



MOLECULAR DETECTION OF *ENTEROBIUS VERMICULARIS* AND *ASCARIS LUMBRICOIDES* IN SEWAGE WATER FOR AL-DIWANIYAH CITY HOSPITALS, IRAQ.

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Abstract

Health institutions put daily large quantities of pollutants supposed to be paid to the wastewater treatment units of hospitals, but some hospitals neglect the maintenance of processing units and others do not have treatment units, which leads to discharge of heavy water to the main sewer system and then reach to the main sewage treatment in the city and it is not designed to treat the sewage of hospitals, causing many problems. The main hospitals in Al- Diwaniyah including Al-Diwaniyah teaching Hospital, Women & Children Hospital and Al- Hussein Hospital have been selected as sites to detect the spread of intestinal parasites especially *Enterobius vermicularis*, *Ascaris lumbricoides* in the wastewater in those hospitals, samples of water collected, one sample each month at the period from the beginning of march/2017 to the end of September /2018. The results of the microscopic examination of (75) sewage water samples taken from the study sites, indicated that the total percentage of water pollution was (53.33%/40) while the results of the molecular examination showed the 18S rRNA gene using polymerase chain reaction technique for (40) positive sewage water samples indicated that the water pollution ratio of the three hospitals was (20%/8). The results of the statistical analysis indicated that there were significant differences in the percentage of sewage pollution among the three hospitals Included by the study.

Key word: *Enterobius vermicularis*, *Ascaris lumbricoides*, PCR , sewage water.

Introduction

Wastewater is one of the most serious public health problems in most countries of the third world. Waste water treatment does not give priority to water. Therefore, domestic and industrial wastes are discharged directly to the receiving aquatic bodies without treatment, this leads to increased pollution of rivers, loss of aquatic life and consumption of contaminated water by animals and plants, which eventually leads to human causing health problems (Dan'Azumi, 2010), The effect of these contaminants in the environment depends largely on the quantity and quality of these contaminants, which must be adequately treated prior to their introduction. Otherwise, direct damage to different levels of organisms will result in a significant change in the diversity and abundance of these organisms (Al-Rubaie, 2002).

The wastewater in hospitals contains organic compounds, residues of detergents, disinfectants, sterilizers, toxic compounds and high load of pathogens

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such as viruses, such as Rotavirus, Adeno virus and bacteria such as *Escherichia coli*, *Vibro cholera*, *Shigellaspp.*, *Salmonellaspp.* and parasites such as *Giardia lamblia*, *Enterobius vermicularis*, *Ascaris lumbricoids*, *Taeniaspp.*, *Ancylostoma spp.*, *Fasciola hepatica* these organisms responsible for many diseases such as cholera, typhoid, diarrhea, skin disease (WHO, 2006).

The method of diagnosis of parasitic infections, especially the genus *Entamoeba spp.*, has always been controversial. Molecular methods in the diagnosis of parasites are sensitive and highly accurate. They can distinguish between strains of a single species, identify pathogens from non-pathogens, study parasitic genetics and virulence factors For pathogens that facilitate the identification and selection of appropriate treatment (Zhang *et al.*, 2015). Due to pollution of sewage water for hospitals with a lot of contaminants, including parasites and therefore put these water to the rivers and then used to irrigate crops. So that, the aim of this study was to

diagnose the eggs of *Enterobius vermicularis* & *Ascaris lumbricoides* in sewage water (Al-Diwaniyah teaching Hospital, Women & Children Hospital and Al- Hussein Hospital) in Al- Diwaniyah city.

Materials and Methods

Collection of samples

The water samples (75 samples, 25 samples from each hospital) were collected (100 milliliters) of the sewage water from three hospitals in Al-Diwaniyah city, including the women and children hospital, AL-Hussein hospital, Al-Diwaniyah teaching hospital, Samples were placed in sterilized tubes with sealed for the purpose of detecting some of the contaminated sewage water parasites, the date of the collection and the collection site were recorded on all collection tubes and transferred to the laboratories of Al-Qadisiyah University, College of Science, Department of environment for the purpose of searching for the presence of some intestinal parasites in them.

Detection of parasites

In search of worm eggs *Enterobius vermicularis* and *Ascaris lumbricoides* in wastewater samples The method of Flotation was used (Sastry & Bhat, 2014; AL-Khalidy, 2015) and the polymerase chain reaction technique according to the method (Jiang *et al.*, 2004), this technique includes three steps: DNA extraction, multiplication and determination of the results of the multiplication.

Primers

The Primers specific to gene (18S rRNA) are designed to diagnose of *Enterobius vermicularis* and *Ascaris lumbricoides* in sewage water samples Using the Genbank-NCBI gene site to obtain the complete sequence of the gene 18S rRNA for each of these

Table 1: The primers used in this study with their nucleotide sequence and the PCR assay.

Primer		Sequence	product	Reference
<i>Enterobius vermicularis</i>	F	TAGGGTTCGACTCCGGAGAG	273bp	(AL-Jabri, 2017)
	R	CGCAGATCCAACACTACGAGCT		
<i>Ascaris lumbricoides</i>	F	AGGGACAAGCGGTATTCAGC	168bp	(AL-Jabri, 2017)
	R	AATTCCTCGATCCCGATCACG		

Table 2: Microscopic diagnosis of *Enterobius vermicularis* and *Ascaris lumbricoides* in Sewage Water.

Parasites	%	Positive number	Total number examined	Sites
<i>Ascaris lumbricoides</i> + <i>Enterobius vermicularis</i>	52	13	25	Women & children hospital
<i>Enterobius vermicularis</i>	40	10	25	AL-Hussein hospital
<i>Ascaris lumbricoides</i> + <i>Enterobius vermicularis</i>	68%	17	25	Al- Diwaniyah Teaching Hospital
	53.33	40	75	Total

χ^2 calculated:3.909

χ^2 tablets:5.991

$P \leq 0.05$

parasites. Using the Primer 3 plus program, the primers were designed and processed by the Korean company Bioneer as in table 1.

Statistical analysis

All results in this study were statistically analyzed to determine the significant differences at the probability level PdH 0.05 using the Chi-Square (Schiefer, 1980).

Results and Discussion

The results of the microscopic examination of (75) sewage water samples taken from the study sites, which included Al- Diwaniyah Teaching Hospital, Women & Children Hospital ,Al-Hussein Hospital in AL-Diwaniyah city, indicated that the total percentage of sewage water pollution was (53.33% /40).

The higher percentage of pollution in the sewage water of Al- Diwaniyah Teaching Hospital was (68%/ 17). This percentage represented the eggs of *Ascaris lumbricoides* + *Enterobius vermicularis*, while the percentage of water pollution in Women & children hospital was (52%/13)this percentage represented the eggs of *Ascaris lumbricoides* + *Enterobius vermicularis*. The contamination of the AL-Hussein Hospital sewage water by (40%/10) this the percentage represented the eggs of the *Enterobius vermicularis* the results of the statistical analysis indicated that there were significant differences in the percentage of sewage pollution among the three hospitals Included by the study as in table 2, Fig. 1, 2. The results of the current study Approach to study of (BenAyed *et al.*, 2009; Layla *et al.*, 2010) Which confirmed the spread of eggs of many worms and protozoa in sewage water, the eggs of *Ascaris* are consider the most pathogenic and more resistant to the environment as they can survive in the appropriate environmental conditions for (5) years and cause infection

(Sanguinetti *et al.*, 2005), while the results of this study differ from the results of study of (Veridiana *et al.*, 2012) Which pointed to the presence of a number of parasites such as *Ascaris* sp. (9.55%) in the sewage water, the sewage water contains large numbers and species of pathogenic parasites that include

Table 3: Molecular diagnosis of *Enterobius vermicularis* and *Ascaris lumbricoides* in Sewage Water.

Parasites	%	Positive number	Total number examined	Site
<i>Ascaris lumbricoides</i>	7.69	1	13	Women &
<i>Enterobius vermicularis</i>	7.69	1		children hospital
<i>Enterobius vermicularis</i>	20	2	10	AL-Hussein hospital
<i>Ascaris lumbricoides</i>	5.88	1	17	Al- Diwaniyah
<i>Enterobius vermicularis</i>	17.64	3		Teaching Hospital
	20%	8	40	Total

 χ^2 calculated: 1.74

 χ^2 tablets: 5.991

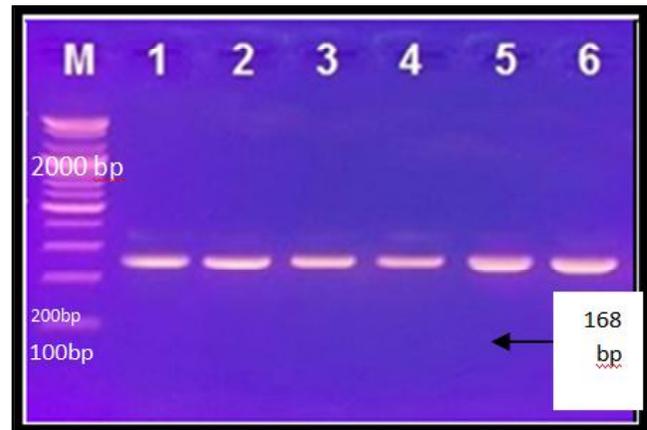
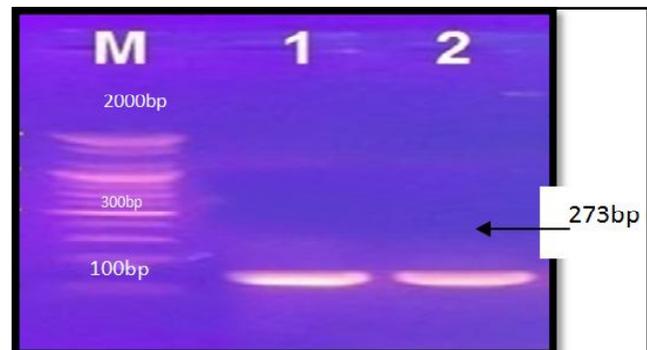
 $P \leq 0.05$

bacteria, viruses, protozoa and worms (FAO, 1992) also (Metcalf and Eddy Inc, 1995) has shown that these parasites are transmitted to wastewater by a human being who is ill or carries the disease, (Scott *et al.*, 2000) explained that wastewater can contain a wide range of parasites such as *Ascaris*, *Schistosoma*, Tape worm, that have a negative impact on human health and the environment. The results of the molecular examination showed the 18S rRNA gene using polymerase chain reaction technique for (40) positive sewage water samples

**Fig. 1:** Egg of *Ascaris lumbricoides* 100X.**Fig. 2:** Egg of *Enterobius vermicularis* 100X.

for microscopic examination indicated that the sewage water pollution ratio of the three hospitals was (20%/8), *Ascaris lumbricoides* were observed by (7.69%, 5.88%) in the water of Women & children hospital, Al- Diwaniyah Teaching Hospital Respectively, While *Enterobius vermicularis* was observed with (7.69%, 20%, 17.64%) in Women & children hospital, AL-Hussein hospital, Al-Diwaniyah Teaching Hospital Respectively. The results of the statistical

analysis indicated that there were significant differences in the percentage of sewage pollution among the three hospitals Included by the study as in table 3, Fig. 3, 4. The results of current study agree with the results the study of (Sydney *et al.*, 2017) Which pointed to the presence of *Enterobius vermicularis* in the wastewater using PCR technology after removal it by physical

**Fig. 4:** The electrophoresis to the 18S rRNA gene for the diagnosis of *Ascaris lumbricoides*, where M represents Marker ladder (2000-100bp) and the numbers (1-2) represent some of the positive samples for the 18S rRNA gene (168bp).**Fig. 3:** The electrophoresis to the 18S rRNA gene for the diagnosis of *Enterobius vermicularis*, where M represents marker ladder (2000-100 bp) and the numbers (1-6) represent the positive samples for the 18S rRNA (273bp).

methods, also agree with the study of (Lucrecia *et al.*, 2017) Which referred to the presence of eggs *Ascaris lumbricoides* in the wastewater by using Polymerase chain reaction, The reasons for the differences in the percentage of sewage contamination of the *Ascaris lumbricoides*, *Enterobius vermicularis* using microscopic and molecular methods may be attributed to accuracy, sensitivity and specificity of polymerization chain reaction, this is Agree with what he mentioned (Azab *et al.*, 1993).

Conclusion

Through the current study, we found that Sewage water in AL-Diwaniyah city hospitals is contaminated with *Enterobius vermicularis* and *Ascaris lumbricoides*.

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