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DIFFERENTIAL DIAGNOSIS OF *Entamoeba*spp IN STOOL SAMPLES USING POLYMERASE CHAIN REACTION

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ABSTRACT

Objective: Diagnosis of *E. histolytica* and *E. dispar* by conventional PCR and determine epidemiology for both parasites by conventional PCR

Methods: (40) stool sample from suspected patients with *E. histolytica* /*E. dispar* and (50) sample of healthy individuals as a control, were examined by direct smear method and wet preparation method to differentiated for infection with *E. histolytica* and *E. dispar* by CP5 and ED-1 genes.

Result: The results showed that (72.5%) were positive for CP5 gene for *E. histolytica*and (27.5%) was negative for CP5 gene but positive for the specific primer (Ed-1) of *E. dispar***.**

Conclusion: Our finding suggests that CP5 gene could be using as a specific diagnosis gene for differentiation between *E. histolytica* and *E. dispar*.

KEYWORDS: Conventional PCR and Determine Epidemiology, Diagnosis Gene

INTRODUCTION

Several members of the genus *Entamoeba* infect humans. Among these only *E. histolytica* is considered pathogenic and the disease it causes is called amebiasis or amebic dysentery. Humans are the only host of *E. histolytica* and there are no zoonotic reservoirs. *E. dispar* is morphologically identical to *E. histolytica* and the two were previously considered to be the same species **(Wiser, 2010)**.

This parasite has a very simple life cycle in which the infective form is the cyst that is considered a resistant form of the parasite. The asymptomatic cyst passers and the intestinal amoebiasis patients are the natural transmitters; they excrete cysts in their feces, which can contaminate food and water sources. Cysts are round structures around 10–16 μm in diameter **(Ximénez*et al.*, 2011).**

Amebiasis occurs worldwide; the prevalence is disproportionately increased in developing countries because of poor socioeconomic conditions and sanitation levels. Infection with *E. dispar* occurs approximately 10 times more frequently than infection with *E. histolytica* Areas with high rates of amebic infection include India, Africa, Mexico, and parts of Central and South America. The overall prevalence of amebic infection may be as high as 50 percent in some areas **(Peterson***et al*., 2011).

Results of several studies on detection and differentiation of *E. histolytica, E. dispar, E. moshkowski* and other harmless amoebae in clinical specimen using PCR showed the potential use of molecular methods in the diagnosis of amoebiasis **(Liang *et al*., 2009).**

A study which involved 218 stool samples has demonstrated the use and role of PCR in differentially diagnosing pathogenic *E. histolytica* (51) from morphologically resembling non- pathogenic *E. dispar* (39) (Hunt, 2011). which otherwise by conventional microscopy cannot be differentiated. Significance and advantages of DNA based techniques over other methods in identifying the parasites quantify and provide important information on formulating and implementing the parasite control programs in both human and animal is highlighted in a recent article by **Hunt (2011).**

MATERIALS AND METHODS

Fecal Sample Collection

The current study was conducted in the period from September 2015 till march 2016, all samples (200) were collected from hospitals in Babylon province, all patients undergo full history and full information were obtained from the patient.

Stool samples were taken from each patient and collected in sterile containers for microscopic examination (wet mount). And positive sample were frozen for DNA detection by conventional PCR.

Wet mount preparation method, with an applicator stick picked up a small amount of specimen butting on clean sterilized slide and mixed with a drop of saline and use cover slip to get a clear vision and examine in 40x, 100x and Identification of the parasite by its motile and size.

Extraction of DNA from Stool Samples

Procedure of DNA extraction according to manufacture procedure of favorgen kit (korea)

Statistical Analysis

Statistical analysis of the results was done by using Chi- square, $p<0.05$ as the lowest limit significance, (SPSS).

Results

Incidence of CP5 Genes for Patients Infected with *E. Histolytica* By Using Conventional PCR

Out of (40) stool samples, only (29) sample showed positive for CP5 gene marker for *E. histolytica*. however, (11) samples (27.5%) showed negative for this gene, moreover (11) stool samples showed also positive for (Ed) primer which is specific for *E. dispar*. so, only (11) samples gave positive for *E. dispar* which is similar to *E. histolytica* morphologically as shown in table (1).

Table 1: Percentage of *E. histolytica* and *E. dispar* Infection by Conventional PCR

No. of Total Samples	No. of Positive Samples for CP5 Gene of *E. Histolytica*	%	NO. of Positive Samples for *E. Dispar*	%
40	29	72.5	11	27.5

In figure (1) showed PCR product for CP5 primer at the size 950bp is shown for *E. histolytica*. While figure (2) showed PCR product of *E. dispar* ED-1primer at a size 174bp is shown for *E. dispar*.

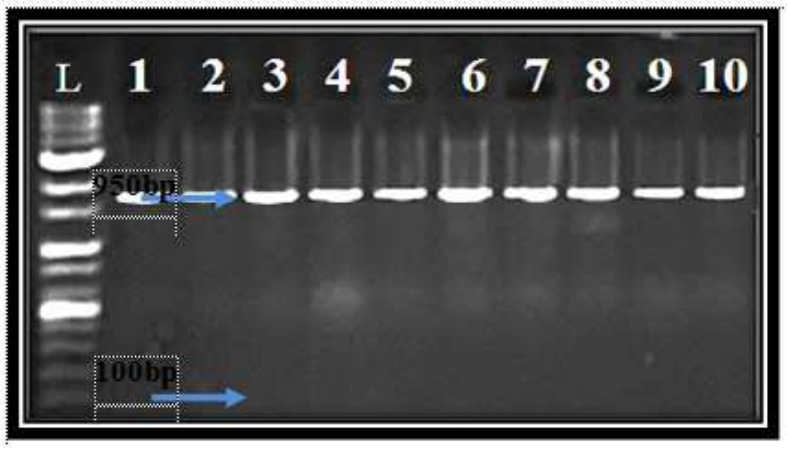

Figure 1: Agarose Gel Electrophoresis Shown PCR Product of *E. Histolytica* CP5 Gene, L: Ladder. Lane 1-10 Present Some Positive Samples Extracted as 950bp PCR Product Size

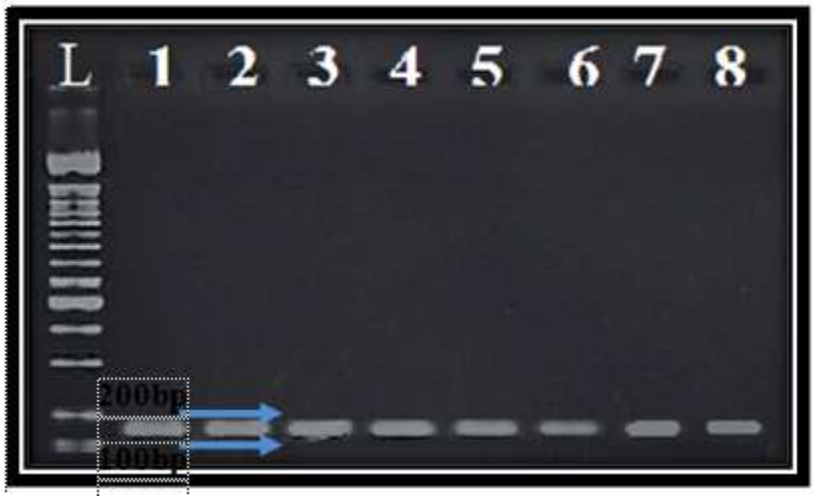

Figure 2: Agarose Gel Electrophoresis Shown PCR Product of *E. Dispar* ED-1primer, L: Ladder. Lane 1-8 Showed the Positive Samples Extracted As 174bp PCR Product Size

Distribution of *E. Histolytica* and *E. Dispar* According To Age Group by Conventional PCR

The result of current study showed non -significant differences at level of $p \geq 0.05$ between all age groups depended on molecular assay for both parasites, that the highest incidence of infection for *E. histolytica* occurs in age group (1-10) years was 41.3 %, while the lower percentage was 3.4 % for (21-30) years, as in table (2), in comparison to infection rate for *E. dispar* the highestinfection occurs in age group (1-10) years was 36.36 %, while the lower percentage was 9.09 % for (21-30,51-60) yearsand no infection recorded for age group(41-50) years as showed in in table (3)

Table 2: Incidence of *E. Histolytica* Infection According to Age Group by Conventional PCR Technique

Age	No. Of Positive Cases	Percentage (%) For Infected Patients	Chi-Square
1-10	12	41.3	P value 0.05 = 0.9824
11-20	8	27.5	
21-30	1	3.4	
31-40	2	6.8	
41-50	2	6.8	
51-60	4	13.7	
total	**29**	**100**	

*non-significant differences at $p \geq 0.05$

Table 3: Incidence of *E. Dispar* Infection According to Age Group by Conventional PCR Technique

Age	No. of Positive Cases	Percentage (%) for Infected Patients	Chi-Square
1-10	4	36.36	P value 0.05 = 0.213
11-20	3	27.27	
21-30	1	9.09	
31-40	2	18.18	
41-50	0	0	
51-60	1	9.09	
total	**11**	**100**	

*non significant differences at $p \geq 0.05$

Distribution of *E. Histolytica* and *E. Dispar* According to Gender by Conventional PCR

The present study showed the infection Percentage in male 59 % higher than female 41 % Also the highest incidence of infection for *E. dispar* occurs in male 73 % than female 27 %, The statistical analysis result shows non-significant difference in infection rate between male and female for both parasites as showed in table (4) and (5).

Table 4: Incidence of *E. Histolytica* Infection According to Gender by Conventional PCR Technique

Gender	No. of positive cases	Percentage (%) from infected patients	Chi-square
male	17	59	P value 0.05 =0.448
female	12	41	
total	**29**	**100**	

*non-significant differences at $p \geq 0.05$

Table 5: Incidence of *E. Dispar* Infection According to Gender by Conventional PCR Technique

Gender	No. of Positive Cases	Percentage (%) From Infected Patients	Chi-Square
male	8	73	P value 0.05 =0.367
female	3	27	
total	**11**	**100**	

*non-significant differences at $p \geq 0.05$

Distribution of *E. Histolytica* and *E. Dispar* According to Residence by Conventional PCR

The result of table (6), showed the percentage of *E. histolytica* infection in rural regions was 69 % higher than urban regions 31% while in table (7), the percentage of *E. dispar* infection in rural regions was 64 % higher than urban regions 36 % by using conventional PCR, with non-significant differences between rural and urban regions for both parasites.

Table 6: Incidence of *E. Histolytica* Infection According to Residence by Conventional PCR Technique

Residence	No. of Infection	Percentage (%) From Infected Patients	Chi-Square
Rural areas	20	69	P value 0.05 = 0.579
Urban areas	9	31	
total	**29**	**100**	

Non-significant differences

Table 7: Incidence of *E. Dispar* Infection According to Residence by Conventional PCR Technique

Residence	No. of Infection	Percentage (%) From Infected Patients	Chi-Square
Rural areas	7	64	P value 0.05 = 0.763
Urban areas	4	36	
total	**11**	**100**	

non-significant differences

Distribution of *E. Histolytica* and *E. Dispar* According to Annual Months of the Study by Conventional PCR

The distribution of *E. histolytica* infections according to months of year was showed in table (8), the most frequent infection was in June and July (16.6, 33.3)% respectively, and lower frequent was in October, November, December, and March 3.3% while in January and February no infections found, with significant differences at $p \leq 0.05$ between the months of the current study.

The high infection rate for *E. dispar* was inMay and July 36.36% respectively, and lower frequent was in September 9.09 % while in January, February, March, and April no infections found, non-significant differences found, table (9).

Table 8: Incidence of *E. Histolytica* Infection According to Annual Months of the Study by Conventional PCR Technique

Month	No. of Infection	Percentage (%) From Infected Patients	Chi-Square
Sep.(2015)	2	6.8	P value 0.05 = 0.0212
Oct.	1	3.4	
Nov.	1	3.4	
Dec.	1	3.4	
Jan.(2016)	0	0	
Feb.	0	0	
Mar.	1	3.3	

Apr.	2	7	
may	4	14	
Jun.	5	17.2	
Jul.	**10**	***31**	
Aug.	3	10.3	
Total	**29**	**100**	

*Significant differences at p≤ 0.05

Table 9: Incidence of *E. Dispar* Infection According to Annual Months of the Study by Conventional PCR Technique

Month	No. of Infection	Percentage (%) From Infected Patients	Chi-Square
Sep.(2015)	1	9.09	P value 0.05 = 0.912
Oct.	0	0	
Nov.	0	0	
Dec.	0	0	
Jan.(2016)	0	0	
Feb.	0	0	
Mar.	0	0	
Apr.	0	0	
may	4	36.36	
Jun.	2	18.18	
Jul.	4	36.36	
Aug.	0	0	
Total	**11**	**100**	

non-significant differences

DISCUSSIONS

Incidence of CP5 Genes for Patients Infected with *E. Histolytica* by Using Conventional PCR

The result of current study for virulence factor cysteine proteinase CP5, showed that (29) sample (72.5)% was *E. histolytica* and (11) sample (27.5)% was negative for CP5, while examined by specific primer for *E. dispar* diagnosis and was all positive for the primer, and also concluded from the results that the virulence factor CP5 found only in *E. histolytica* and didn't found in *E. dispar*, and that referred to pathogenicity of *E. histolytica*, also referred to ability to use CP5 as a diagnostic and differentiation between pathogenic entamoeba*E. histolytica* and non-pathogenic *E. dispar*.

The current results agree with other study, amolecular method with a single-PCR for amplification of a part of CP5 gene enabling to differentiate the pathogenic species, *E. histolytica*, from the non-pathogenic species, *E. dispar*, CP5 gene found in *E. histolytica* isolates from 22 positive; including 20 non-dysentery samples isolated in laboratory of public health center located in Tabriz and Bandar abbas. The new diagnostic method reported here can aid in easier and less costly identification of *E. histolytica* by routine laboratories compared to other methods and may help the health care system by avoiding use of unnecessary drugs in patients infected with *E. dispar* (Rostamighalehjaghi*et al*., 2010)

Another study showed that CP5 and CP1 genes founded in *E. histolytica* and not in other nonpathogenic species like *E. dispar*, in AlnajafAlashrafprovince, (40) sample (25)% were positive for real time PCR to detect CP5 and CP1 genes in *E. histolytica* (Al-Torfi, 2014).

A study has identified that EhCP5 coupling with goblet cell αvβ3 receptors can initiate a signal cascade involving PI3K, PKCδ and MARCKS to drive mucin secretion from goblet cells critical in disease pathogenesis, and identified the key virulence factor in live *E. histolytica* that elicits the fast release of mucin by goblets cells as cysteine protease 5 (EhCP5) whereas, modest mucus secretion occurred with secreted soluble EhCP5 and recombinant CP5 (Cornick*et al*., 2016).

Cysteine proteases of the protozoan parasite *E. histolytica* are key of virulence factors involved in overcoming host defences. These proteases are cathepsin-like enzymes with a cathepsin-L like structure, but cathepsin-B substrate specificity. In the host intestine, amoeba cysteine proteases cleave colonic mucins and degrade secretory immunoglobulin IgA and IgG rendering them ineffective. They also act on epithelial tight junctions and degrade the extracellular matrix to promote cell death. They are involved in the destruction of red blood cells and the evasion of neutrophils and macrophages and they activate pro-inflammatory cytokines IL-1 beta and IL-18. In short, amoeba cysteine proteases manipulate and destroy host defenses to facilitate nutrient acquisition, parasite colonization and/or invasion. Strategies to inhibit the activity of amoeba cysteine proteases could contribute significantly to host protection against *E. histolytica*(Kissoon-Singh*et al.*, 2011).

Cysteine peptidases of *E. histolytica*(EhCPs) are important in amoebic invasionprocess. Up to now, about 50 CPgenes have been characterized in genome of *E.histolytica*though some including ehcp1, ehcp2, ehcp-5 and EhCP-A7 are the major CPs. However, only gene products from five of these genes, EhCP1, EhCP2, EhCP3, EhCP5, and EhCP112, have been identified in cultured trophozoites(Lidell*et al*.,2006 ; Bellos*oet al*.,2004 ; Bruchhaus *et al*.,2003).

In another target for the PCR amplification was a small region (135 bp) of the SSU rRNA selected to increase the sensitivity of the test. Sixty-eight specimens tested positive by PCR, 2 for *E. histolytica* and 66 for *E. dispar*. For detection of *E. dispar*, ELISA performance was lower than that of microscopy, while PCR was much more sensitive than microscopy. Given the low proportion of *E. histolytica* cases, test performance for this species is difficult to assess. However, for differentiation, PCR performed well on simulated samples, while ELISA gave a discordant result for one of the two samples PCR positive for *E.histolytica* during the study. Also the search report confirms that *E. dispar* infection is significantly higher among travelers and underlines the possibility of acquiring *E. histolytica* infection in regions that are not areas of endemicity (Gonin and Trudel, 2003).

In Iran there was a 100% correlation between the results from the TechLab*E. histolytica* II stool antigen kit and those from nested PCR. It concluded that *E. dispar* is much more common in asymptomatic cyst passers in Iran and that antigen detection and PCR are comparable diagnostic modalities (Mohammadi *et al*., 2006).

The investigation we carried out to determine prevalence and spatial distribution of these infections shows the importance of these parasites in Babylon province, Determination of spatial distribution of these parasites will help to focus delivery of chemotherapy in this area.

These two species of protozoa are present with substantial prevalence in this area, although their spatial

distribution is not focused in any one place, determination of the population segments with the highest levels of infection will help to target the chemotherapeutic fight.

In Turkey, diagnosis of amebiasis depends on microscopical examination with saline and iodine staining by technicians in most of the diagnostic laboratories. Trichrome staining method is only performed in certain specialized parasitology laboratories, The incidence of *E. histolytica/E. dispar* by microscopy was found to be 0-17% and 2.5-13% and in the Sanliurfa province, respectively, and differentiation between *E. histolytica* and *E. dispar* in 83 PCR positives was revealed in 6 (7.2%) of the samples to be positive for *E. histolytica* versus 72 (86.7%) positive for *E.dispar*. Five (6.0%) samples were found to be co infected with *E. histolytica* and *E. dispar*(Zeyrek*et al*., 2013).

Relation of *E. Histolytica* and *e. Dispar* Infection with Age Group According to Conventional PCR

Most epidemiologic studies in developing countries carried out for amoebiasis is either based on microscopy alone or culture/ microscopy used as a screening tool, have poor sensitivity and specificity and thus fails to figure out its true magnitude. The purpose of this study was to assess the true prevalence of amoebiasis in Babylon province by PCR assay.

The highest incidence of infection with *E. histolytica* in the present study, was occurs in age group (1-10) years was (41.3) %, while the lower percentage was 3.4% for (21-30) years. And the highest incidence of infection for *E. dispar* occurs in age group (1-10) years was (36.36) %, while the lower percentage was (9.09)% for (21-30,51-60) years. With non -significant differences at level of $p \geq 0.05$ between all age groups.

The present study agree with studies, which reported age group (1-10) years was (25)% while the lower percentage was 5% for (21-30) years by real time PCR in Al -Najaf province (Al- Torfi,2014).

These result agree with the study of ma'ala(2015) in babylon province, which refer that the highest infection was for age group (1-10) year 29.3% and minimum infection percentage was 8.3% for age group (21-30,41-50) year.

Our result disagree with other studies as a study of Al- yassarre(2004) which recorded infection rate 11% for children 7< year in Al- Eskandarya also the study of Al-Muhana (2013) in Al najaf province 85.7% for age less than one year.

In Bangladesh The highest isolation rate of *E. histolytica* and *G. lambliaoccurred* in children aged 49-60 months (38.1%,80.9%) respectively, and the highest isolation rate for *Cryptosporidium* spp. occurred in children aged 13-24 months. Low infection rate was found at the age group of 13-24 months for *E. histolytica* and *G. lamblia*. Infection with *E. histolytica* (P=0.037) and/or *G. lamblia*(P=0.028) showed a steady increase with age (Ahmed *et al*., 2016).

In stool samples collected from public hospitals and primary schools in Venda in Africa examined by ELISA and a nested polymerase chain reaction (PCR). *E. histolytica* was detected in 37/197 (18.8%) and 1/47 (2.1%) samples, whereas 50/197 (25.3%) and 4/47 (8.5%) had *E. dispar* in the hospitals and schools, respectively. The age groups most infected were 0–2 years (33%) followed by 20–29 years (27%). *E. histolytica* was significantly associated with diarrhea (77.4%), and with the presence of lactoferrin (85.7%) in the stools, indicating intestinal inflammation (Samie*et al*., 2006)

Twenty one of 25 ALA cases diagnosed were in the age range of 30-60 year. The two paediatric cases included in a study were negative for amoebic a etiology (Dinoop*et al*., 2016).

Poor living condition, previous history of infection in a family member, unhygienic toilet facility, children in age group <15 year, participants having lower levels of education and daily laborers were identified as significant risk factors for amoebiasis(Nath*et al*., 2015).

Studies have shown that molecular assays are highly effective and sensitive for the detection of parasitic infections, regardless of the type of infection and the submitted sample. These tests can also be used in studies of animal models, drug efficacy, and vectorial capacity (Al- Torfi, 2014).

Another important aspect of molecular methods is their applicability to epidemiological studies, because such studies involve genetic diversity of populations and geographical distribution of parasitic diseases, susceptibility to infections and possible mutations, besides correlation between hosts and clinical manifestations, thus enabling a better understanding of the behavior of a disease among a given population (Nath*et al*., 2015).

Although high cost is still a limiting factor for the use of molecular techniques, these tests are increasingly being used in clinical diagnosis, treatment monitoring, and epidemiological studies of parasitic diseases affecting people worldwide. Their use provides detailed knowledge on the morphology, genetic characteristics and behavior of parasitic disease in the affected populations (Nath*et al*., 2015).

The opinion of the researchers who agreed with the results of the current study with their findings that the high rate of infection in this age group due to many reasons that category are the most movement and activity and thus be the most touch with the external environment factors and of little attention to personal hygiene and taking into account the health conditions as well as a play with others outside the home and eating fruits and vegetables unwashed and not washing hands thoroughly before eating and after defecation, also most children had less immunity in their bodies to fight against these parasites vice versa the adult person.

Our result agree with a study southwest of Iran among the 655 recruited patients, eleven subjects with *E. histolytica* / *E. dispar* isolates (1.7%) were identified by microscopy methods and Ten of the positive isolates (90.9%) were identified as *E. histolytica* by PCR and one isolate (9.09 %) was positive for *E. dispar*. Most of the patients with gastrointestinal disorder were in the groups of 0-9 years. Among them, 11(1.7%) isolate were identified as *E. histolytica/E.* dispar complex by microscopic examination (Pestehchian, *et al*., 2011)

E. dispar frequency was 7.12 %, *E. histolytica* frequency was 3.55 %in Mexican school children, and the frequency of intestinal parasite infection was not associated with age or gender, with a similar distribution for males and females. With regard to the risk factors in the schools, a hygiene index was established based on the conditions of the sanitary facilities, the cleanliness of bathrooms, the availability of trash cans, the presence of clean common areas, overcrowding conditions and access to health authorities and Eight different genotypes were obtained for *E. dispar* isolates with the molecular marker NKD3-D5. None of the cases in which the species *E. histolytica* was developed symptoms attributable to an invasive for disease. And the parasitized condition appeared to have no significant impact on the development or nutritional status of affected children. Also Genotype 1, which corresponds to the reference strain *E. dispar* SAW760, considered a non-pathogenic amoeba, was the most prevalent (Rojas *et al*., 2016).

Relation of *E. Histolytica* and *E. Dispar* Infection with Gender Group According to Conventional PCR

The present study showed the infection Percentage in male 59% higher than female 41%, Also the highest

incidence of infection for *E. dispar* occurs in male (73)% than female (27)%, with non-significant difference in infection rate between male and female.

May be attributed the causes of infection higher in males to many factors, including environmental and behavioral linked according to sex, the number of researchers explained that the reasons for this is due to male behavior in dealing with the environment around them and they are the most group movement and touchable with external factors and staying for long periods away from home for business purposes as well as eating the food and drink from the crowded restaurants and hawkers **(**ma'ala,2015;Salman, 2012).

Klein (2004) and Stanley (2003) referred to the effect of steroid hormone androgen in male and estrogens in female which lead to change the genetic behavior and that cause to change the ability of an individual to fight the parasitic infections of amoebiasis.

The present study agree with studies, which reportedthe infection Percentage in male 65% higher than female 35% (Al- Torfi, 2014), alsowith Al-Ebrahimi (2013) who found that *E. histolytica* more common in men than women, in the study of ma'ala(2015) found the infection rate was higher 63.8% in male than female 36.2%. In a study in northeastern Brazil the infection rate for female was 49.8 % and for male 50.2% (Calegar*et al*., 2016).

And also disagree with other study, Mengistu*et al* (2007), shawed that the infection rate was higher in female than male, this due to poor sanitary conditions and other socioeconomically factors. Also in the study of (Samie*et al*., 2006) distribution of Entamoeba infections was higher in female 9%than male 6.5%.

In the study of Rojas *et al*. (2016). The frequency of Entamoeba parasite infection was not associated with gender, with a similar distribution for males and females differences in gender between *E. histolytica* and *E. dispar*children and not statistically significant; this suggests that the main sources of infection for the children may be outside the family environment. The other place where children spend most of their time is the school this circumstance points out to the existence of wide spread sources of intestinal parasites in the community environment**.**

Our result agree a study in Colombia included direct microscopic examination frequency of the complex *E. histolytica/E. dispar/E. moshkovskii* was 18.8% (34/181) and PCR showed a frequency of 49.1% (89/181), discriminated as 23.2% (42/181) that were positive for *E. dispar*, 25.4% (46/181) for *E. moshkovskii* and 0.55% (1/ 181) for *E. histolytica*. Also, mixed infections were detected between *E. dispar* and *E. moshkovskii* at 4.42% (8/181) of the samples (López*et al.*, 2015).

Relation of *E. Histolytica* and *E. Dispar* Infection with Residence According to Conventional PCR

The result of current study showed the percentage of *E. histolytica* infection in rural regions was 69 % higher than urban regions 31 % by using conventional PCR, these result agree with the study of ma'ala(2015) which found the infection rate was higher inrural 69.0%than urban regions 31 % by using Real time PCR, also the study of Al-Torfi (2014) referred that the infection was higher inrural 67.5%than urban regions 32.5 % by using Real time PCR.

The opinion of the researchers who agreed with the results of the current study with their findings that the high rate of infection inrural area is due to the mortality and morbidity of amoebiasis and considered a big health issue in recent years, also amoebiasis influence individuals with low socioeconomic status, in addition to contaminated water in rural

areas.

The percentage of *E. dispar* infection in rural regions was(64) % higher than urban regions (36) % by using conventional PCR, these results agree with studyin Canada included results as Fifty-four samples containing *E. dispar*, 1 sample containing *E. histolytica*, and 40 samples negative for both species were identified by ELISA. Among the 40 negatives, 9 samples displayed rare or few *E. histolytica* or *E. dispar* organisms at microscopic examination (results which were confirmed by PCR). ELISA was thus less sensitive than microscopy or PCR. This report also confirms that *E. dispar* infection is significantly higher among travelers and underlines, and in rural areas (57)% than urban areas(40)%(Gonin and Trudel, 2003).

According to our results suggest that PCR should be useful as a reference test for sensitive differentiation of both species and to contribute to physicians' decision in treatment of *E. histolytica* or *E. dispar* infected patients.

In Nhue River in Vietnam as a rural area, water are intensively used in agriculture, socio-economic and personal hygiene factors determine infection with *E. histolytica*, rather than exposure to human and animal excreta in agricultural activities, In particular, the transmission routes via contaminated hands play a major role, documented in our study with a more than three- fold risk increase if hands are not washed properly. In contrast, the transmission routes via contaminated food are not of relevance. And no any association between an *E. histolytica* infection and consumption of raw vegetables, leftover food from previous days and different types of drinking water. Close contact with domestic animals was associated with an important risk increase the infections (Duc*et al.*, 2011).

In a rural area from Central Colombiathe result was23.2% (42/181) positive for *E. dispar*, 25.4% (46/181) for *E. moshkovskii* and 0.55% (1/ 181) for *E. histolytica*. Also, mixed infections were detected between *E. dispar* and *E. moshkovskii* at 4.42% (8/181) of the samples (López*et al.*, 2015).

The frequency of *E. histolytica* and *E. dispar* infection was analyzed in a rural community in the state of Morelos, Mexico, using polymerase chain reaction (PCR). Sociodemographic variables as risk factors for the infection were assessed. Results highlighted the number of individuals with intestinal parasites (43.1%) in the community, indicating extensive fecal. A high frequency of *E. histolytica* asymptomatic infection, higher than *E.dispar* infection (13.8% versus 9.6%), was detected by PCR **(**Ramos *et al*., 2005).

Relation of *E. Histolytica* and *E. Dispar* Infection with Months of the Study According to Conventional PCR

In the current study an apparent seasonal tendency was recognized in the monthly prevalence of *E. histolytica* infection was in June and July (16.6, 33.3) % respectively, and lower frequent was in October, November, December, and March 3.3% while in January and February no infections found.

The high infection rate for *E. dispar* was inMay and July (36.36)% respectively, and lower frequent was in September (9.09)% while in January, February, March, and April no infections found

Our results agree with many studies, A study in Al-Emam Ali hospital in Babylon, High rate of infection with *E. histolytica* was found in September (41.93%) and low rate of infection in May (14.06%) (Ali, 2015).

Humans infected with *E. histolytica* (patients and healthy carriers) are the only reservoir of the species and the only source of environmental contamination *via* faecal shedding of cysts. Infection of other mammals (apes, cats, dogs,

pigs) is infrequent, as these hosts act as neither reservoir nor source of infection. The lifetime of cysts in the environment varies depending on environmental conditions and can be as much as several months, Consequently, the cysts of *E. histolytica* and *E. dispar* can contaminate the environment and persist for several weeks in wastewater and on agricultural products irrigated by it. Water and plants in contact with soil or irrigated by sprinklers are the main environmental sources of the hazard, American Water Works Association (2006).

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