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# MOLECULAR DETECTION OF *ENTAMOEBA* SPP. BY USING MULTIPLEX PCR IN THI-QAR PROVINCE

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**ABSTRACT : The existence of *Entamoeba histolytica* as a parasite of human intestinal is a severe problem, especially in developing countries. *E. histolytica* often infect as a commensal within the large intestines without clinical manifestations. Amoebic infections in Iraq are common. Multiplex Polymerase Chain Reaction (PCR) technique was used for 90 samples infected with amoebiasis diagnostic by optical microscopy. DNA extraction of the parasite was evaluated by using specific primer for *E. histolytica* and another for *E. dispar* (573bp and 390bp), respectively. The results of the diagnostic with PCR show distinguish of amoebiasis to 50 (55.5%) infected with pathogenic *E. histolytica* and 27(30%) infected with nonpathogenic *E. dispar* with mixture infection 13(14.4%). Also the high rate with using PCR test show in the age group 1-10 years with no significant between male and female.**

***Key words :*** Amoebiasis, *E. histolytica, E. dispar,* PCR, Thi-Qar.

## INTRODUCTION

*Entamoeba histolytica* is a parasitic protozoan, belong to the genus *Entamoeba* that infected humans and other primates, where they have many species (*E. dispar*, *E. coli*, *E. hartmani*, *E. polecki* and *E. gingivalis*). The prevalence of *E. histolytica* could be infected about 50 million people worldwide each year. About a hundred thousand die each year, mostly from liver abscesses or other complications (Yakubu *et al*, 2003).

*Entamoeba histolytica* is causative agent of amoebiasis or amoebic dysentery (Sateriale *et al*, 2011). The pathogenic nature of *E. histolytica* was first reported by Fedor Losch in 1875, but it was not given its Latin name until Fritz Schaudinn described it in 1903. *E. histolytica*, its name suggests (*histo–lytic* = tissue destroying) is pathogenic; infection can be asymptomatic or can lead to amoebic dysentery or amoebic liver abscess (Ryan and Ray, 2004; Shirley *et al*, 2018).

The detection of *E. histolytica* infection depended on microscopic examination for stool samples but the microscopy is difficult to differentiate between pathogenic *E. histolytica* and non-pathogenic *E. dispar* (Fotedar *et al*, 2007). PCR are more dependable to distinguish between the species. Stool PCR is regarded as an appropriate substitute for the detection of *Entamoeba* spp. infections.

Recently, there are several methods used to distinguish of *E. histolytica* are depended on detection of *E. histolytica*-specific antigen and DNA in stool samples. Numerous molecular methods detection tests, like conventional PCR and real-time, have been developed for the diagnosis and differentiation of *Entamoeba* spp. in clinical samples. These molecular approaches have ran to a review the epidemiology and detection of amebiasis in relation of prevalence ( Fotedar *et al*, 2007).

## MATERIALS AND METHODS

### Collection of stool samples

A total of 110 stool sample collection (by sterile containers) from patients with diarrhea in Thi-Qar province from the (General AL-Hussein, General Bent Al- Huda, Al- Musawe, Suq shoyokh, Al chibash, Al Rifiae and Al shatra) hospitals. The collection samples is carried out the period between September 2019 to May 2020. These samples were divided into two parts the first portion was for the microscopic to detected the presence of parasites while the other portion of 200 mg stored directly at -20°C for molecular analysis by conventional PCR (Sabet *et al*, 2009).

The DNA was extracted from the 200mg stool samples by using (DNA extraction a commercial Kit

presto™ stool DNA Macrogen Company, Korea). The extraction was performed according to the manufacturers instructions. The extra DNA was amplify with PCR using specific primer (Table 1) and following the program mention in Table 2.

**Table 1 :** Primers of *Entamoeba histolytica* and *Entamoeba dispar* used in current study

| Primer | | Sequence (5'-3') | Product Size |
|---|---|---|---|
| 18S ribosomal RNA gene *Entamoeba histolytica* | F | ACGAGGAATTGGGGTTCGAC | 573bp |
| | R | AAATGCTTTCGCTCTCGTGC | |
| 18S ribosomal RNA gene *Entamoeba dispar* | F | ATTGGAGGGCAAGTCTGGTG | 390bp |
| | R | AAATGCTTTCGCTCTCGTGC | |

**Table 2 :** DNA amplification in the thermocycler program.

| PCR step | Temperature | Time | Repeat |
|---|---|---|---|
| Initial denaturation | 95 c | 5 min | 1 |
| Denaturation | 95 c | 30 sec | 30 cycle |
| Annealing | 58 c | 30 sec | 30 cycle |
| Extension | 72c | 1 min | 30 cycle |
| Final extension | 72 c | 5 min | 1 |
| Hold | 4 c | More than 5 min | _ |

The DNA products run in Agarose gel 1.5% to check for the specific band.

## RESULTS

### Results showing differentiation between *E. histolytica*, *E. dispar* and Mixed infection PCR test

Ninety suspected patients with amoebiasis in Thi-Qar province were examined by molecular test (polymerase chain reaction PCR). Out of 90 the test shown 50(55.5%) gave positive result infected with *E. histolytica*, 27 (30%) infected with *E. dispar* and 13(14.4%) have mixed infection. Table 3 shows the infected with *E. histolytica* had higher value 55.5% infection with significant differ than other infection (Figs. 1, 2, 3).

### PCR result according to the sex

Table 4 shows the female have significant positive rate 18(66.6%) and 9(69.2%) infected with *E. dispar* and mixed respectively than male 9(33.3%) and 4(30.7%), while female have slightly high rate 26(52%) than male infected with *E. histolytica*.

The results diagnosis with PCR shows the infected with *E. histolytica* 50(55.9%) have high rate than other infection.

**Table 3 :** Results of PCR test showing differentiation between *E. histolytica, E. dispar* and mixed infection.

| Parasite | Total | PCR+ve | | PCR-ve | |
|---|---|---|---|---|---|
| | | No. | % | No. | % |
| *E. histolytica* | 90 | 50 | 55.55 | 40 | 44.44 |
| *E. dispar* | 90 | 27 | 30 | 63 | 70 |
| Mixed | 90 | 13 | 14.44 | 77 | 85.55 |
| X2 | 6.175(S) | | | | |
| P value | 0.008 | | | | |

S: significant difference at P < 0.05

### PCR results according to region

Table 5 shows the higher rate with significant ($p<0.05$) infection with the *E. histolytica* in region of Thi-Qar province by using PCR test in (Sugshoyokh, Chibash and Rifiae) 20(40%), 15(30%) and 10(20%), respectively, and the lower infection in Shatra and Nassirya. Infection with *E. dispar* have higher rate in (Sugshoyokh and Al-shatra).

The higher rate of infected with mixed infection was recorded in (Sugshoyokh and chibaysh) 5(38.4%) and 4(30.7%) respectively and the lowest infection in (Al-Nassirya and Al-rifiae).

**Table 4 :** PCR examination according to sex.

| Sex | *E. histolytica* | | *E. dispar* | | Mixed | |
|---|---|---|---|---|---|---|
| | +veNo. | % | +veNo. | % | +veNo. | % |
| Male | 24* | 48 | 9** | 33.33 | 9** | 30.76 |
| Female | 26* | 52 | 18** | 66.66 | 18** | 69.23 |
| Total | 50 | 55.5 | 27 | 30 | 13 | 14.4 |
| X2 | 0.823 NS | | | | | |
| P value | 0.006 S | | | | | |

(S) represents the significant difference at the value of p < 00.05. (NS) represents the non significant difference at the value of p > 00.05; sign.: **, non-sign.: *

**Table 5 :** PCR results according to region.

| Area | *E. histolytica* | | *E. dispar* | | Mixed | |
|---|---|---|---|---|---|---|
| | +veNo. | % | +veNo. | % | +veNo. | % |
| Niasrya | 2* | 4 | 2* | 7.40 | 1* | 7.69 |
| Rifiae | 10** | 20 | 5** | 18.51 | 1* | 7.69 |
| Chibaysh | 15** | 30 | 4* | 14.81 | 4** | 30.76 |
| Suqalshoyokh | 20** | 40 | 10** | 37.03 | 5** | 38.46 |
| Shatra | 3* | 6 | 6** | 22.22 | 2* | 15.38 |
| Total | 50 | 55.5 | 27 | 30 | 13 | 14.4 |
| X2 | 0.0521(S)** | | | | | |
| P value | 0.003 | | | | | |

(S) : significant difference at p < 0.05; sign.: ** , non-sign.: *

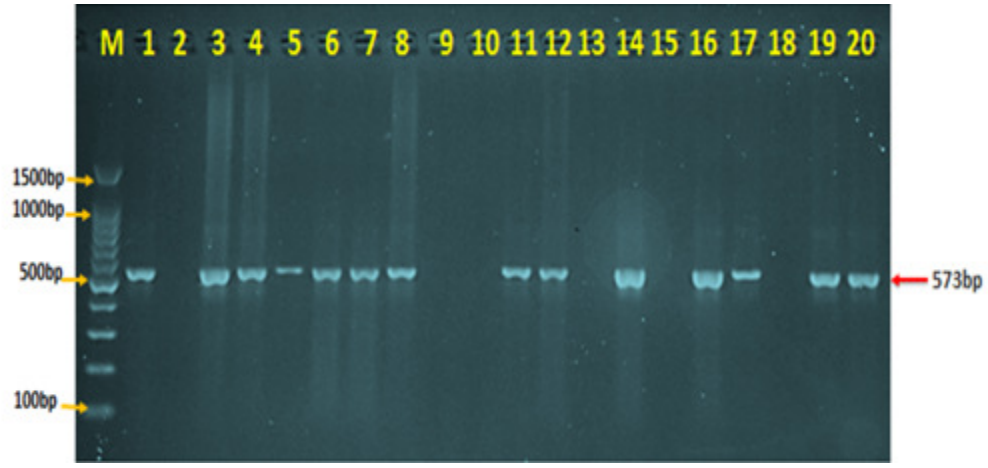


**Fig. 1 :** PCR amplification of *E. histolytica* 573bp fragment gen in Agarose gel product analysis of human stool samples.

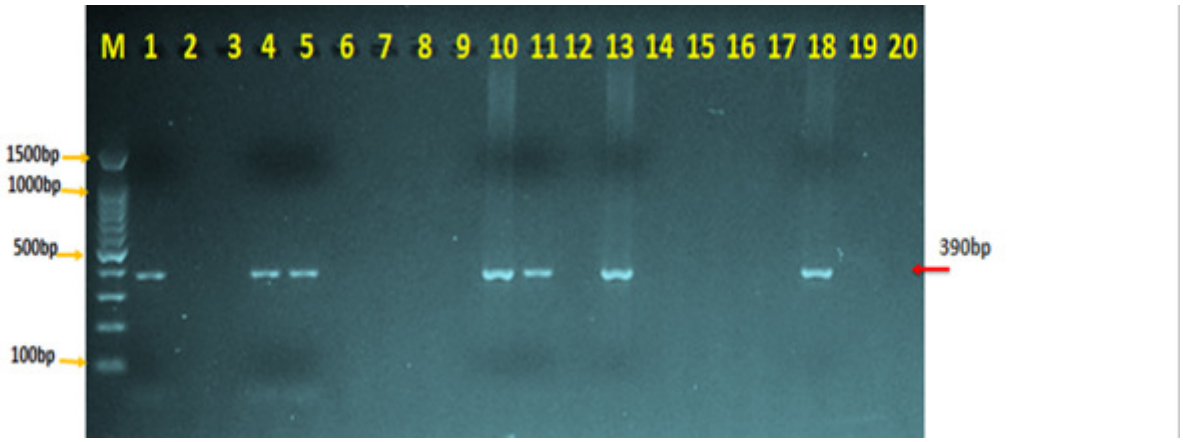


**Fig. 2 :** PCR amplification of *E. dispar* 390 bp fragment gen in Agarose gel product analysis of human stool samples. M: marker (1500-100bp) , Lane:(2,3,6,7,8,12) negative control.

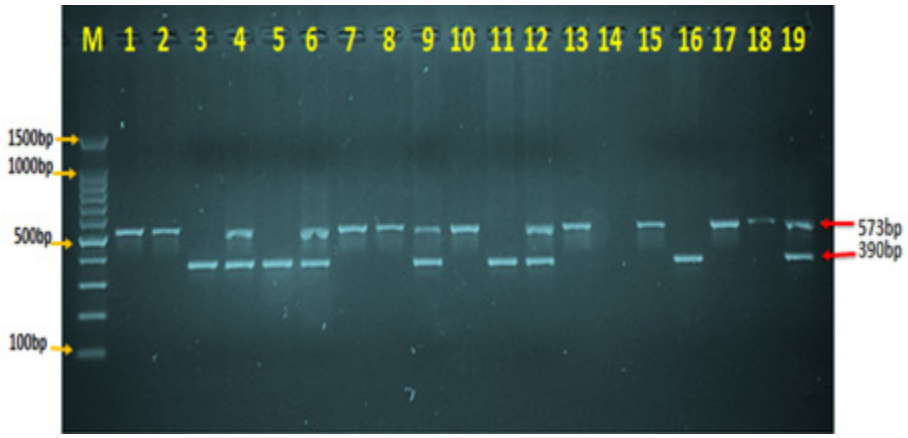


**Fig. 3 :** Multiplex PCR amplification of *E. histolytica,*573 bp., *E. dispar* 390 bp fragment gen in Agarose gel product analysis of human stool samples. M: marker (1500-100bp)

### PCR results according to age

The positive results of PCR test shows the higher rate infection with *E. histolytica* was in age group (>0-1) years 15(30%), and the lower rate in age group more than 40 years 6(12%). More ever the higher rate of infection with the *E. dispar* in age groups (>0-1) years 9(33.33%) with lower infection in age more than 40 year. In mixed infection the higher rate represent in age group (0-10) years (Table 6).

## DISCUSSION

*Entamoeba histolytica* is a protozoan parasite-infected human intestine, which responsible for causing amoebic dysentery, About 100000 people may die yearly worldwide due to this parasite infection (Walsh, 1986). Morphologically *E. histolytica* was similar to

**Table 6 :** PCR examination in 90 stool samples according to age.

| Age group | *E. histolytica* | | *E. dispar* | | Mixed | |
|---|---|---|---|---|---|---|
| | +veNo. | % | +veNo. | % | +veNo. | % |
| 0-10 | 15* | 30 | 9** | 33.33 | 5** | 38.46 |
| 11-20 | 12** | 24 | 6** | 22.22 | 2* | 15.38 |
| 21-30 | 8* | 16 | 8** | 29.62 | 2* | 15.38 |
| 31-40 | 12** | 24 | 2* | 7.40 | 3* | 23.07 |
| > 40 | 6* | 12 | 2* | 7.40 | 1* | 7.69 |
| Total | 50 | 55.5 | 27 | 30 | 13 | 14.4 |
| X2 | 0.421(S) | | | | | |
| P-value | 00.005 | | | | | |

(S) represents the significant difference at the value of $p < 0.05$; sign.: ** , non-sign.: *

nonpathogenic strain *E. dispar,* which found in the human intestine, these features make a diagnosis to distinguish between these two parasites difficult by using optical microscopic examination (Blessmann *et al*, 2002).

Over the last decade, there has been an incredible development in molecular-based diagnostic methods to detect *E. histolytica*. Many studies have documented the ability of the PCR method to differentiate between pathogenic *E. histolytica* than non-pathogenic *E. dispar* infection (Tanyuksel *et al*, 2005).

So, the primary benefit of using PCR is the opportunity of difference between *E. histolytica* and *E. dispar* in area where the occurrence of other *Entamoeba* species is common (Santos *et al,* 2007). PCR is most accurate to know the epidemiology of *E. histolytica* and *E. dispar* infection, PCR and ELISA had equivalent sensitivities when accomplished directly on fresh stool samples.

In the present study out of 90 infected with amoebiasis detected with microscopy, PCR stool examination verified 50(55.5%) infections of *E. histolytica* and 27(30%) *E. dispar*, showing large wrongly detected amoebic infections in our country. *E. histolytica* is mostly detected on microscopy of stool, but this method is neither sensitive nor specific to differentiate between *E. hitolytica* and *E. dispar.* Also, microscopy is very greatly depending on the ability of the technician. A study remarkable by Petri *et al* (2000) suggested that the stool's microscopic examination might not be utilized for diagnosing amoebiasis because it is a method with low-sensibility, specificity as well as presenting false-positive results. Also, dysentery caused by enteritis including viruses, bacteria, and other agents must be indicated (Evangelopoulos *et al*, 2000; Ramos *et al*, 2000; Blessmann *et al*, 2002; Petri, 1999; Stanley, 2003) and possible might be miss-diagnosed as amoebic colitis in the case when microscopy is the only utilized approach.

Freitas *et al* (2004), has been an agreement with our studies suggested that the PCR can be regarded as one of the most tools for differentiation between *Entamoeba* spp. Another work, carried out in Ethiopia with 108 samples of stool, showed that just single samples is *E. histolytica*, whereas 77 *E. dispar* in the case when using PCR. Also, the remaining 30 samples have been indicated to be negative for the two species (Kebede *et al*, 2004).

Similar to a study by Tasawar *et al* (2010), no significant difference was detected in the current study between the rate of *E. histolytica* in males (48%) and females (52%). In Iran, the molecular epidemiology regarding the *Entamoeba* complex, indicated that positive PCR results reported 396 (83%), 55 (12%) and 11 (2.4%) out of 480 were *E. dispar*, *E. histolytica* and *E. moshkovskii*, respectively (Haghighi *et al*, 2018). In Yemen and United Arab Emirates, *E. histolytica*, *E. dispar* and *E. moshkovskii* were identified in 44.2%, 34.4% and 39.9% of the 276 PCR-positive products (Al-Areeqi *et al*, 2017) and 13.3%, 6.7% and 3.3% of the 120 samples, respectively (ElBakri *et al*, 2013).

## CONCLUSION

The PCR showed distinguish of amoebiasis infected with pathogenic *E. histolytica* and *E. dispar*. Also, the high rate is shown in the age group 1-10 years with no significant between males and females.

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