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Gene expression of *Entamoeba histolytica* in diarrhetic patients from Wasit province

Thuraya Khaled Abdulwahed
Medical Physics Department, Kut University College, Wasit, Iraq, 52001
Email: Thuraya.khaled@alkutcollege.edu.iq

Abstract
In order to identify the amoebiasis-causing *Entamoeba histolytica* in people who were suffering from diarrhea, this study made use of molecular detection technologies. We gathered stool samples from one hundred people who were experiencing symptoms of diarrhea and classified them according to their age and gender. The findings of an inspection using a direct microscope showed that ninety percent of the samples had *entamoeba* germs. According to the findings of a microscopic inspection, the rate of infection with *Entamoeba* was much greater in females (86.73 percent) than in men (70.8 percent), and this difference was significant at the level of 0.05 for the P value. The investigation also indicated significant infection rates in the age categories of 5-11 years and more than 50 years (respectively 85.23 percent and 78.81 percent), but a reduced infection rate in the age group of more than 2 years (43.45 percent). The nested multiplex polymerase chain reaction, in which the small subunit gene was used, was able to establish that the infection rate for *E. histolytica* 91 was greater (91 percent). The prevalence of the *Entamoeba histolytica* infection is much higher in females (89.23 percent) than it is in males (82.35 percent). Both the older age group (>2) and the younger age group (28-48 years) had an infection rate that was one hundred percent attributable to E. histolytica. Based on gender, age, and the results of nested multiplex PCR, this gave the proportion of people that were infected with *Entamoeba histolytica.*
Keywords: *Entamoeba histolytica*, diarrhea, PCR, Gene Expression

Introduction
Entamoeba is a kind of protozoan parasite that may infect both vertebrates and invertebrates. Entamoeba can also cause disease in humans. *Entamoeba* are capable of infecting both animal and plant hosts and causing sickness (1). *Entamoeba species* that have been discovered in the lumen of the human gut; however, there are up to twenty-four *Entamoeba species* that have been identified across the world. *Entamoeba histolytica* is a protozoan parasite that is responsible for a disease that is the third biggest cause of death in the world, after malaria and schistosomiasis in terms of prevalence. *Entamoeba histolytica* causes the condition known as Entamoebiasis (2, 3). There are around more than 200 million people throughout the world who are afflicted by the condition, and between 50000 and 120000 people every year lose their lives as a direct consequence of the ailment (4, 5). Two of the most important factors that contribute to the rapid spread of the disease around the world are the presence of unclean water and the absence of sufficient sanitation systems in developing nations, both of which are contributing factors. In addition, it is becoming more common in these areas to disregard the symptoms of parasitic illnesses, even if they may point to a potentially life-threatening condition (6). Due to the fact that amoeba are the organisms

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responsible for causing amoebiasis, the condition is classified as a parasitic illness intestinal disease caused by the parasite E. histolytica that causes major mortality and morbidity (7). The Wasit region has been the site of a number of investigations into distinct species of *Entamoeba*, including one that was carried out by who utilized the polymerase chain reaction (PCR) method in order to identify and differentiate between *Entamoeba* species. Other researchers have also conducted their research in this region (8, 9). As a direct result of the objectives in this research are to make use of nested multiplexing in order to determine the rate of infection induced by *Entamoeba histolytica* based on factors such as age and gender.

Material and methods

Sample collection

Patients in the Wasit province who exhibited symptoms of diarrhea between the dates of January 4, 2021 and November 1, 2021 were eligible to have their fecal specimens taken, and there are a total of one hundred of these specimens. These patients were seen at various healthcare facilities in the Wasit province, including medical labs, medical health centers, General Alzahraa, and a few clinics and hospitals. These people have been examined at a selection of Waist's plethora of medical facilities, which can be found all around the province. The samples, after being taken from the site in which they were acquired in Eppendrof tubes, were stored in cold storage containers after being transported from the region in which they were obtained.

Microscopical detection

In the laboratory, the specimens were cut into two parts, and each portion was assigned a weight that was nearly exactly the same as the weight of the other section (1.5-3 gm). The original sample was put in an Eppendrof tube and maintained at a temperature of minus twenty degrees Celsius in order to extract DNA from it. This has to be done in order to successfully extract the DNA. Mount smear techniques often called for the use of adequate amounts of distilled water, with the typical amount falling between 15 and 25 milliliters.

PCR assay

In order to extract DNA from the stool samples, a Presto TM Stool DNA Extraction Kit was utilized, which was acquired from a company that does business under the name Geneaid/Korea. The kits that are often used in the execution of this method are normally quite a bit more compact than the kit that was before being used. The following is a synopsis of the procedures that were carried out in line with the instructions that were given by the manufacturer: After placing 200 milligrams of feces, 900 milliliters of ST1 buffer, and a short vortex inside of a Bead beating tube containing ceramic beads, the combination was allowed to incubate for five minutes at -70 degrees Celsius, and then it was vortexed for 10 minutes while at room temperature. After that, the substance was centrifuged for two minutes at a speed of 9,000 x g while it was allowed to remain at room temperature (RT).

PCR de-inhibition

The sample was allowed to incubate at a temperature of four degrees Celsius for a total of five minutes. After that, the sample was centrifuged at 15,000 x g for three minutes at room temperature in order to remove any insoluble particles and PCR inhibitors that could have been present in it. The Inhibitor Removal Column was found in the clear supernatant that was

left behind after a 2 ml centrifuge tube was used. Within the picture, the ring in purple that represents this column may be seen. A total of one minute of centrifugation at 15,000 x g was performed on the column at room temperature before it was discarded. The centrifugation lasted for one minute. The flow-through that was collected from a centrifuge tube that carried 2 milliliters of material was stored so that it may be used for studies on DNA binding.

Binding and washings

Following the addition of a total of 800 microliters of ST2 buffer into the flow-through, the contents of the flow-through were subjected to a vigorous mixing technique for duration of five seconds. This procedure lasted for the duration of the experiment. The duration of this procedure included the whole of the flow-through. After that, a 1.5 mL Collection tube was given a green ring, also referred to as a green column, and it was placed inside of the tube. The green column was another name for the ring. This process was carried out three times in all. In the green column was a mixture of samples that had a total volume of 800 microliters when they were injected. Microliters served as the unit of measurement for the volume of the injection. Following that, the flowthrough was separated at a velocity of 18,000 x g for one minute, during which time the temperature was held constant at room temperature. Position the green Column within the Collection Tube, which has a fluid-holding capacity of 1.5 milliliters and can accommodate the Column. After that, the other parts of the sample combination have to be shifted with the help of the green Column. In order to properly separate the components, they were centrifuged for one minute at 18,000 x g while the temperature was maintained at room temperature during the process (RT). To the green column was added the ST2 Buffer, which had a combined capacity of three hundred and fifty liters. After that, they each continued on their own paths for the next thirty seconds while being exposed to an acceleration that was 16,000 times more than their average acceleration caused by gravity in RT. Following the removal and disposal of the flowthrough, the green column was reinserted into the 1.5 ml Collection tube in order to facilitate a second round of collection. This was done so that the sample could be taken. This was done so that we could collect the sample in its entirety, and it was successful. It was concluded that a total of 500 microliters of Wash Buffer needed to be added to the green Column. This was a complete volume increase. After that, they went through an experience that was comparable to 18,000 times their body weight in g, and then for the next thirty seconds, they were kept apart at R. The green Column has to be reinserted into the 1.5 ml Collection Tube as soon as the flow-through has been thrown away. In order to dry the column matrix, the dry green Column collecting Tube was separated at 18,000 x g for three minutes while the temperature was maintained at room temperature. This was done while the temperature was kept at room temperature.

Qualification of DNA

Using a Nanodrop spectrophotometer, an investigation and evaluation of the quality of the genomic DNA that had been isolated from samples of feces were carried out. The samples had been taken from different individuals. This operation was carried out as planned. The following techniques, which are described below, illustrate how to use this instrument to

explore and assess the quality of DNA. This is accomplished by measuring the absorbance of the sample at a variety of wavelengths (270-280 nm). x After the Nanodrop program has been activated, choose the application that satisfies your requirements in the way that comes the closest to fulfilling those requirements (Nucleic acid, DNA). In the course of the process of cleaning the measuring pedestals, we discovered that we needed to go for dry wipes on a number of occasions. To properly reset the instrument, carefully pipette two microliters of free nuclease water over the surface of the bottom measurement pedestals. This will ensure that the device is properly calibrated.

PCR reaction

Gene aid, a firm that has its headquarters in Korea, was kind enough to provide the three extraction kits that were necessary for the process of extracting DNA from human feces and urine. In addition, forward and reverse primer pairs were employed in order to complete amplification of the 19S rRNA gene by employing nested multiplex PCR. This was done so that the gene may be replicated more than once. This was done in order to establish beyond a reasonable doubt the existence of the gene. The initial primer pair was created in such a way that it allowed for the identification of 900 base pairs (bp) of the 19S rRNA gene in the *Entamoeba*. Primers were used in tandem, and then that tandem was put to use in the analysis of (*E.histolytica*). This research, which used a one plus primer design, drew its information from the NCBI-Genebank as its primary source of data. This primer, which contains 800 base pairs and may be used in nested PCR investigations including E. histolytic as well as nested PCR tests comprising Entamoeba spp, was kindly donated by the Macroge corporation in Korea (532bp). Primers and probes are two examples of additional PCR reaction components that may be kept in a standard Maxime PCR PreMix container. These components are called primers and probes, respectively. In addition, the PCR component that is outlined in the table that is positioned directly above this one is included inside this tube. In addition, the PCR component that is outlined in the table may be found included inside this tube (dNTPs, pH: 9.0, KCl, MgCl2, stabilizer, tracking. dye, Tris-HCl and Taq DNA polymerase). Following that, a PCR tube was inserted inside of an Exispin vortex centrifuge, and the centrifuge was used to spin the tube at a speed of 4000 rpm for a length of three minutes. This process was repeated three times. This procedure was carried out a total of three times. Following that, the samples were put into a PCR thermal cycler to be processed. The abbreviation for "polymerase chain reaction" is which stands for "polymerase chain reaction. The components of the PCR master mix that are described in the table that is located above are then placed in a typical Maxime PCR PreMix, which is an example of a mixture that includes all of the additional components that are required for the polymerase chain reaction. Following this step, the PCR master mix is ready to be used. After the PCR master mix has been assembled, this next step may then be carried out. The table that is located just above this one has an explanation of the many components that make up the PCR master mix (dNTPs, pH: 9.0, KCl, MgCl2, stabilizer, tracking dye, Tris-HCl and Taq DNA polymerase). After that, the PCR tubes were spun for three minutes at a speed of four thousand revolutions per minute inside of an Exispin vortex centrifuge. Following that, the samples were loaded into a PCR thermal cycler and allowed some time to complete their respective processes.

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Estimation analysis

After all of these steps had been carried out, the PCR's findings were analyzed using an agarose gel electrophoresis after everything was finished: To produce agarose gel with a concentration of one percent, 2X TBE was dissolved in a water bath maintained at one hundred degrees Celsius for fifteen minutes prior to the temperature being lowered to fifty degrees Celsius. This step was performed in order to manufacture the gel. This procedure was carried out so that the gel could be produced successfully. Following that, three microliters of ethidium bromide dye were added to the agarose gel solution so that it could be colored. This was done so that the gel could be used. After finishing the meticulous process of positioning the comb in the tray, the agarose gel solution was then poured into the tray. This procedure was repeated a number of times until the tray could hold no more stuffing. Once allowing the comb to first set for fifteen minutes at room temperature while the agarose gel solution was being made, it was carefully removed from the tray after the initial setting period had concluded. This was done at the same time as the solution for the agarose gel was being prepared. This therapy was administered a total of three times over the course of the investigation. Position 6 on the gel tray that was placed inside of the electrophoresis chamber was adjusted in order to make room for the inclusion of a 2X TBE buffer. The chamber accommodates the gel tray within its confines. The PCR product was added to each of the wells in the comb at a concentration of 10 microliters, with the exception of the first well, which got only 4 microliters of the PCR product. Four microliters of the PCR product. This was due to the fact that the first well served as a control for the experiment (120bp Ladder). After that, there was an electrical current source that lasted for the whole of one full hour, had 120 volts, and 90 AM. This current source was there after the event in question. In order to get vision into the findings of the PCR, a transilluminator that emits ultraviolet (UV) light was used (10).

Results

Microscopical findings

Our study demonstrated that the percentage of infections caused by *Entamoeba spp.* was significantly higher in females (86.73 percent), in comparison to males (70.8 percent). Because of the way nature works, females are more likely to handle food preparation and household chores, which can expose them to water or food that has been contaminated by *entamoeba* cysts. This difference in infection rate between females and males can be attributed to a wide variety of factors, including hormones and the fact that nature works in such a way that females are more likely to handle food preparation and household chores. The most recent revision's findings revealed that the highest number of infectors was found in the age group of 5-11 years old, as well as in the age group of more than 49 years old. *Entamoeba species* were found to be responsible for these findings. A rate of infection that was 85.9 percent was found in both of these age groups. The age group of less than 2 years old had a lower infection rate than the other age groups, which had a percentage of 63.23

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percent. When viewed under a microscope, trophozoites of the parasite *Entamoeba histolytica* could be seen in both female and male positive samples (figure 1, 2, 3).

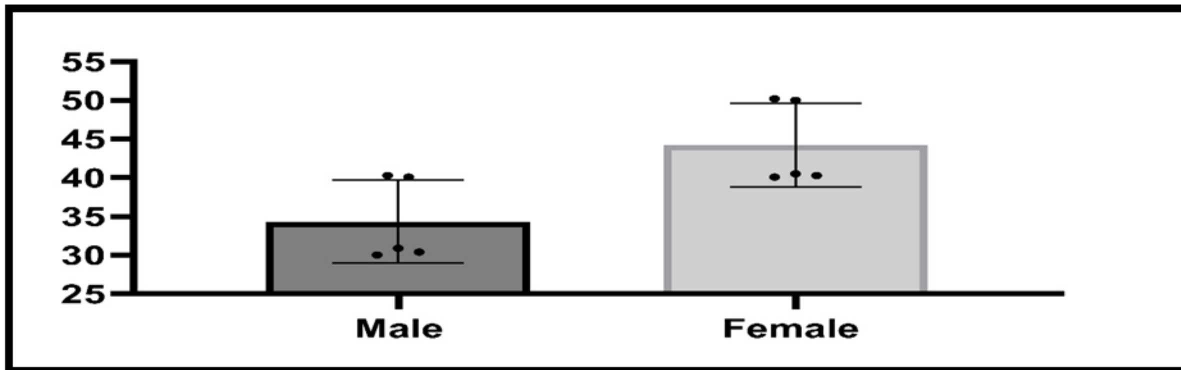

Figure 1: microscopical detection *Entamoeba histolytica* could be seen in both female and male positive samples.

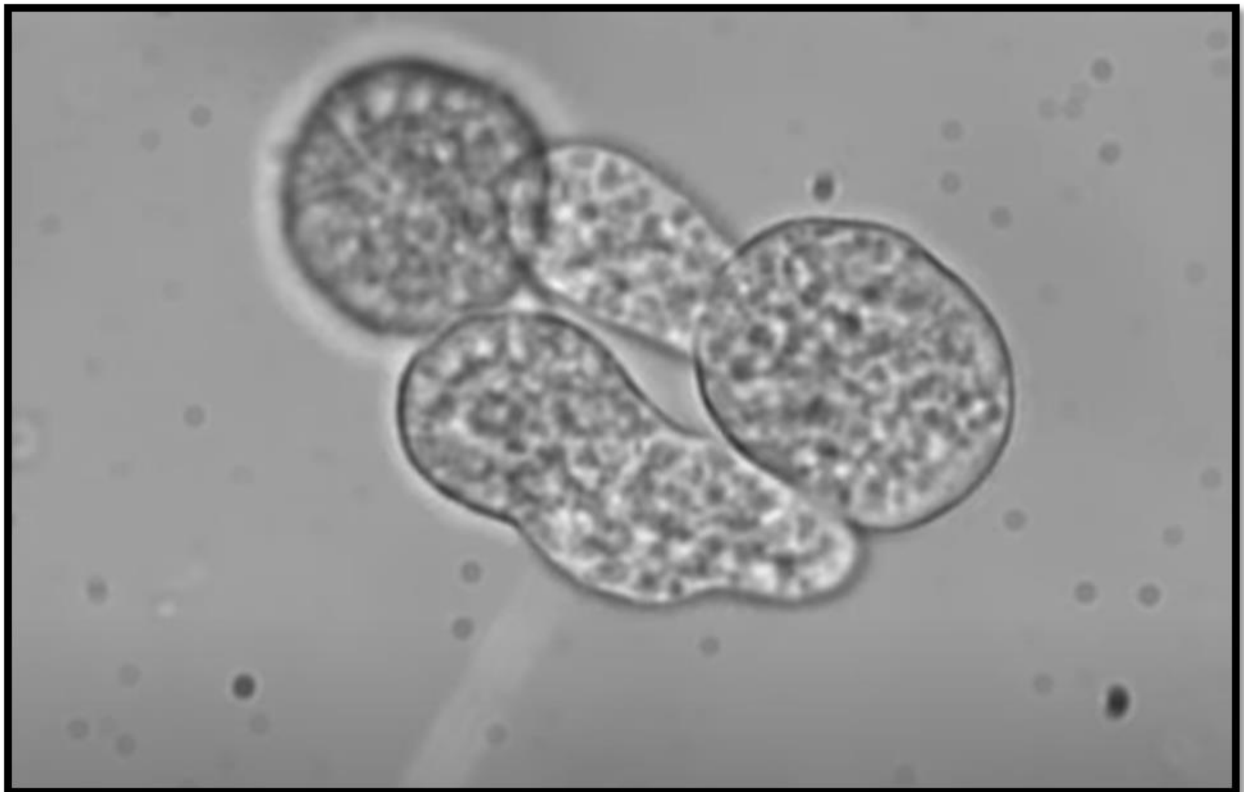

Figure 2: microscopical detection *Entamoeba histolytica* could be seen in male positive samples.

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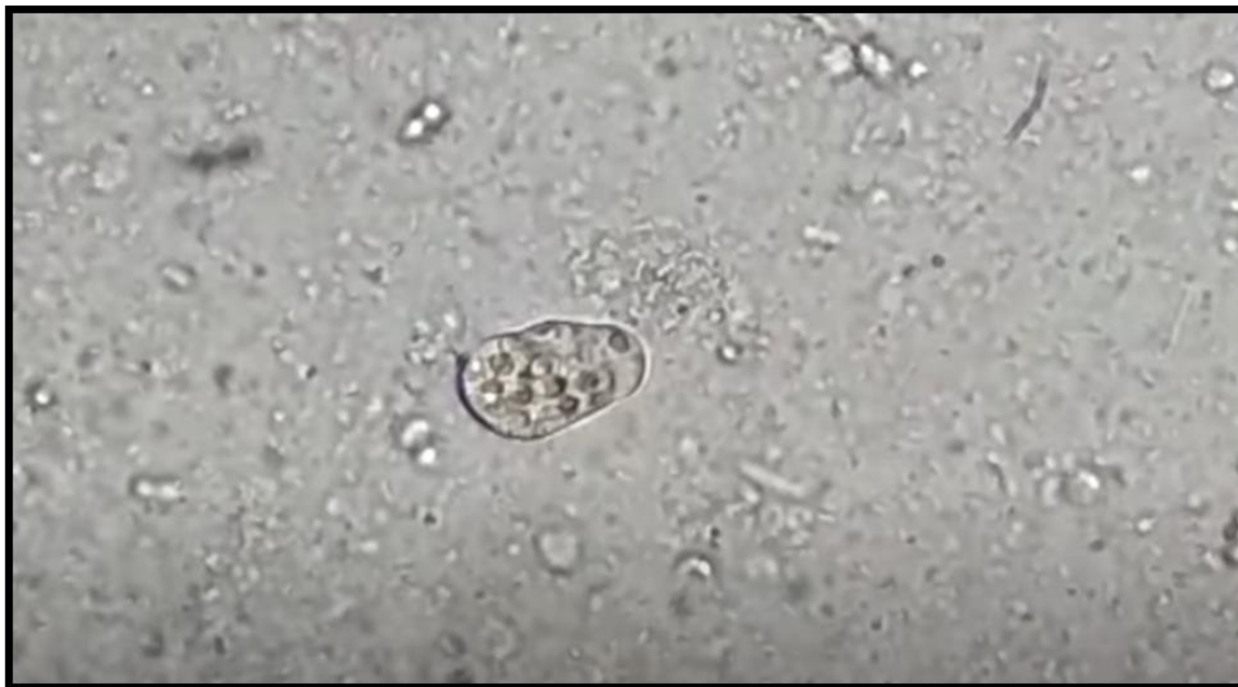

Figure 3: microscopical detection *Entamoeba histolytica* could be seen in female positive samples.

Molecular finding

The first round of PCR for DNA samples showed that 79 out of 100 stool samples successfully amplified the 19sRNA gene using nested multiplex PCR. A success rate of 79 percent can be calculated from this. In spite of the fact that Entamoeba infections are typically quite mild, there are certain strains that have the potential to attach themselves to the bowel wall, which can lead to severe extra bowel pathogens and amoebic colities. In spite of this, amoebic dysentery continues to be observed in clinical settings (Table 1, Figure 4).

Table 1: molecular detection*Entamoeba histolytica* could be seen in both female and male positive samples.

Gender	Samples of study	Infected results (+)	Total Percentage
Male	54	59.2	70.8%
Female	46	64.23	86.73%
Significancy	P. value = 0.002		

P. value <0.005

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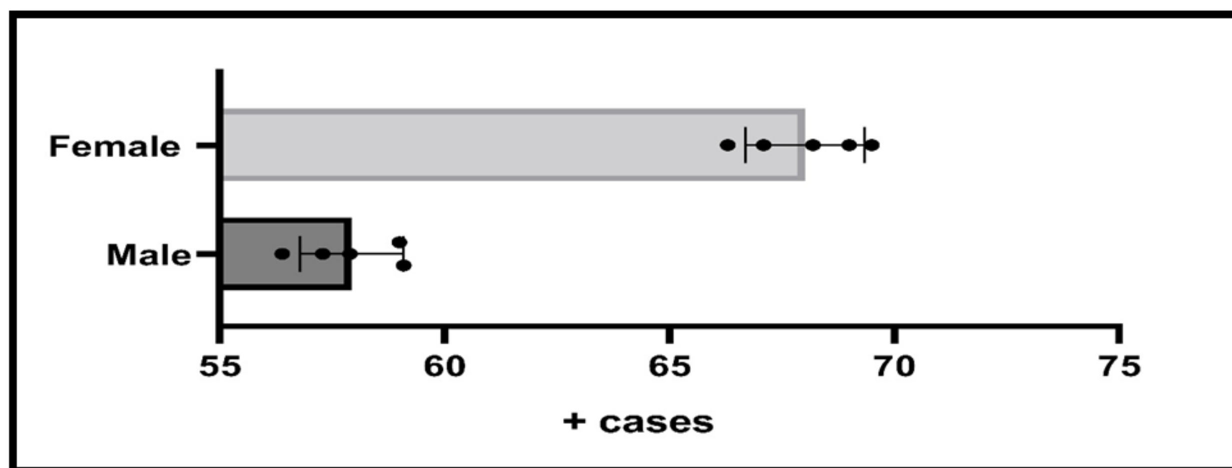

Figure 4: molecular detection*Entamoeba histolytica* could be seen in both female and male positive samples.

Discussion

The findings of our study are in line with those that were recounted, which indicated that the waist province in Iraq had a higher ratio of females to males (11). On the other hand, the findings of the WASIT revealed that females had a significantly higher infection rate than males (86.73 percent), as compared to the results of the test conducted on males (70.8 percent), (12). Despite the findings of a recent study which demonstrated that the percentage of Entamoeba spp infections was more prevalent in females (44.1 percent) compared with males, the researchers concluded that males were equally susceptible to contracting the parasite (22.3 percent), (13). Females are more likely to be exposed to water or food that has been contaminated by Entamoeba cysts when they are cooking or cleaning (14). This difference in infection rate between females and males may be due to a number of factors, including hormones or the fact that nature works differently for females, which means that females are more likely to become infected when they are exposed to water or food that has been contaminated by Entamoeba cysts (15). Entamoeba species discovered in According to the findings of the most recent round of research, the age range of 5-11 years old and the age range of more than 49 years old each had a percentage of 65 percent in terms of the number of infectors that were discovered within their respective populations (16). The age group of less than 2 year old had a lower infection rate overall, with a percentage of 45.3 percent. They found a higher prevalence of infection among children aged 5 to 14 (31 percent), but a lower prevalence among children aged 0 to 2 years old. This is in agreement with what we already knew (11 percent), (17). In a similar fashion, many of the researchers scored more prevalence infection of Entamoeba spp in young children's they in Iraq in Yemen, that they recorded high prevalence of infection (50.2 percent) in age of less than ten years but did not agree with recorded lower infection rate (7.7 percent) in age of over 45 years (18). This was the case despite the fact that they recorded lower infection rate in age of over 45 years (19). Would be described by the effect of various aspects for the children as a result of their activities, which are the result of them caring less about their own sanitation (20). These activities are the result of the children spending less time cleaning up after themselves (21). Additionally, the areas of their bodies that are exposed have the highest concentrations of nutrients, which

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come from a wide variety of sources (22). Others may be resistant to infectious organisms due to the use of medicines that target a variety of pathogen (23). While a high infection rate in older age may be the cause of the detail as the elderly individuals showing to types of infections among life them like weakened immune systems, this may not be the case for all elderly people (24). Despite the fact that a high infection rate in older age may be the root cause of the detail, this holds true (10, 25). In the first round of PCR for DNA samples, the 19sRNA gene was successfully amplified in 86.8 percent of the stool samples using nested multiplex PCR, as indicated by the results of that round of testing (26). Even though Entamoeba infectious are not typically dangerous in the majority of cases, there are strains some of which can attach to the bowel wall, resulting in severe extra bowel pathogens and amoebic colitis (27). These conditions can be caused by certain strains of Entamoeba. In terms of the clinical picture, amoebic dysentery is always present. Using nested PCR, the researchers found that there were 55 percent positive samples out of a total of 80 samples in Baquabah city (28). These findings are consistent with the findings that were found in Iraq, which recorded a lower percentage than ours did in this city (29). despite the fact that the nested multiplex PCR method recorded an even higher percentage (88 percent), respectively (30).

Conclusion

The most recent report found that patients who suffered from diarrhea had a higher rate of infection with Entamoeba histolytica. This information was found in accordance with the findings of a previous study. According to the findings of the research, the female participants had a significantly higher infection rate with E. histolytica than the male participants did; however, when the two groups were compared using the P0.05 threshold, there was no significant difference found between them. Even though the microscopic examination method is a reliable test for the detection of *Entamoeba* spp. in diarrheal patients, there was a high probability of incorrectly diagnosing amoebic dysentery as being caused by Entamoeba histolytica. This was due to the fact that the microscopic examination method relied on the observation of a small number of organisms. This was because the bacteria were much too small to be seen by the human eye even when viewed through a microscope. According to the findings of this study, the infection rate of E. histolytica existed at a large prevalence in people whose ages ranged from less than 2 years old to between 25 and 49 years old. The median age of the participants in this study was 25 years old.

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