Available online at http://qu.edu.iq/journalsc/index.php/JOPS

ISSN 2411-3514 ONLINE
ISSN 1997-2490PRINTED

Al-Qadisiyah Journal Of Pure Science (QJPS)

Vol. 23, No. 04, pp. 47 –54, Year-2018

INVESTIGATION OF PARASITIC INFECTION AMEBIASIS AMONG A POPULATION IN AL-SAMAWAH CITY/ AL-MUTHANNA PROVINCE BY USING MOLECULAR METHOD

REAL-TIME POLYMERASE CHAIN REACTION

Dunnia Abdullah Barakat

Department of the Environment, College of Science, University of Al- Qadisiyah, Iraq

Dunnia.Barakat@qu.edu.iq

Doi : 10.29350/jops.2018.23.4.914

ABSTRACT: *Entamoeba histolytica* parasite is widely distributed throughout the world, but prevalence rates are highest in developing countries . *E.histolytica* infection ,Amebiasis, is consistently present among the poor developing countries, this study aim to investigate the amoeba parasites in the city of Al-Muthanna province using microscopic and molecular methods to explain the spread of the disease in this city because of its importance to the health of individuals in society where we use microscopic examination and PCR technology to investigate the amoeba parasites and found the percentage of amoeba parasites was $_{69.2307690}$ % and the negative samples were $_{30.769231}$% from the 286 total sample, a significant difference below the significant level p≤00.05 in the incidence of parasitic infection.

KEYWORDS: *Entamoeba histolytica*, Amebiasis, Al-Muthana, Real-Time PCR.

1- INTRODUCTION

Parasites are pathogens that have no effect on bacteria, viruses, and fungi, as they have been told by the doctor about the subject during their lives (Garcia *et al*., 2003; Al-abodi, 2018a), the primary pathogen parasites belong to the genus *Entamoeba* and that the infection of amoeba species either results ulceration in its walls of large intestine and deep ulcer may be lead to peritonitis (Shaker *et al*., 2018; Al-abodi, 2018b), and the formation of colonies harmless in the intestines or invasion of the walls of the colon or the destruction of the host tissue, for example lung, liver, brain, causing the disease Amebais Amebiasis It is noteworthy that most types of amoeba Parasites are common in the bowel cavity and do not cause human diseases (Hamzah *et al*, 2006; Debbie-Ann *et al*., 2018).

The primary parasite *E. histolytica* is the primary cause of amoeba. It is believed that amoebae disease has occurred in several steps and in multiple processes. Therefore, many studies have attempted to solve the mechanical problem of the virulence factors (Al-Mayali and Al-abodi, 2017), molecules and cells that work on

Available online at http://qu.edu.iq/journalsc/index.php/JOPS

ISSN 2411-3514 ONLINE
ISSN 1997-2490PRINTED

Al-Qadisiyah Journal Of Pure Science (QJPS)

Vol. 23, No. 04, pp. 47 –54, Year-2018

them, the diagnosis of *E. histolytica* was based on a microscopic examination of the primary parasite, particularly in the case of bloody diarrhea within half an hour to an hour after the arrival of samples to the laboratory. The presence of *erythrophagocytic trophozoites* in the fecal samples indicates an amoebic condition. (Hamzah and Jameel, 2017; Zeb *et al*., 2018). The researchers (Gonzalez-Ruiz *et al*., 1994) considered this examination to be 100% qualitative to diagnose *amoeba*. However, there are few cases of bloody diarrhea, and in the absence of the active phases of red blood cells, the sensitivity of the microscope is limited because of its inability to distinguish between the amoeba-containing samples of the tissue and those containing the most variable amoeba times, In recent years, the molecular biological technician in the diagnosis and investigation of amoeba the condition of the tissue, the current study aims to investigate the amoeba parasites in the city of Al-Samawah, Al-Muthanna province using microscopic and molecular methods to give a clear picture of the spread of the disease in this city because of its importance to the health of individuals in society.

2-Materials and methods

The samples of faeces were collected from patients were complaining of intestinal disorders, abdominal pain and diarrhea cases in Al-Samawa Educational Hospital, Children's Hospital and some external medical laboratories in the city center and its suburbs for the period from 1 March to 1 September 2018, the different age groups ranged from 1 year - more than 50 years. The fecal samples were collected in a small sterile plastic container with a size of 20 ml with a wide face and a tight lid to keep the moisture of the sample and cart with a small paper with the sample number and date of collection Of the samples were examined with a light microscope, the samples were examined in a direct wet smear method according to (TanyuKsel *et al*. 2005; Al-Abodi and Al-zayadi, 2017).

DNA extraction from fecal samples was performed using the Stool Genomic DNA extraction kit and equipped with Korean Bioneer (Al-abodi *et al*., 2015). The extraction process was carried out according to the company's instructions, and then we use of the Nanodrop spectrophotometer (THERMO. USA) to detect and measure the concentration of DNA and RNA where DNA is detected by DNA concentration (ng \ µlDNA) and purity measurement DNA by reading the wavelength absorption range between 280-260 nm, Real-Time PCR technique was performed using the initiators and sensors of 18S rRNA genes responsible for the diagnosis of the *Entamoeba parasite species* from human stool samples, the design of primers and probes for gene 18S rRNA responsible for the diagnosis of parasite species Entamoeba in samples of human feces by using the polymerase chain reaction in real-time Real technology-Time PCR was used gene bank Genbank-NCBI site to get the complete sequence of the gene 18S rRNA gene for each type of parasite species Entamoeba Using Primer3plus, the primers were designed

Available online at http://qu.edu.iq/journalsc/index.php/JOPS

ISSN 2411-3514 ONLINE
ISSN 1997-2490PRINTED

Al-Qadisiyah Journal Of Pure Science (QJPS)

Vol. 23, No. 04, pp. 47 –54, Year-2018

and the prefixes were prepared by the Korean company Bioneer: According to a study (Al-abodi, 2016).

Table (1): The primers used in this study with the sequence Nucleotide.

Primer	Sequence	
E.H-ssrRNA primer	F	GAATTGACGGAAGGGCACAC
	R	AACTAAGAACGGCCATGCAC
E.H-ssrRNA probe	FAM-CAGGAGTGGAGCCTGCGCTT-BHQ1	

The Real-Time PCR reaction components mentioned in the table (1) were then placed into 0.2ml white tubes of real-time PCR and then all tubes were transferred to th e vortex centrifuge (Exispin) centrifuge at 3000 rpm for three minutes and then placed in a Real-Time PCR device.

2-1-Statistical analysis

All the results obtained in this statistical study were subjected to the Chi-square test and showed the probability level ($p \leq o.o5$) (Petri and Watson, 2004).

3-Results :-

The samples were collected for 286 Patients who had diarrhea. The samples were collected for six months From January to John ,2018 at Al Samawah Teaching Hospital. As detailed in table (2), The results showed that, (198) positive cases that were divided into three age groups to facilitate their study. Men between the ages of 1-30 years of age, 93 men, followed by 63 women of the same category, the ratio was equal for both sexes in the age group (30-60), which is 19. in the category (60-90) three cases in men and one case In females, the percentages of the injury as shown in table (2), While the number of negative cases was (88).

Table (2) :-Number of positive cases of amoeba parasite by age group.

age categories	Sex		total	Percentage
	No. of male	No. of female		
(1-30) years	93	63	156	78.79
(30-60) years	19	19	38	19.19
(60-90) years	3	1	4	2.02
Grand total			198	100%

A total of 286 Faeces samples were tested for diarrhea and gastrointestinal disorders. the results showed that only 198 samples of amoeba parasites, the percentage of amoeba parasites was % 69.2307690 and the negative samples were *30.769231 A significant difference below the significant level 00.05 in the incidence of parasitic infection as shown in Table(3)

Table (3) Infection rate of amoeba parasites using the wet smear method

Parasite	Number of samples	Percentage
Positive samples of Amoeba parasites	198	69.2307690 %
Negative samples of Amoeba parasites	88	30.769231 %
Grand total	286	100.00 %

Available online at http://qu.edu.iq/journalsc/index.php/JOPS

ISSN 2411-3514 ONLINE
ISSN 1997-2490PRINTED

Al-Qadisiyah Journal Of Pure Science (QJPS)

Vol. 23, No. 04, pp. 47 –54, Year-2018

4-Discussion:-

The current study recorded the percentage of amoeba parasite infection reached (69.2307690 %) this percentage is almost similar to that, Bank *et al.*, 2006, its (7.06%) when studying intestinal parasites among school students in Nigeria. Kumar *et al.*(2009) reported a (16.1%) incidence of intestinal parasites among school students in Guantanamo, And Sehgal *et al*. (2011), when they studied a group of school students and pregnant women who recorded an infection rate of 42.8% for amoeba While Ayalew *et al*, (2011), who recorded 27.3% infection in northern Ethiopia (kalukalu) . in the study of the spread of intestinal parasites shows the students of the University of Polytechnic in Nigeria the proportion of amoeba parasites 64.5%, while the current study rate is lower than what was recorded,In the study of intestinal infections in India, Davane *et al* (2012) reported the incidence of amoeba parasites 66.6%,while the rate of infection was higher than that of Gelaw *et al.* (2013) among school students in Northeast Ethiopia with 13.8% and While Wegayehu *et al* (2013)reported that 11.4% were infected with intestinal parasites in southern Ethiopia.

Molecular methods of diagnosis are more accurate and very sensitive methods (Al-abodi, 2018c), the results of the molecular examination showed to the presence of this type in **198** samples (**69.2307690** %) of the total number of positive samples of **286** samples. Figure (1) shows the inflation curve of the real time PCR results of the positive samples of *E. histolytica* 18s r RNA gene.

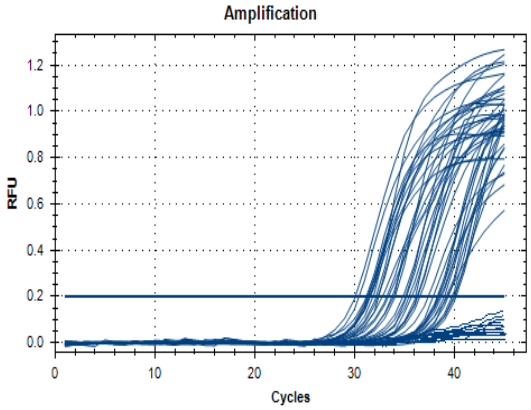

Fig.(1): The Amplification plot illustrates the Real-Time PCR test of the positive results of an *Entamoeba histolytica* parasite by an 18s r RNA gene.

The current study reported different rates of infection for the three species of parasite under study, with an infection rate of type *E. histolytica*. this is consistent with gender dominance with (Ngui *et al*., 2012) in a study in Malaysia using PCR technique to investigate Three based on the 16SrRNA gene that the highest percentage was *E.histolytica* (7.5%), in Iraq, the results of the present study are in agreement with a study by Al-Khafaji (2014) in Al Diwaniyah province who recorded a percentage of *E.histolytica* type of infection was 47%, However, the results of this study were not agree with other studies of the highest incidence of *E.dispar* and the lowest proportion of *E.histolytica*, as in (Ali *et al.* 2003), in Bangladesh using traditional PCR Of the types of amoeba parasite depending on gene SSU_rRNA with the highest percentage of infection of the species *E.dispar* and reached 35.8%, In a study by Parij and Khairnar (2005),,

Available online at http://qu.edu.iq/journalsc/index.php/JOPS

ISSN 2411-3514 ONLINE
ISSN 1997-2490PRINTED

Al-Qadisiyah Journal Of Pure Science (QJPS)

Vol. 23, No. 04, pp. 47 –54, Year-2018

in India using the NCR-based nested technique using the SSUrRNA gene in the three species with the highest incidence of *E.dispar* at 8.8%, and with (Fotedar et al. 2007) in Sydney, Australia using the traditional PCR gene for 18SrRNA of *E.histolytica* 3.4%. , While Abid *et al.* (2013) was in disagreement in his study in Tikrit where the highest percentage of infection was did not agree in Tikrit in his study that the highest percentage of infection was of the type *E.dispar* by 83.33% in addition to the percentage of mixed collection *E. dispar* + *Entamoeba moshkovskii*parasite was 1.85% and no recorded infection of the species Which was found in (AL_Shalah ,2014) in Babil province using Elisa technique, which found *E.dispar* had the highest infection rate at 87.9%, while *E. histolytica* recorded a 22% infection rate and no gender infection was recorded. *Entamoeba moshkovskii*, In terms of value, The current study reported incidence of *E.histolytica*, was 74% which is in agreement with Ngui *et al.(*2012) who reported 75%in Malaysia,it did not agree with other studies. The current rate was higher than that of Ali *et al.* (2003) in Bangladesh, which was 15.6%, and (Parija and Kahenar ,2005) in Iran, which was 1.7% and (Fotar *et al.*, 2007) in Sydney, Australia, from 3.4% and (Mojarad *et al.*, 2009) in Iran at 3.45% and with (Kheirandish *et al.*, 2011)in Iran where they did not score any *E.histlytica* infection, And did agree with (Al-Bakri *et al.*, 2013) In the United Arab Emirates, 10% as well as (Abid *et al.*, 2013) in Tikrit, where they recorded no injuries and recorded (AL_Khafaji, 2014) in Diwaniya from 47%.

5-Conclusions:-

Entamoeba histolytica parasite is widely distributed throughout the world , At the city of Al-Samawah, Al-Muthanna province The results recorded a wide spreading of this parasite , among 286 samples, there are 198 cases During a period of six months. The highest incidence of infection(93 cases) in males for the age group 1-30 years and followed by (63 cases) in women in the same age group .

References:

Abid, S.J. Davies, P. Waines, M. Emery, M. Castex, G. G ioacchini, O. Carnevali, R.Bickerdike, J. Romero, D .L.(2013). MerrifieldDietary synbiotic application modulates Atlantic salmon (*Salmo salar*) intestinal microbial communities and intestinal immunity.Fish Shellfish Immunol., 35, pp. 1948-1956.

Al-Abodi H R J and Al-zayadi W A A (2017) The use of traditional methods and the most recent molecular methods to investigate the presence of parasite Giardia lambia in infants with diarrhea and study some factors affecting its spread in Qadisiyah governorate. Al-Kufa University Journal for Biology 9(1), 365-373.

Al-Abodi, H. R. J. (2018a). EFFECT AND SPREAD OF GIARDIA PARASITE ON CHILDREN IN

Available online at http://qu.edu.iq/journalsc/index.php/JOPS

ISSN 2411-3514 ONLINE
ISSN 1997-2490PRINTED

Al-Qadisiyah Journal Of Pure Science (QJPS)

Vol. 23, No. 04, pp. 47 –54, Year-2018

PRIMARY DEVELOPMENT STAGES IN SOUTHERN IRAQ. Biochem. Cell. Arch. Vol. 18, No. 2, pp. 1537-1541.

Al-Abodi, H. R. J.; Al-Mayali, H. M. H. and Ali, M. J. (2015). Molecular study to most important virulence factor of Entamoeba histolytica in Diwaniyah province. International Journal of Advanced Research , Volume 3, Issue 8, 231-236.

Al-Abodi, H. R. J. (2018b). Pathological Effects Associated with Parasitic Infections. *Indian Journal of Natural Sciences*, Vol.9 /Issue 51, 16146-16151.

Al-Abodi, H. R. J. (2018c). Suspicion in the form of infection is the basis for selecting the appropriate method for examining the toxoplasmosis disease of bends that have no symptoms from patients. Int. J. Adv. Res. 6(9), 655-662.

ALI, I.K ., HOSSAIN , M.B . , ROY , S. , ET AL . (2003) INTAMOEBAMOSHKORIS INFECTION IN CHILDREN IN BANGLADESH . EMERG . INFECT . DIS ., 9 (3) . 9ABSTRACT) .

Al-abodi, H. R. J. (2016). Phylogenetic sequeincing for some species of Entamoeba sp. in Al-Qadisiya Province. Ph.D. thesis, College of Sciences, University of Al-qadisiyah.

Al-Bakri1,A., Samie, A., Ezzedine S. and Odeh, R. (2014) Genetic variability of the Serine-Rich Entamoeba histolytica protein gene in clinical isolates from the United Arab Emirates. Tropical Biomedicine 31(2): 370–377

Al-Khafaji , Arshed Shaker . (2014) Molecular characterization of Entamoeba moskoviskic as the new recording in Diwaniya by using single round polymerase chain reaction PCR . M.Sc. Thesis college of medicine . Al-Qadissiay University.

AL-MAYALI, H. M. H. AND AL-ABODI, H. R. J. (2017). MOLECULAR CHARACTERIZATION OF ENTAMOEBA SPP. IN AL-QADISIYA PROVINCE, IRAQ. AL-KUFA UNIVERSITY JOURNAL FOR BIOLOGY, VOL.9 / NO.1 : 374-386.

AL-SHALAH, S. A. J. (2014). DIAGNOSTIC STUDY OF ENTAMOEBA HISTOLYTICA AND ATTENUATED OF PARASITIC BY GAMMA RADIATION. M.SC. THESIS, COLLEGE OF MEDICINE, UNIVERSITY OF BABYLON, IRAQ.

Ayalew A, Debebe T, Worku A. (2011). Prevalence and risk factors of intestinal parasites among Delgi school children, North Gondar, Ethiopia. J Parasitol Vector Biology. 3:75–81.

Banks, J. C., Palma RL and Paterson AM**.** **(**2006). Cophylogenetic relationships between penguins and their chewing lice. US National Library of Medicine National Institutes of Health;19(1):156-66.

Available online at http://qu.edu.iq/journalsc/index.php/JOPS

ISSN 2411-3514 ONLINE
ISSN 1997-2490PRINTED

Al-Qadisiyah Journal Of Pure Science (QJPS)

Vol. 23, No. 04, pp. 47 –54, Year-2018

Davane, M. S., Suryawanshi N. M., Deshpande, K. D. A. (2012). prevalence study of intestinal parasitic infections in a Rural hospital. Int J Recent Trends Sci Technol. 2(1):1–3.

Debbie-Ann, T. S., Laura F., Koji, W. and Shannon M. (2018). A Review of the Global Burden, New Diagnostics, and Current Therapeutics for Amebiasis. *Open Forum Infectious Diseases*, 5(7), ofy161.

Dunnia A. Barakat And Forat A. Hady.(2017). Effect Of Frozen Meat In The Hormonal Imbalance In Male In The Diwaniyah City. World Journal Of Pharmaceutical Research .Volume 6, Issue 3.

Fotedar, R.; Stark, D.; Beebe, N.; Marriott, D., Ellis, J. &Harkness, J. (2007). Laboratory diagnosis techniques for *Entamoeba histolytica*. J. Clin. Microbiol. Rev., 20: 511-532.

Garcia LS, Shimizu RY, Novak S, Carroll M, Chan F. (2003). Commercial assay for detection of *Giardia lamblia* and *Cryptosporidium parvum* antigens in human fecal specimens by rapid solid-phase qualitative immunochromatography. J Clin Microbiol;41:209–12.

Hamzah, H. M. and Jameel, H. R. (2017). Epidemiology study of Entamoeba spesies in AL-Qadisiya governorate. Al-qadisiyah Journal for pure science, Vol.9, No. 3.

Hamzah , Z. Petmiter , S. Mangthin , M ., Leelayoova , S. and Chavalitsh –Winkoon , P. (2006) . differential detection of *Entamoeba histologica* , *Entamoeba dispar and E.Moshicoviskii*by single –round PCR assay .J. clin . microbial ., 4 : 3196 -3200.

Gelaw A., Anagaw B., Nigussie B., Silesh B., Yirga A., Alem M. Endris M. and Gelaw B.(2013). Prevalence of intestinal parasitic infections and risk factors among schoolchildren at the University of Gondar Community School, Northwest Ethiopia: a cross-sectional study. *BMC Public Health*, **13**:304.

Gonzalez-Ruiz , A. ; Haque, R. ; Aguirre , A. ; Castanon , G. ; Hall , A. ; Ruis-Palacios , G. ; Miles , M. A. ; Warhurst , D. (1994) . Value of microscopy in the diagnosis of dysentery associated with invasive *Entamoeba histolytica* . J. Clin. Pathol. 47:236-239.

Kumar A, Molli PR, Pakala SB, Bui Nguyen TM, Rayala SK, Kumar R. PAK thread from amoeba to mammals. J Cell Biochem. 2009;107:579–585.

Kheriandish, F., Tarah, M., Haghighi, A., Nazemalhosseini-Mojarad ,E, Kheriandish M. (2011). Prevalence of intestinal parasites in bakery workers in Khorramabad, lorestan Iran. Iran J Parasitol;6:76-83.

Mojarad, E. N.,Haghighi A.,Kazemi B.,Rostami Nejad M.,Abadi A.,Zali M.R.High genetic diversity among Iranian *Entamoeba dispar* isolated seed on the noncoding short tandemrepeat locus D-A Acta Trop.2009,111:133-136.

Ngui, R., Angle, L., Fakhrurrazi, S. A., Lian, Y. L. A., Ling, l. y. and brahim, J. (2012).Diffrerntiating*Entamoeba histolytica* ,

Available online at http://qu.edu.iq/journalsc/index.php/JOPS

ISSN 2411-3514 ONLINE
ISSN 1997-2490PRINTED

Al-Qadisiyah Journal Of Pure Science (QJPS)

Vol. 23, No. 04, pp. 47 –54, Year-2018

Entamoeba dispar, *Entamoeba moshkoviskii* using nested polymerase reaction (PCR) in rural communities in Malaysia. Parasites and vectors, 5: 187.

Parija, S.C. and Khairnar, K .(2005). Entamoeba moshkovskii and Entamoeba dispar associated Infections in Pondicherry, India. Entamoeba histolytica cell movement: A central role for self-generated chemokines and chemorepellents .J. Health.Popul. Nutr., 23:292-295.

Shaker, E. M., Al-Shaibani, K. T. M. and Al-abodi, H. R. J. (2018). Effect of Alcohol extract of green tea plant *Camellia sienensis* as a therapeutic of parasite *Entamoeba histolytica. Plant Archives* Vol. 18 No. 1, pp. 953-959.

Sehgal, W. Buermann, R. Harrigan, C. Bonneaud, C. Lois eau, A. Chasar, I. Sepil, G. Valkiūnas, T. Iezhova, S . (2011).SaatchiSpatially explicit predictions of blood parasites in a widely distributed African rainforest bird .Proc. R. Soc. Lond. B Biol. Sci., 278 pp. 1025-1033.

Tanyuksel, M.; H. Yilmaz; M. Ulukanligil; E. Araz; M. Cicek; O. Koru; Z. Tas and W.A. Petri.(2005). Comparisonof (microscopy and enzyme – linked immune sorbent assay) for the diagnosis of amoebiasis. Exp. Parasitol., 110 : 322-326 .

Wegayehu, T. Tsalla T. , Seifu B. , and Teklu T. (2013). Prevalence of intestinal parasitic infections among highland and lowland dwellers in Gamo area, South Ethiopia. BMC Public Health.; 13: 151.

ZEB, A., QURESHI, A.W., KHAN, L., ABU MANSOOR, U. S.AND IRFAN, M. (2018). PREVALENCE OF *ENTAMOEBA HISTOLYTICA* IN DISTRICT BUNER KHYBER PAKHTUNKHWA,PAKISTAN .JOURNAL OF ENTOMOLOGY AND ZOOLOGY STUDIES:6(1): 185-188.