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Molecular Detection of *Entamoeba histolytica* in Human and Cattle

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Abstract | The purpose of this research is to determine how common *Entamoeba histolytica* is in Babylon City, Iraq, among both people and animals. One hundred samples were taken from humans and one hundred from cattle around the province, for a grand total of 200 fecal samples. PCR and microscopy were performed to assess the genetics and diversity of the *Entamoeba histolytica*. The ITS1 region of the parasite's small subunit rRNA gene was amplified using a PCR. in conjunction with microscopy. The microscopy results revealed the existence of parasite cysts and trophozoites in both human and cattle fecal samples. The PCR results indicated that the genus *Entamoeba* was detected in 43% (43/100) of the human fecal samples (27 in male and 16 in female). Moreover, the results showed that the high frequency of *E. histolytica* was in children less than 5 years old (48.6%) as compared to patient from 5-10 years and patient more than 10 years where a rate of 38.2% and 41.4% was observed, respectively. For cattle, PCR indicated 59% positivity based on the ITS gene. A total of 13 were positive in male and 46 were positive in female. On the other hand, the high frequency (63.2%) of *E. histolytica* was recorded in animals from 2 years old and above as compared with those less than 2 years (56.5%). In conclusion, the molecular approaches employed in the present investigation shown a remarkable degree of sensitivity and specificity.

Keywords | *Entamoeba histolytica*, Cattle, PCR, Babylon, Human and Diarrhea

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INTRODUCTION

Internal protozoa of the genus *Entamoeba* can cause an infection known as amoebiasis. Following malaria and schistosomiasis in terms of human mortality, *E. hist*olytica is ranked as the third most dangerous parasite (Lourenço, 2023; Baig *et al*., 2024). Amoebiasis typically spreads in underdeveloped nations due to a lack of sanitation, low hygiene standards, and overcrowding in the housing market. In contrast, people who have traveled to regions where the disease is common are the principal vector for its spread in developed countries (Fletcher *et al*., 2012). Many cases arise from persons who are carriers of the illness, referred to as cyst passers, and who excrete the parasite in their feces, either in a fully mature or partially formed state (Cui *et al*., 2019). Monkeys, dogs, cattle, and potentially pigs are naturally affected by *E. histolytica*, although the likelihood of human exposure from these species is quite low compared to humans themselves (Hossain *et al*., 2023). Global frequency of *E. histolytica* infection is significant, particularly in tropical and subtropical regions. Amoebiasis is expected to impact five hundred million worldwide (Roro *et al*., 2022). Recently a study has approximated that amoebiasis affects fifty million individuals on a yearly basis, resulting in an estimated 100,000 deaths per year (Flaih *et al*., 2021; Gupta *et al*., 2022). *Entamoeba* spp. is a type of protozoan that lives in the intestines and can cling to and damage the tissue lining the intestines. The life cycle

of this organism consists of two forms: Trophozoites and cysts. Infection is acquired through the consumption of water or food that has been contaminated with cysts. The trophozoites in the small intestine undergo excystation to reach maturity and establish themselves in the colonic area, where they then attach to the mucosal layer of the large intestines. *E. histolytica* is the causative agent of both intestinal and extra-intestinal infections (Al-Yasari *et al*., 2019; Vaisusuk and Saijuntha, 2021; Hamzah *et al*., 2020). PCR-based methods often demonstrate higher sensitivity and specificity in comparison to microscopy tests (Markussen *et al*., 2023). This study was conducted in Babylon City, Iraq to assess the efficacy of a multiplex PCR method for the molecular detection of *Entamoeba histolytic*a in both humans and cattle.

MATERIALS AND METHOD

Sampling

In various parts of Babylon Governorate, Iraq, feces samples were taken from 100 humans and 100 animals. Containers containing the samples were sealed and sent cold to the Parasitology Laboratory at the College of Veterinary Medicine at Al-Qasim Green University in Babylon, Iraq. The collection period for these samples was from October 2023 to April 2024.

Microscopic inspection

The condition of the feces and the presence of any visible blood or mucus were meticulously recorded in each sample. Parasite cysts and their vegetative stages were detected using a wet direct dye using iodine dye.

PCR analysis

The DNA was extracted after each 200 mg fecal sample was washed in 1 ml of sterile Phosphate Buffer saline with a pH of 7.2. The samples were then centrifuged at 14,000 × g for 5 minutes, following the instructions of the QIAGEN kit from Hilden, Germany. Primers used in this investigation were F: GGCCGTTCTTAGTTGGTGGA and R: GTGTGTACAAAGGGCAGGGA for a product of 374bp (Albanese *et al*., 2019; Mosa *et al*., 2022). 0.2 microliters of DNA polymerase at 5 units per microliter, 2 microliters of DNA at 100 nanograms, 0.6 microliters of each primer at 40 micromoles, and 16.5 microliters of PCR-water were utilized for the PCR reaction component. Thermocycler settings included 92°C denaturation, 40 cycles of denaturation, annealing, and extension (72°C for 45 seconds followed by 72°C for 5 minutes), and 72°C for 5 minutes of final extension.

Statistical assessment

According to Schiefer (1980), statistical analysis was performed on all samples in this study using chi-square to evaluate if there were significant differences for the analyzed variables. Significant differences were defined at the probability level (P≤0.05).

RESULTS AND DISCUSSION

Microscopically findings

The morphology and motility of the trophozoite were examined under the microscope, and the nuclei in both the cyst of the parasite and the trophozoite were stained with iodine Figure 1.

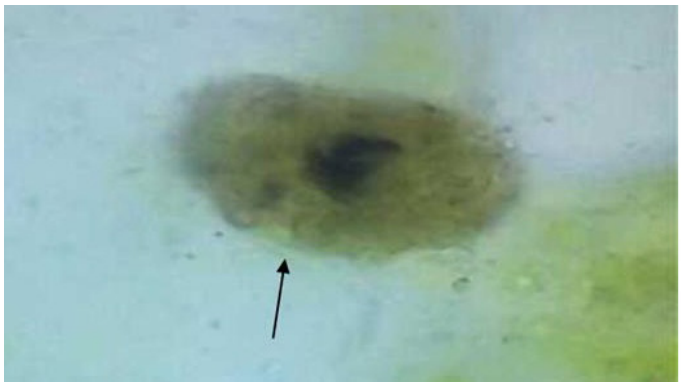

Figure 1: The histolytic cyst stage of *E. histolytica*, stained with iodine (40X), was isolated from feces.

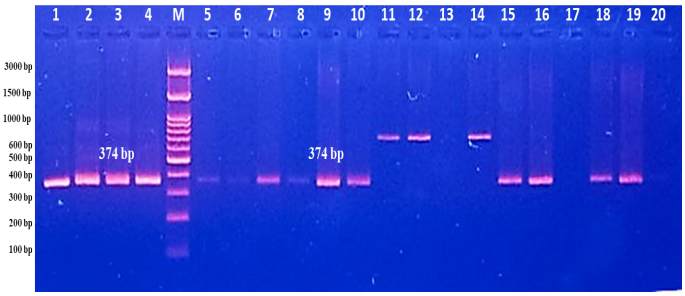

Figure 2: The PCR amplification results of internal transcribed spacer1 gene (ITS1) of *Entamoeba histolytica* isolated from human stool. Agarose gel picture appears the PCR product bands with molecular weight of 374 bp. (M) refers to (3000 bp) DNA ladder, (1) positive control. (2) Negative control. (3-20) some of PCR results of stool samples.

PCR technique

We used PCR on DNA samples that had a concentration of 400 to 710 ng/µl and a purity level of 1.6 to 1.8. Out of 100 human feces samples, 43% tested positive for the ITS gene, according to the total results of the PCR technique (Figure 2). The correlation between frequency and sex of patient showed that out of 54 male 27 were positive (27/54) 50% of infection, while in female were (16/46) 34.8% as described in Table 1. Moreover, the results shown in Table 2 showed that the highly frequency of *E. histolytica* were in children less than 5 years old 48.6% (18/37). While in patient from 5-10 years and patient more than 10 years

were 13/34(38.2) and 12/29(41.4), respectively.

Table 1: The frequency of *E. histolytica* in feces of human diagnosed by PCR.

Percentage of total (%)	Positive	Total cases	Sex
50	27	54	Male
34.8	16	46	female
43	43	100	Total
2.346868			X^2
0.125535NS			P value

Table 2: The age correlation with the frequency of *E. histolytica* of fecal human samples.

Percentage of total (%)	Positive	Total cases	Age
48.6	18	37	Less than 5 years
38.2	13	34	From 5 years to 10 years
41.4	12	29	More than 10 years
43	43	100	Total
0.827671			X^2
0.661110 NS			P value

For cattle, the total results of PCR technique showed that, out of 100 cow stool samples (59%) were positive for (ITS) gene as depicted in Figure 3. The correlation between frequency of E. histolytica and sex of animal showed that out of 25 male 13 were positive (13/25) 52% of infection, while in female were (46/75) 61% as described in Table 3. On the other hand, the results in Table 4 showed that the highly frequency of *E. histolytica* were in animals from 2 years old and above 63.2% (24/38). While the animals aged less than 2 years the frequency were 35/62(56.5).

Table 3: The frequency of *E. histolytica* in cattle that diagnosed by PCR.

Percentage of total (%)	Positive	Total cases	Sex
52	13	25	Male
61	46	75	Female
59	59	100	Total
0.675210			X^2
0.411241 NS			P value

Table 4: Age correlation with frequency of *E. histolytica* in cattle fecal samples.

Percentage of total (%)	Positive	Total cases	Age
56.5	35	62	Less than 2 years
63.2	24	38	From 2 years and above
59	59	100	Total
0.438029			X^2
0.508075 NS			P value

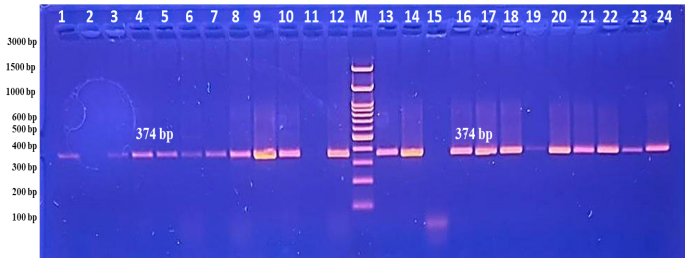

Figure 3: The PCR amplification results of internal transcribed spacer1 gene (ITS1) of *Entamoeba histolytica* isolated from cattle feces. Agarose gel picture appears the PCR product bands with molecular weight of 374 bp. (M) refers to (3000 bp) DNA ladder, (1) positive control. (2) Negative control. (3-20) some of PCR results of stool samples.

The findings of this study indicate that PCR is a highly efficient method for detecting *E. histolytica*. Therefore, employing PCR is a feasible method for distinguishing between distinct species. This approach has superior sensitivity and specificity when compared to microscopy (Hamzah *et al*., 2019). The ITS1 region of the parasite genome is essential for molecular diagnostics, as it is used for both species' identification and overall diagnosis (Paul and Kannan, 2019). López-López *et al*. (2017) have utilized a segment of the ITS1 gene to accurately distinguish and categorize *E. histolytica* and *E. dispar* in their investigation. Five single nucleotide changes were identified in this gene between the two species. The precise detection of *E. histolytica* and *E. dispar* was achieved by various approaches, including real-time PCR. Regrettably, the excessively high cost limits their usage in developing nations, and specific models may generate inaccurately pessimistic results (Calderaro *et al*., 2015; Van Den Broucke *et al*., 2018). In addition, the PCR method based on the ITS1 gene was shown to be extremely efficient in the current study. The user's text is a single period. The findings align with the study conducted by Saba and Meki (2017), which determined that the ITSI gene is the suitable DNA region for identifying *E. histolytica* in the middle Euphrates region of Iraq. The findings of this experiment were like those seen by (Zebardast *et al*., 2014; Mohmood *et al*., 2020). The infection rate was 62.96%, while Iran's infection rate stood at 67.7%. A study conducted by Sajid *et al*. (2024) revealed that 3.2% of Malaysians were exclusively infected with *E. histolytica*, whereas 13.4% had concurrent infections of both *E. histolytica* and *E. dispar*. According to the studies conducted by Abdel-Naim (2012) and Al-Aarif and Al-Jarjary (2023), the infection rate of *E. histolytica* among patients in the outpatient clinic at Al-Zaqaziq University Hospital was 50%, while the infection rate of *E. dispar* was 35%. Emphasizing the efficacy of the primers developed for diagnosing E. histolytica is crucial. The primers successfully amplified the target section in the ITS1 region, leading to the presence of migration products

at the target test. The size of these products was 374 base pairs, as depicted in Figures 2 and 3. This confirms and validates the accuracy of the diagnosis. The discrepancies in the outcomes of the PCR approach can be ascribed to variations in the methodologies employed for DNA extraction from fecal samples and the implementation of PCR, together with potential disparities in the quantity of parasites detected in the feces. Alternatively, the variations in infection rates of these parasites may be attributed to their random spread across numerous countries worldwide, influenced by diverse climatic conditions, cultural practices, and traditional beliefs.

CONCLUSIONS AND RECOMMENDATIONS

Microscopic examination continues to be a dependable technique for verifying the existence of the parasite. Nevertheless, it lacks efficacy in assessing the parasite's pathogenicity or discerning its precise classifications. Hence, it is advisable to employ serological or molecular diagnostic techniques in addition to the findings from microscopic inspection. The molecular and serological methods used in this study demonstrated a high level of sensitivity and specificity.

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NOVELTY STATEMENT

Evaluate the potential of Molecular Detection of Entamoeba histolytica in Human and Cattle.

AUTHOR'S CONTRIBUTION

Qasim Jawad Amer and Mohammed Jawad Kadhim are participatred in research desing, proposal writing, experimental and clinical works and also drafting the manuscript.

Ethical approval

Ethical approval was obtained from the ethical committee (127/2023) in the Al-Qasim Green University, College of Veterinary Medicine.

Conflict of interest

The authors have declared no conflict of interest.

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