

How to Cite:

Jaber, A. H., & Anah, S. A. (2022). Isolation and molecular identification of *Cryptosporidium* spp. from water samples in Al-Diwaniyah province. *International Journal of Health Sciences*, 6(S5), 3194–3199. <https://doi.org/10.53730/ijhs.v6nS5.9328>

Isolation and molecular identification of *Cryptosporidium* spp. from water samples in Al-Diwaniyah province

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Abstract---This study was conducted to isolate *Cryptosporidium* from water sources in Al-Diwaniyah Governorate. Water samples were collected during the period extending from September 2021 to February 2022, with a total of 500 water samples distributed among three water sources, namely, The total percentage of contamination of the three water sources (rivers, purification plants and liquefaction water) with the *Cryptosporidium* parasite was 16%. The results of using the interlaced polymerase chain reaction technique for examining 40 water samples from two different sources (rivers and purification stations) showed the presence of the hsp70 gene with a molecular weight of 370 pb of *C. parvum*, reaching 40%. *C. Parvum* recording in water samples by molecular methods is the first in Iraq.

Keywords---Water, *Cryptosporidium*, Al-Diwanyah, Iraq.

Introduction

Unclean drinking water is one of the most important environmental factors contributing to the spread of human and animal diseases, as it is responsible for 1.9 million human deaths annually (Striepen, 2013). Water is also one of the most important and dangerous sources in spreading parasitic diseases (WHO, 2009) as small numbers of parasite oval sacs are sufficient to cause infection and water pollution (Huntor and Nichols, 2003). *Cryptosporidiosis* from Zoonotic diseases caused by the parasite *Cryptosporidium* spp. It is one of the

eukaryotic protozoan that parasitizes inside cells (Casemore, 2000; Fayer et al., 1997)

The Nested polymerase reaction is an effective way to amplify parts of DNA (Michael and Joseph., 2019). The steps of this method include two stages: first, a specific piece of DNA is amplified with a length that exceeds its target length in the end, and then a specific region is amplified in the area that inflated in the first step

Materials and Methods

500 water samples were collected and distributed to three water sources, namely, river water and water from purification and liquefaction plants. Conventional concentration and separation methods were used to isolate *cryptosporidium* oocysts from water samples according to the parasite method (2009) extracted and then using) Nanodrop spectrophotometer (THERMO USA). hsp and an internal pair bp370.

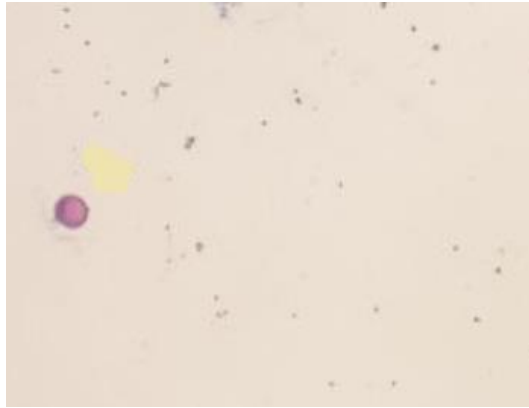
Table 3-1: Primers used to diagnose *Cryptosporidium parvum*

Primers	Sequence 5'-3'		Product size
PCR hsp70 gene	F	TGAGGGTGAGAGAGCCATGA	524bp
	R	GCATACCACCCTCAGCAGAG	
Nested PCR hsp70 gene	F	ATTCCACCAGCACCAAGAGG	370bp
	R	CAGTTTGTTGTGCTCGAGC	

The DNA was extracted according to the method mentioned by (Jiang et al., 2005) and a Nanodrop spectrophotometer (THERMO USA) was used to measure the concentration and purity of the extracted DNA. After preparing the N-PCR mixture using (Go Tag Green PCR Master Kit) in PCR tubes in the tubes attached to the kit and containing the PCR components, the other components were added to the reaction mixture according to the instructions attached to the kit, then the N-PCR reaction products were analyzed by electrophoresis using a gel agarose, at a rate of 5.1%.

Results

The results of the current study of examining 300 samples of water from rivers in Al-Diwaniyah governorate, which included the governorate center (Al-Diwaniyah) in addition to four rivers from the districts and sub-districts (Afak, Al-Hamza, Al-Shamiya, Al-Daghara), which recorded the total percentage of contamination of river water with the parasite *cryptosporidium*. Also, the results of this study showed that the percentage of oocysts of *C. parvum* parasite in filtering plants was 13075% and in liquefied water 3.33% as shown in the figure(3 - 1). Pictured (3-1)



Picture (1-3): Oval sacs of *Cryptosporidium* spp. *Cryptosporidium* isolated from a water sample and stained with Xyl-Nielsen Modified (100X)

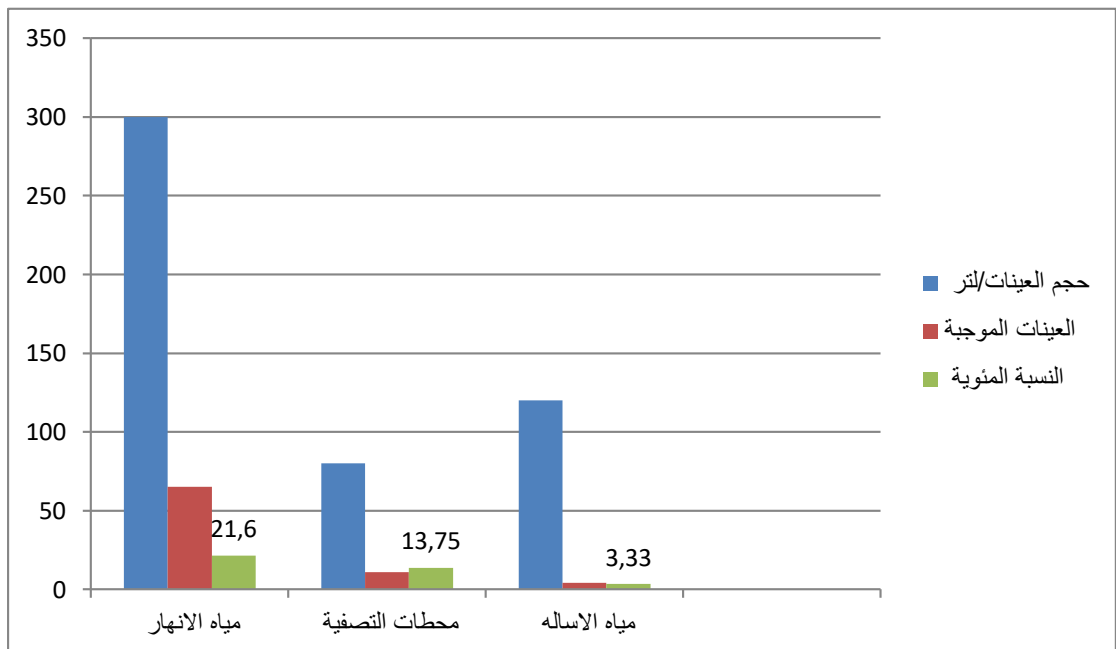


Figure (3-1): Comparison of percentages of *Cryptosporidium* parasite isolated from different water sources in Al-Diwaniyah Governorate



Figure (3-2): Agarose gel electrophoresis image that showed the Nested PCR product analysis of hsp70 gene in *C. parvum* from environmental samples. Where, the Lane (M): DNA marker ladder (1500-100bp) and the Lane (1-20) were showed some positive hsp70 gene in *C. parvum* at 370bp Nested PCR product size.

The results of using the interlaced polymerase chain reaction technique for examining 40 water samples from two different sources (rivers and purification stations) showed that the presence of the hsp70 gene with a molecular weight of 370 pb of the parasite *C. parvum* amounted to 40% as shown in Table (3-2).

Table (3 - 2): Preparation and amplification of the 370bp molecular weight hsp70 gene of *C. parvum* in water samples using N-PCR reaction

percentage %	The number of positive samples	The number of samples examined	water type
46.6	14	30	river water
20	2	10	water pumping stations
40	16	40	the total number
*2.22			Calculated chi-square value
0.136			computed p-value

Discussion

The results of the current study indicated that the total percentage of contamination of the three water sources (rivers, filtration plants and liquefaction water) with the *C. spore* parasite amounted to 16%, and this percentage is close to what Muhammad (2011) found in his study of well water in Mosul Governorate, as he confirmed the contamination of well water with a parasite. *Cryptosporidium*, at a rate of 16.36%, and it differed from what was recorded by Al_Bayatee et al (2012) in Diyala Governorate, who indicated that the percentage of parasite ova in

different water sources, which included (filtration plant water, drinking water, heavy water, ponds and swamps) was 25.25%. It is much higher than the percentage recorded in the current study. The results of this study also showed that the percentage of oocysts of the crypt spore parasite in filtering plants is 13075% and in liquefied water 3.33%, which indicates that it has the ability to penetrate filter systems because of its small size, it used the polymerase chain reaction technique. Nested type N-PCR as a diagnostic method to confirm the results of microscopic examination of water samples containing *C. Parvum* oocysts, as this technique is used to investigate sporozoites and in Different samples even if they contain a small number of copies of the target DNA and use two pairs of primers and the inner pair is the determining factor for the sensitivity and specificity of the test (Albert & fenoy, 1990). The results of using the polymerase overlap chain reaction to amplify the gene hsp70 (370pb of *C. Parvum* parasite) in 40 positive water samples (30 samples from river water and 10 samples from liquefaction plants water), but the amplification occurred in only 16 samples with a total percentage of 40%. The highest amplification rate appeared in river water samples, which amounted to 46%, and the lowest was in the water of liquefaction stations, with an amplification rate of 20%. When statistical comparison, it was found that there were no significant differences in the amplification rates of the hsp70 gene at a probability level of $p < 0.05$. The reason for the high rates of gene amplification in River water samples naturally due to the difference in the number of samples that were subjected to molecular examination.

As for the other water samples in which no amplification of the hsp70 gene occurred, those samples represented other types of the genus *cryptosporidium*, as they gave a positive result, meaning that the amplification of the hsp70 gene occurred during the first cycle of the reaction and no amplification occurred in the second cycle, as the outer pair primers of the same genus (bp) bind to a site of the template DNA strand and this site is common to the group of organisms of the same genus, while the inner pair Inner primers (bp370) binds to a site of the template that is species-specific (sachse & Frey, 2008).

The recording of *C. Parvum* in water samples by molecular methods is considered the first recording of it in Iraq, as local studies did not indicate that it was previously recorded. The current study agreed with some international studies that confirmed the contamination of water sources by the parasite *C. Parvum* by different molecular methods. One of these studies is the study (sharipuzzaman et al (2015) in Bangladesh with a percentage of 25% using PCR technology and study (mahmoudi.,(2020) in the waters of rivers belonging to the city of Gilan in Iran and indicated the presence of this parasite in 10 samples by PCR method.

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