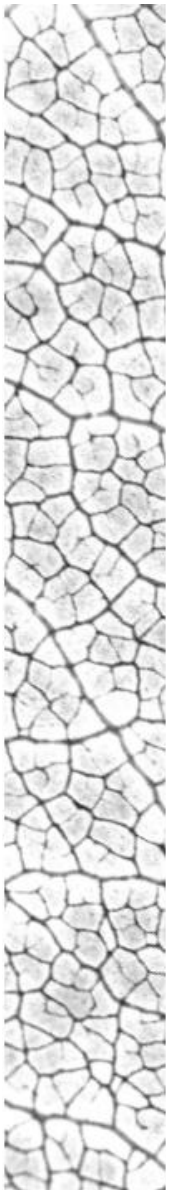

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Detection of *entamoeba histolytica and cyptosporidium parvium* in drinking water of Al-diwaniyah City, Iraq

Khadeeja Abees Hmood AL-Khalidy [1*], Hayder Kamil Jabbar [2]

[1] Department of Environment, College of Sciences, University of AL-Qadisiyah, IRAQ
[2] Department of Medical and Basic Science, College of Nursing, University of AL-Qadisiyah, IRAQ
*Corresponding author: Khadeeja.abees@qu.edu.iq

Abstract

The current study was carried out to detect parasitic contamination depending on presence some intestinal protozoan such as *Entamoeba histolytica* (in cysts phase) and *Cryptosporidium parvium* (in Oocysts phase) in drinking water in some neighborhoods in the city of Al-Diwaniyah (Al-Sadr, Al-Nahdah, Al- Zahra, AL- Orouba, A- Zaytoon, Al-Furat) in the Al- Qadisiyah province, (36) samples of water collected (6-samples from each neighborhood), one sample each month at the period from the beginning of January / 2017 to the end of July/2018, method was used concentration and modified Ziehl-Neelsen. The results of the study indicate that the total percentage of water contamination was 5/36 (13.88%), also indicated that the highest percentage was recorded (33.33%) of *Entamoeba histolytica*-cystsin Al- Nahdah neighborhood, while the lower percentage was (16.66%) in Al-Sadr, Al- Zahra, Al- Orouba neighborhoods, the statistical analysis indicated to significant differences in the percentage of contamination between Al-Nahda and the other neighborhoods at the probability (P≤0.05).

The results of the molecular examination using the conventional polymerase chain reaction indicated that the proportion of the18S rRNA gene (501bp) responsible for the diagnosis of *Entamoeba histolytica* in drinking water was 80% while The molecular examination results indicated that there was no found of the 18S rRNA gene (347bp) which was responsible for d diagnosis of *Cryptosporidium parvium* in drinking water.

Keywords: *Cryptosporidium, Entamoeba,* concentration, modified Ziehl-Neelsen, PCR

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INTRODUCTION

Entamoeba histolytica is one of the most dangerous parasites in humans and is caused amoebic dysentery, which is one of the problems of public health in the tropics and subtropical regions (Miceli, 2004; Lejeune et al., 2009). It is ranked third among the three most dangerous parasites after malaria and bilharzias (Nongyao et al, 2004), *E. histolytica*passes through its life cycle with two main phases: the trophozoite stage and the cyst, the latter represents infective stage, infection in this parasite occur by eating food and drink contaminated with that stage (Linford et al., 2009).

Also there was other protozoan parasite that can cause an infection called Cryptosporidiosis in humans or animals through digestion of Cryptosporidium-Oocysts, via for example contaminated food or drinking water, pollution of public drinking water can cause a simultaneous infection in a big part of the population, So it is necessary to make sure that the drinking water does not contain infectious Oocysts (Betancourt and Rose, 2004; Harfoush, 2020).

Cryptosporidium parvium is one of the most widely distributed intestinal parasites in the world ,It belongs to the genus *Cryptosporidium*, this parasite is called hidden spores, because it is difficult to distinguish spores in the Oocyst (Moon and Woodmansee, 1986). This parasite was an important that causes diarrhea in humans and various animals and affects a wide range in vertebrates such as humans, domestic and wild animals (Soave and Armstrong, 1986), the risk of *Cryptosporidium* lies in the multiplicity of transmission methods. This disease was transmitted by direct contact with infected animals, the contamination of water and food with Oocyst also plays an important role in the transmission of infection, and it is also transmitted through the inhalation of Oocyst in the respiratory tract (Briefs, 2002).

The Aim

The aim of this study was to determine contamination of drinking water with *Entamoeba histolytica* and

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*Cryptosporidium parvium*in some neighborhoods in the city of Al- Diwaniyah, Iraq.

MATERIALS AND METHODS

Collection of Samples

100 milliliters water samples were collected of the residential neighborhoods such as AL-Sadr, Al- Nahdah, Al -Zahra, Al -Orouba, Al-Zaytoon, Al-Furat, these neighborhoods were in AL-Diwaniyah city.Iraq.

Samples were placed in sterilized bottles with sealed for the purpose of detecting the contaminated parasites of drinking water, The date and location of the sample collection were recorded on all collection bottles and transferred to the laboratories of Al- Qadisiyah University / College of Science / Department of environment for the purpose of searching for the presence of *E. histolytica-cyst* and *C.parvium-Oocyst.*

DIAGNOSIS

Microscopic Diagnosis

Samples examination By Concentration, flotation and Modified Ziehl-Neelsen

In search of *E. histolytica-cyst* and *C.parvium-Oocyst* in the drinking water samples, the method of concentration was used according to (Mohsen et al., 2013), which included the filtration of the collected water samples and directly upon arrival to the laboratory using filters filtration, in order to increase the concentration of the parasite on the five filters and put in the suppression of filtration with the continuous replacement of the upper filter with a new filter at the bottom when you notice the low rate of filtering speed resulting from the clogging of the holes so that always remain filter layer consisting of five filters, the filters were then washed with 200 ml of distilled water, the parasites were then isolated by the method of dilution with the graduated sugar solution by placing (9) ml in a test tube and (2) ml of sheathers solution (SG 1.27) were added, the tubes were then placed in the Centrifuge in 3000 cycles / Minutes, the floating part was placed on a glass slide then placed cover of slide then fixed with 70% methanol ,then staining using Iodine solution and the modified ziehl-Neelsen technique (Henriksen and Pholenz, 1980), then all these slides were examined under the microscope with 10X and 40Xmagnification force for diagnosis of, of *Entamoeba histolytica*- cysts *Cryptosporidium parvium*-Oocysts.

Molecular Diagnosis

DNA extraction

DNA was extracted from the samples which gave a positive result of the microscopic examination, which numbered 5 samples according to the method that mention in (Rebecca et al., 2003), using the Genomic DNA Extraction Kit Produced by Korean company Bioneer.

Table 1. The primers used in this study with their nucleotide sequence and product of PCR

Primer		Sequence	product
Entamoeba histolytica	F	GGCCGTTCTTAGTTGGTGGA	501bp
	R	GTGTGTACAAAGGGCAGGGA	
Cryptosporidium parvium	F	GGTGACTCATAATAACTTTACGG	347 bp
	R	ACGCTATTGGAGCTGGAATTAC	

Table 2. The PCR reaction mixture

PCR master mix	Volume
DNA template	5µL
Forward primer 10pmol	1.5µL
Reverse primer 10pmol	1.5µL
PCR water	12µL
Total	20µL

Table 3. PCR Thermo cycler conditions

PCR Step	Repeat cycle	Temperature	Time
Initial denaturation	1	95 C	5 min
Denaturation	30	95 C	5 sec.
Annealing		58 C	30 sec
Extension		72 C	3 min
Final extension	1	72 C	10 min
Hold	-	4 C	Forever

Conventional PCR technique

PCR technique was performed using 18S rRNA gene responsible for diagnosis *Entamoeba histolytica* and *Cryptosporidium parvium* in drinking water using the gene bank site to obtain the full genes sequence using the Primer 3plus program, the primers were designed and processed by Korean Pioneer Company as in **Table 1**.

Preparation of PCR master mix

The PCR reaction mixture Prepared according to the company's instructions using the AccuPower® PCR Master Mix kit as in **Table 2**.

Statistical Analysis

Data was analyzed using the statistical software Spss version 10.5 software where the chi-square test under probability level P≤0.05, was used to determine the significant differences as mentioned in Niazi (2001).

RESULTS AND DISCUSSION

Microscopic Diagnosis

Entamoeba histolytica and Cryptosporidium spp were water-borne pathogenic Protozoan's spread through fecal-oral transmission which cause several health problems, to include gastrointestinal diseases associated with the consumption of contaminated water, the results indicated in **Table 4** to the contamination of the drinking water in residential neighborhoods in Al-Diwaniyah city with intestinal parasites, it was noted that the total pollution rate was 13.88% (five positive samples of the total of 36 samples) where the presence of *Entamoeba histolytica*- cystin one sample in Al- Sadr and Al-Zahra in rate (16.66%),two samples in Al-Nahda with (33.33%) and one sample in Al-Orouba, which is contaminated with *Cryptosporidium parvium*- Oocyst *and Entamoeba histolytica*- cystin rate (16.66%), while

Table 4. Numbers and percentages of *C. parvium, E. histolytica*in the water of neighborhoods in the city of AL-Diwaniyah

neighborhoods	N. Examined	N. Positive	Registered parasites	%	N. Negative	%
Al-Sadr	6	1	*Entamoeba histolytica*	16.66	5	83.33
Al-Nahda	6	2	*Entamoeba histolytica*	33.33*	4	66.66
Al-Zahra	6	1	*Entamoeba histolytica*	16.66	5	83.33
Al-Orouba	6	1	*Cryptosporidium parvium* + *Entamoeba histolytica*	16.66	5	83.33
Al-Zaytoon	6	0	-	0	6	100
Al-Furat	6	0	-	0	6	100
Total	36	5	-	13.88	31	86.11

*refer to significant from other at P≤0.05
א2 calculated: 3.947 א2 tablets: 2.920 א

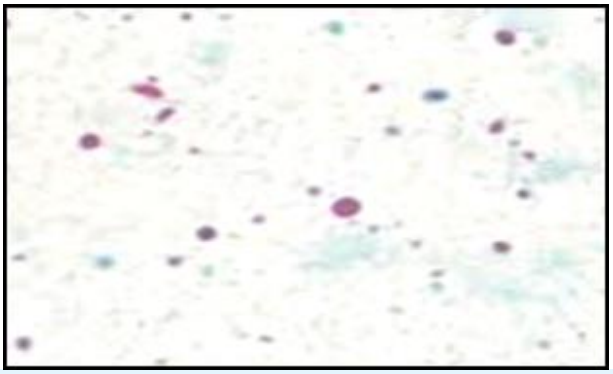

Fig. 1. *Cryptosporidium parvium*oocysts in water stained with Ziehl-Neelsen modified acid-fast (400X).

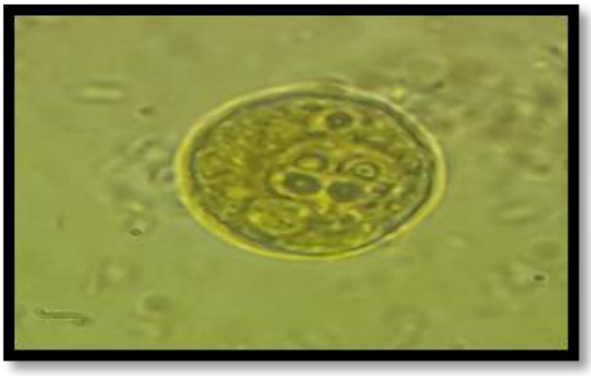

Fig. 2. *Entamoeba histolytica*-cysts in water stained with Iodine solution (400X)

the above parasites were not recorded in Al-Zaytoun and Al-Furat neighborhoods.

The percentage (13.88%) in our study it was less than the percentage recorded in a study (AL-Khalidy and Hmood, 2016), in Al- Diwaniyah city when she recorded (51.76%) during the examination of 170 sample by using a flotation method, and less than the percentage in a study (Reynolds et al., 1999), which recorded the percentage recovery of Oocysts in water ranging from (82·3 % to 86·3%) by using IMS procedure.

The presence of *C. parvium-Oocysts, E. histolytica-Cysts* (as in **Figs. 1** and **2**) in the water samples in this study may be due to the extension of the old basins of drinking water near the heavy water basins, in addition to the fact that these basins were very old, which caused them to erosion, leak and mix their water with heavy water (Pankaj Haribhau Chaudhary, Mukund Ganeshrao Tawar 2019).

E. histolytica -cysts are ubiquitous in surface waters worldwide, their concentrations were reported to be in the range of 0.01 to 100 cysts per liter (WHO, 2002), and they survive for up to 2 months in water at 8°C (Meyer and Jarroll, 1980), they were reported to be strongly resistant to disinfection, including chlorination, and difficult to remove by standard filtration (WHO, 2002), in contrast to the case for other waterborne pathogens.

Cryptosporidium-oocysts that can be found in treated wastewater could have been inactivated, and lost their ability to infect humans (Taran-Benshoshan et al., 2015), also the climate change may be affect the concentration of Oocysts in water and the risk for pollution of drinking water (Lake et al., 2005).

Molecular Diagnosis

E.histolytica and Cryptosporidium Parvium

The results of the present study using the conventional polymerase chain reaction indicated that the highest contamination ratio in drinking water was(80%/ 4 out of 5) This was represented *E.histolytica*, as in **Fig. 3**, which represents the positive number of water samples during molecular assay where the 18S rRNA gene(501bp)appears in four samples While the molecular detection did not show the presence of the 18S rRNA (347 bp) as in **Fig. 4** which was responsible for d diagnosis of *Cryptosporidium parvium*, also noted that the rate of contamination of drinking water in this study was higher than the percentage of 28.7% mentioned in a study (Ezatollah et al., 2015), also the results of the present study differ from the results of a study (Thulasi et al., 2016) and it was closely to the results of a study (Arash et al., 2015).

CONCLUSION

Through the current study, we found the drinking water in some neighborhoods in the city of AL-Diwaniyah, was contaminated with two types of intestinal parasites were *Entamoebahistolytica*-cyst and *Cryptosporidiumparvium*-Oocyst.

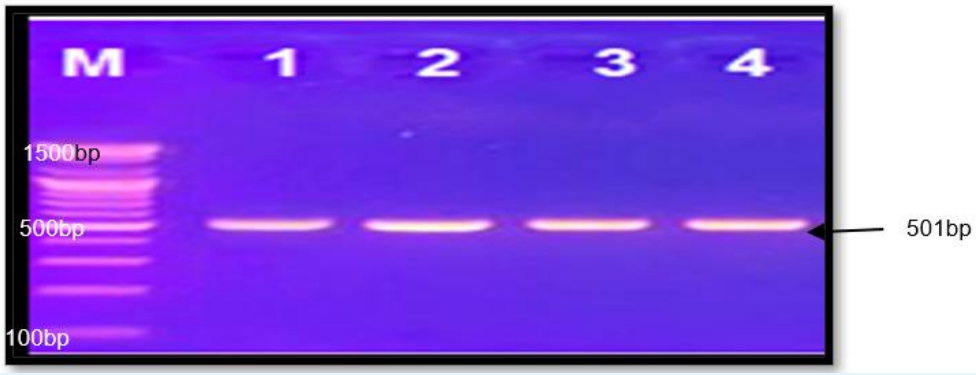

Fig. 3. Electrophoresis of agarose gel containing the product of the 18S rRNA gene test for *E.histlytica,(*M) represents Marker ladder100-1500bp,the numbers from (1-4) represent some positive samples for the 18S rRNA gene.

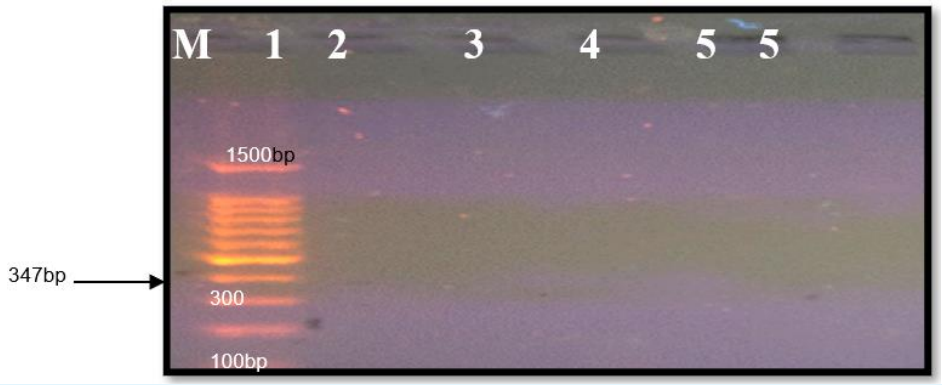

Fig. 4. Electrophoresis of agarose gel no found the 18S rRNA gene (347 bp) of Cryptosporidium parvium (M) represents Marker ladder 100-1500 bp, the numbers from (1-5) represent negative samples for the18S rRNA gene

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