journal of microbiology, Immunology and Infection*, 47(5), 377-380.
2. Ahmed, N. M., Al-Niaeemi, B. H., & Dawood, M. H. (2020). Epidemiological and clinical aspects about the endemic intestinal parasites in reviewers of Mosul General

- hospitals and healthcare centers/Nineveh governorate-Iraq. *EurAsian Journal of BioSciences*, 14(2).
3. Al Laham, N., Elyazji, M., Al-Haddad, R., & Ridwan, F. (2015). Prevalence of enteric pathogen-associated community gastroenteritis among kindergarten children in Gaza. *Journal of biomedical research*, 29(1), 61.
 4. Al Saqur, I. M., Al-Warid, H. S., & Albahadely, H. S. (2017). The prevalence of *Giardia lamblia* and *Entamoeba histolytica*/dispar among Iraqi provinces. *Karbala International Journal of Modern Science*, 3(2), 93-96.
 5. Alanazi, A. (2017). The prevalence of intestinal parasitic protozoan among patients in Ad-Dawadimi General Hospital, Saudi Arabia. *Tropical biomedicine*, 34(2), 453-60.
 6. Al-Areeqi, M. A., Sady, H., Al-Mekhlafi, H. M., Anuar, T. S., Al-Adhroey, A. H., Atroosh, W. M., ... & Surin, J. (2017). First molecular epidemiology of *Entamoeba histolytica*, *E. dispar* and *E. moshkovskii* infections in Yemen: different species-specific associated risk factors. *Tropical Medicine & International Health*, 22(4), 493-504.
 7. Ali, J. K. (2015). Prevalence of *Entamoeba histolytica* and *Giardia lamblia* parasites among patients attending Al-Emam Ali hospital in Al-Mashrooh province, Babylon. *Kufa J Vet Med Sci*, 6, 30-4.
 8. Al-Jawabreh, A., Ereqat, S., Dumaidi, K., Al-Jawabreh, H., Abdeen, Z., & Nasereddin, A. (2019). Prevalence of selected intestinal protozoan infections in marginalized rural communities in Palestine. *BMC public health*, 19(1), 1-11.
 9. Al-Zubadi, W. F., Al-Masoudi, H. K., & Abdul-Lateef, L. A. (2021). Detection and Sequencing of Iron Superoxide Dismutase Gene in *Entamoeba histolytica* Isolated from Patients with Diarrhea in Iraq. *Archives of Razi Institute*, 76(5), 1165-1171.
 10. Bansal, A., Bansal, A. K., Bansal, V., & Kumar, A. (2016). Liver abscess: catheter drainage v/s needle aspiration. *International Surgery Journal*, 2(1), 20-25.
 11. Bazzaz, A. A., Shakir, O. M., & Alabbasy, R. H. (2017). Prevalence of two gastrointestinal parasites *Entamoeba histolytica* and *Giardia lamblia* within Samarra city, Iraq. *Advances in Bioscience and Biotechnology*, 8(11), 399-410.
 12. Dhubyan Mohammed Zaki, Z. (2021). The Prevalence of *Entamoeba histolytica* and *Giardia Lamblia* Associated with Diarrhea among Children in Ibn Al-Atheer Hospital in Mosul City-Iraq. *Archives of Razi Institute*.
 13. Dincer, M., Kahveci, K., Doger, C., & Yarici, A. K. (2017). Long-term care hospitals in Turkey: a review. *Eastern Mediterranean Health Journal*, 23(8), 564-570.
 14. Feiz Haddad, M. H., Maraghi, S., Ali, S. A., Feiz Haddad, R., & Nasser Zadeh, R. (2018). Intestinal parasitic infections frequency in referred patients to a large teaching hospital, Khuzestan, Southwest, Iran, 2017. *Tropical Biomedicine*, 35(4), 915-925.
 15. Flaih, M. H., Khazaal, R. M., Kadhim, M. K., Hussein, K. R., & Alhamadani, F. A. B. (2021). The epidemiology of amoebiasis in Thi-Qar Province, Iraq (2015-2020): differentiation of *Entamoeba histolytica* and *Entamoeba dispar* using nested and real-time polymerase chain reaction. *Epidemiology and Health*, 43.

16. Hamad, N. R., & Ramzy, I. A. (2012). Epidemiology of *Entamoeba histolytica* among children in Erbil province, Kurdistan Region-Iraq. *Journal of research in Biology*, 1, 057-062.
17. hamady obeid Al-Taei, A. (2019, September). The prevalence of intestinal parasite among the attending peoples to Al-Hashimyah hospitals for seven years, Babylon province, Iraq. In *Journal of Physics: Conference Series* (Vol. 1294, No. 6, p. 062022). IOP Publishing.
18. Hawash, Y. A., Dorgham, L. S., Amir, E. A. M., & Sharaf, O. F. (2015). Prevalence of intestinal protozoa among Saudi patients with chronic renal failure: a case-control study. *Journal of tropical medicine*, 2015.
19. Hussein, K. R., Dahis, H. N., & Mshhwt, A. K. A. (2015). Parasitic infections causing diarrhoea among children less than six years at Al-Nasiriyah province. *University of Thi-Qar Journal*, 10(3), 10-17.
20. Jaffar, Z. A., Shammari, A. J. K., & Merdaw, M. A. Z. (2021). Incidence of intestinal parasitic infections among random samples at Al-Aziziyah hospital in Wasit province/Iraq. *Baghdad Science Journal*, 18(2), 0217-0217.
21. Jaleel Rhadi, H. A. (2021). Epidemiology of *Entamoeba histolytica*, *Giardia lamblia* and *Blastocystis hominis* in Basra Province\Iraq. *Indian Journal of Forensic Medicine & Toxicology*, 15(3).
22. Junaidi, J., Cahyaningsih, U., Purnawarman, T., Latif, H., Sudarnika, E., Hayati, Z., & Muslina, M. (2020). *Entamoeba histolytica* Neglected Tropical Diseases (NTDs) Agents that Infect Humans and Some Other Mammals: A Review. In *E3S Web of Conferences* (Vol. 151, p. 01019). EDP Sciences.
23. Kadir, M. A., El-Yassin, S. T., & Ali, A. M. (2018). Detection of *Entamoeba histolytica* and *Giardia lamblia* in children with diarrhea in Tikrit city. *Tikrit Journal of Pure Science*, 23(6), 57-64.
24. López, M. C., León, C. M., Fonseca, J., Reyes, P., Moncada, L., Olivera, M. J., & Ramírez, J. D. (2015). Molecular epidemiology of *Entamoeba*: first description of *Entamoeba moshkovskii* in a rural area from Central Colombia. *PLoS One*, 10(10), e0140302
25. Malaa, S. F. A., Al Tufaili, R. A. N., Hamza, D. M., & Ajem, R. (2019). A Phylogenetic Study of *Entamoeba Histolytica* Isolated from Patients in the Babylon Hospital of Iraq Based on 18S Ribosomal RNA Gene. *Indian Journal of Public Health*, 10(8), 51.
26. Marenga, G., Traficante, S., Ragonici, S., Vincenzi, C., Rocchetti, M., De Rito, G., ... & Messineo, D. (2019). Successful diagnosis of a longstanding Giant amoebic liver abscess using contrast-enhanced ultrasonography (CEUS): a case report in a Western country. *The American journal of case reports*, 20, 493.
27. Mezeid, N., Shaldoum, F., Al-Hindi, A., Mohamed, F. S., & Darwish, Z. E. (2021). Prevalence of intestinal parasites among the population of the Gaza Strip, Palestine.

28. Nath, J., Ghosh, S. K., Singha, B., & Paul, J. (2015). Molecular epidemiology of amoebiasis: a cross-sectional study among North East Indian population. *PLoS neglected tropical diseases*, 9(12), e0004225.
29. Nowak, P., Mastalska, K., & Loster, J. (2015). *Entamoeba histolytica*-pathogenic protozoan of the large intestine in humans. *Journal of Clinical Microbiology and Biochemical Technology*, 1(1), 010-017.
30. Rahi, A. A., & Majeed, L. (2019). Epidemiological study of intestinal protozoa at Wasit province
31. Ralston, K. S., & Petri Jr, W. A. (2011). Tissue destruction and invasion by *Entamoeba histolytica*. *Trends in parasitology*, 27(6), 254-263.
32. Regan, C. S., Yon, L., Hossain, M., & Elsheikha, H. M. (2014). Prevalence of *Entamoeba* species in captive primates in zoological gardens in the UK. *PeerJ*, 2, e492.
33. Rockwell, R. L. (2002). *Giardia lamblia* and Giardiasis with Particular Attention to the Sierra Nevada.
34. Royer, T. L., Gilchrist, C., Kabir, M., Arju, T., Ralston, K. S., Haque, R., ... & Petri Jr, W. A. (2012). *Entamoeba bangladeshi* nov. sp., Bangladesh. *Emerging infectious diseases*, 18(9), 1543.
35. Schiefer, W. C. (1980). *Statistics for the biological sciences*. 2nd ed. Addison-Wesley publishing, California, London
36. Schuster, F. L., & Visvesvara, G. S. (2004). Amebae and ciliated protozoa as causal agents of waterborne zoonotic disease. *Veterinary parasitology*, 126(1-2), 91-120.
37. Yilmaz, H., & Abdullah, A. (2017). Prevalence of Intestinal parasites (*Entamoeba* species and *Giardia lamblia*) in Duhok and Erbil cities. Northern Iraq. *J Microbiol Exp*, 4(6), 00132.
38. Zeb, A., Qureshi, A. W., Khan, L., Mansoor, A., Ullah, S., & Irfan, M. (2018). Prevalence of *Entamoeba histolytica* in district Buner Khyber Pakhtunkhwa, Pakistan. *Journal of Entomology and Zoology Studies*, 6(1), 185-188.