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VIROLOGICAL EVALUATION OF DRINKING WATER IN EGYPT

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ABSTRACT

This study aims to evaluate the inlet and outlet water of the Nile River at El-Rayah El-Menofy in three drinking water stations during the four seasons at 2007-2009. The water samples were ultrafiltered to concentrate coliphages, enteric and H5N1 viruses. Coliphages infecting *Escherichia coli* were detected in both of sewage polluted samples and chlorinated water samples especially in warmer seasons, however, maximum counts were recorded during summer and the minimal were detected in winter. The viruses obtained by ultrafiltration process were negatively stained with 2% phosphotungestic acid. Electron microscopy revealed that the phage particles had an isometric head and long-contractile tail. Some particles appeared to have a short tail with full heads, the bacteriophage resembles those belong to the Myoviridae family. Enteroviruses were detected using RT-PCR and real-time-RT-PCR in inlet water of El-Bagour and Shibin El-Kom stations in summer season, while H5N1 was not detected in all sites throughout four seasons. The viruses obtained by ultrafiltration process were negatively stained with 2% phosphotungestic acid. Electron microscopy revealed that human virus particles were of 24-30 nm in diameter and an isometric structure. The virus resembles those belong to the picornaviridae family.

Keywords: Coliphages, Drinking water, El-Rayah El-Menofy, Enteroviruses, H5N1, Nile River, Pollution, RT-PCR, real-time-RT-PCR.

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INTRODUCTION

In Egypt, the Nile River is the main source of drinking water. The Nile unfortunately, receives heavy loads of industrial, agricultural and domestic wastes. Drinking water must meet specific criteria and standards to ensure that water supplied to the pipes is safe and free-from pathogenic microorganisms as well as hazardous compounds **(WHO report, 1993)**. At the North of Cairo at Delta Barrage, the Nile River bifurcates into two branches namely Damietta and Rosetta and four Rayahs (Canals) namely El-Nassery, El-Behery, El-Menofy and El-Toufeky **(Abdel Aziz, 2005)**.

More than 140 different types of virus are known to be excreted in faces by infected persons. Several of these viruses have been associated with disease transmitted by water. Viruses exist in natural water or tap water with a much lower concentration because viruses can not reproduce in water. 'Even in low concentrations, they are still capable of causing diseases when ingested. Enteric viruses may cause severe waterborne diseases and many outbreaks due to contaminated drinking water such as paralysis (poliovirus), meningitis (Echovirus), myocarditis (Coxsackievirus), or infectious hepatitis A viruses (HAV) **(Fong and Lipp, 2005)**.

Many workers studied the viral content, the physical and chemical characters of the Nile River at El-Rayah El-Menofy and the distribution of some heavy metals in the branch. In addition, studies dealing with the bacteriological quality indicators of the River waters and water treatment stations were recorded **Sadeghi *et al.*, (2007) and El-Adly *et al.*, (2008)**. This study was aimed to determine the seasonal variations in microbial pollution indicators and to evaluate coliphages as an indicator for presence of water borne viruses pollution, elucidate the occurrence of coliphages and enteric viruses throughout El-Rayah El-Menofy and water treatment stations.

MATERIALS & METHODS

Water samples:

Water samples were collected from February 2007 to November 2009, from subsurface layer of three stations (El-Bagour (Ba); Menof (Me) and Shibin El-Kom (Sh)) at El-Rayah El-Menofy.

Water treatment stations receive the Nile River water from El-Rayah El-Menofy. The treatment processes in all examined water are the same including pre-chlorination, flocculation, sedimentation, sand filtration and finally post chlorination.

For enteric and H5N1 viruses detection, water sample (40 liters in clean container) was collected from each site. Ten ml per 40 liters of 2M magnesium chloride was added to increase the stability of the viruses in the samples during transportation. While sodium thiosulfate (BDH chemicals Ltd Poole England) was added to the chlorinated samples to a final concentration of 5 mg/l, to inactivate chlorine and transported in an ice box to the laboratory for analyses within 8 hours.

Detection of coliphages in water samples:

Coliphages were detected using the method described by **(Othman, 1997).**

Bacterial viruses assay:

Coliphages were qualitatively and quantitatively assayed by the spot test and the over layer agar techniques (plaque assay technique) according to method of **Othman (1997).**

Purification of coliphages:

Dextran sulfate-polyethylene glycol system was used for phage purification according to method of **(Watanabe *et al.*, 1970).**

Virus concentration:

Viruses were concentrated by ultrafiltration of 40 liters of each water sample using a hollow fiber cartridge (Amersham BioScience, VFP-50-E-9A model, NRC). The porosity of the ultrafiltration cartridge was 50 KDa and 8400 cm^2 surface area in which almost all viruses will be trapped **(Kfir *et al.*, 1995).**

After finishing filtration of the whole sample, the adsorbed viruses on the filter were re-concentrated as described by **Katzenelson *et al.*, (1976).** The cartridge water content was mixed with beef extract powder to give a final concentration of 3% in water (Lab-lemco powder, Oxoid), the pH was adjusted to 3.5 by 1N HCl. The virus eluted were collected and centrifuged at 5000 rpm for 30 min (BOECO, Germany, M-240). The produced pellets were re-suspended in 1m sterile DEPC-treated water (RNase free water) and 0.15 M Di-sodium hydrogen phosphate (Na_2HPO_4) and the concentrated

samples were decontaminated by adding 1% antibiotic-antimycotic mixture (PAA Laboratories Gmbh, Austria). The virus suspensions were stored at -80°C for further investigation.

Transmission electron microscope:

The isolated viruses by ultrafiltration process were examined as described by **Accolas and Spillmann (1979)**. The viruses were stained with a 2% (W/V) solution of phosphotungstic acid (PTA), pH 6.5 and examined in TEM (Model Jeol JEM-1010) operated at 60 KV at Regional Center for Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt.

Molecular detection of enteric and H5N1 viruses:

Viral RNA extraction was performed in a biosafety level 2 laboratories *Dept. of Water Pollution Researches, National Research Centre, Dokki,* Cairo, Egypt with enhanced containment practices. Ribonucleic acid was extracted using viral RNA kit (QIAamp, Qiagen®, Hilden) according to the instructions of the manufacturer. The purified viral RNA was stored at -80°C for later analysis and confirmed spectrophotometerically at A_{260}/A_{280} (UV-2401 PC UV-Vis spectrophotometer, Molecular Biology Lab. NRC) as well as agarose gel electro-phoresis was performed. The RNA yield was determined by the following equation:

$$\text{RNA yield (mg/ml)} = \frac{\text{O.D at 260 nm}}{\text{Extinction coefficient}} \text{ X dilution factor}$$

Primer synthesis:

Oligonucleotide primers for H5N1:

Hemagglutinin (H5) gene approximately 320 bp was amplified using the following designed forward (F) and reverse (R) primers based on previously published sequence of A/Chicken/Hong Kong/y388/97 (H5N1) HA **(accession number AF098542)** using DNA star program:

H5-kha-1: 5'-CCTCCA GARTATGCMTAYAAAATTGTC-3'

H5-kha-3: 5'-TACCAACCGTCTACCATKCCYTG-3'

Neuraminidase (N1) gene approximately 615 bp was amplified using the following forward (F) and reverse (R) primers according to **Wright, (1995)**

N1-F: 5'-TTGCTTGGTCGGCAAGTGC-3'

N1-R: 5'-CCAGTCCACCCATTTGGATCC-3'

Oligonucleotide primers for enteroviruses:

Enteroviruses (EV1 and EV2) primers approximately 153 bp was amplified using the following designed forward (F) and reverse (R) primers was amplified using the following forward (F) and reverse (R) primers according to **Chapman *et al.*, (1990)**

EV1-F: 5'-
CACCGGATGGCCAATCCA -3'
EV2-R: 5'- TCCGCCCCC ATG -3'

Poliovirus 1 Mahoney strain (PV1 and PV444), Partial sequence fragment of approximately 293 bp was amplified using the following designed PV primers according to **Shieh *et al.*, (1996) (accession number V01148)** primers PV444 was used for the reverse transcriptase (RT) and both of primers P1 and PV4444 were used for the polymerase chain reaction.

P1-F: 5'-
CGTTATCCGCCTATGTACTT -3'
PV444-R: 5'-
CATTCAGGGGCCGGAGG -3'

The primer sets were obtained from Rous-associated virus 2 (RAV-2, TakaRa Biomedicals, Japan), and adjusted to approximately 200 nano moles with TE buffer (10mM Tris-HCl, pH 7.4, 1Mm EDTA, pH 8.0), and stored at – 20°C.

RT-PCR for enteroviruses detection:

The mixture of 9 µl viral RNA tempelate, 1 µl reverse primer (200 nMol/µl) and 5µl DEPC-treated water was first heated at 70°C in the heating block of the DNA-thermal cycler (Biometra, Goettingen, Germany) for 5min to allow opening the helices of the RNA template. Such mix was then chilled on ice followed by adding 1 µl of 10mM dNTPs, 1µl RNase inhibitor (40U), 5µl of 5X RT-buffer, 2µl DEPC-water and 1µl M-MLV-RT (20U) then the tube was returned to the heating block of the DNA-thermal cycler. The cDNA synthesis program included reverse transcription stage at 42°C for 90 min linked to RT-inactivation stage at 94°C for 10 min.

The procedure cDNA was used for PCR reaction by mixing the following reagents to a final volume of 80: 2 µl of 10 mM deoxinucleoside triphosphates (dNTPs), 2 µl of each sense and antisense primer, 8 µl of 10 x PCR buffer containing 2mM $MgCl_2$, 1 µl (5 U) TaKaRa Ex *Taq* DNA polymerase and 47 µl of DEPC-treated water. The PCR mixture was subjected to 40 cycles 94°C, 50°C, 72°C each for 1 min

followed by one cycle of 72°C for 10 min.

Amplification of cDNA H5 and N1 virus:

In 750 µl eppendorf tube, 5 µl of the cDNA were mixed with 1 µl of 10 mM of dNTPs, 1 µl of each of the F and R primers (200 nMol/µl), 10 µl of 10X *Taq* buffer, 2 µl of 25 mM $MgCl_2$, 1 µl (5U) *Taq* DNA polymerase and 29 µl DEPC-treated water giving a final reaction volume of 50 µl. The tube was placed in the DNA-thermal cycler and the amplification program included the following stages: initial denaturation at 94°C for 3 min linked to 40 cycles each consisted of 3 stages including denaturation at 94°C for 1min, annealing for 1min (at 58°C for H5 and at 55°C for N1) and extension at 72°C for 1min. The last cycle was linked to a final extension step at 72°C for 10 min followed **(Wright, 1995).**

Real-time-RT-PCR:

The rt-RT-PCR reaction was performed in a biosafety level 2 laboratories, *Dept. of Water Pollution Researches, NRC, Dokki,* Cairo, Egypt and carried out using Power SYBR® Green master Mix (Applied biosystem, Cambridge, UK), a reporter dye is used. SYBR green has the capability to bind to double stranded DNA upon amplification and emit fluorescence. Such fluorescence could be quantitively detected by fluorescence detector in the smart cycler (Cepheid, Sunnyvale, USA). Data are produced as sigmoidal-shaped amplification profile TaqMan when using a linear scale, in which the fluorescence is plotted against the number of cycles. The threshold cycle (C_T) serves as a tool for calculation of the starting template amount in each sample. This is the cycle in which there is the first detectable increase in fluorescence. Determination of C_T values is carried out slightly differently on Applied Biosystems and Light Cycler systems.

RESULTS

Coliphages were detected in 24 samples at El-Rayah El-Menofy (raw water and chlorinated water samples) using spot test technique. The results in **Table (1)** showed that phages specific for *E.coli* were found in twelve of tested samples (7 inlets and 5 outlets in both of El-Bagour, Menof and Shibin El-Kom sites) especially in warmer seasons (summer, spring and autumn). Whereas, no coliphages were

detected in all examined samples in winter season. Presence of bacteriophages specific for *Escherichia* isolates was confirmed by the spot test. As shown in **(Figure 1)** lysis of bacterial lawn by bacteriophage can be clearly seen.

Using single plaque isolation technique, two single isolates of phages specific for *E.coli* strain HB1D1 were obtained. The isolated phages formed circular, clear plaques of about 3–5mm in diameter. These two phage isolates were designated C1 and C2 (the letter C refer to coliform bacteria *E.coli* and numbers 1 and 2 are the isolates numbers).

A large amount (1000ml) of high titre phage lysate was prepared from the isolated coliphages by liquid propagation culture technique. The titre of C1 and C2 phages were $3X10^2$ and $3X10^{10}$pfu/ml, respectively. Dextran sulfate-polyethylene glycol two phase systems were used to obtain a purified and concentrated phage preparation. About 63.2, 1.92 and 16.8 of PE, Dextran sulfate and NaCl, respectively were added to the phages lysate in separating funnel and mixed well. About twenty five milliliters of turbid phase were collected from the separating funnel after precipitation with PEG, and about 2ml of intermediated phase (Cake) were collected by centrifugation of the turbid phase.

Viruses obtained by ultrafiltration were negatively stained with aqueous solution of 2% phosphotungestic acid (ATP) in aqueous solution brought to pH=7 with NaOH. Electron microscopy revealed that the phage particles have isometric heads and long-contractile tails. Some particles appeared containing short tail with full heads **(Figure 2).** The bacteriophage resembles those of the Myoviridae family of International Committee on Taxonomy of Viruses (ICTV) **(Othman, 1997 and El-Arabi, 2003)**.

To assess the quality of the isolated RNA by mini spin column protocol, two raw water samples were confirmed by gel electrophoresis on 2% agarose gel. The isolated RNA had an $A_{260/280}$ ratio of 1.9. The RNA yield was 50µg/200µg concentrated samples.

Viruses which produced by ultrafiltration of raw and chlorinated samples, were

examined by TEM. RNA was extracted by mini spin column reagent followed by RT-PCR and real-time-RT-PCR and tested for both of enteroviruses and H5N1. The results showed that two sites were positive for enteroviruses among all sites especially in warmer seasons (summer season) namely El-Bagour inlet (Ba*) and Shibin El-Kom inlet (Sh*). Whereas, H5N1 was not detected in all sites through all seasons.

Viral concentrate was negatively stained with uranyl acetate (2% W/V), for 1 min and 2% phosphotungestic acid in aqueous solution brought to pH 7 with NaOH. Electron microscopy revealed that the virus particles are of isometric structure and their diameters ranged between 24 to 30nm. The virus resembles those of picornaviridae family of International Committee on Taxonomy of Viruses (ICTV) as showing in **(Figure 3).**

In this study, 24 water samples were ultrafilterated and tested for the presence of enteroviruses and H5N1 using real-time-RT-PCR TaqMan probe labeled with the FAM fluorescent dyes. The results revealed that enteroviruses were detected in two water samples through all seasons especially in warmer seasons (summer season) in both of two sites namely El-Bagour inlet (Ba*) and Shibin El-Kom inlet (Sh*) as detected by RT-PCR and real-time-RT-PCR. Moreover, the results revealed that cross-amplification signal and cut off the threshold (C_T) curve as indicated in **(Figure 4).**

When procedure was used to investigate the water quality of 24 samples collected from both of raw and chlorinated water samples through four seasons. The results showed 2 bands of sizes ~153 bp and 293 bp for enteroviruses in water samples of two sites. The other 22 water samples were negative for enteroviruses through all seasons. For H5N1 detection in all 24 samples by both of RT-PCR and rt-RT-PCR, none was positive for H5N1, two water samples were positive for enteroviruses by both of RT-PCR and rt-RT-PCR **(Figure 5).**

The results of RT-PCR, real-time-RT-PCR, gel electrophoresis assay and TEM indicate that poliovirus isolates were all derived from the Sabin vaccine strains, suggesting that El-Rayah El-Menofy Nile River water, Egypt, is a wild-poliovirus free area.

Table 1. Qualitative and quantitative assay of coliphages in raw (inlet) and chlorinated (outlet) water samples collected from different sites along the four seasons.

Seasons / Sites	Summer		Spring		Autumn		Winter	
	Phage assay (After enrichment)		Phage assay (After enrichment)		Phage assay (After enrichment)		Phage assay (After enrichment)	
	Qualitatively	Quantatively (pfu/ml)	Qualitatively	Quantatively (pfu/ml)	Qualitatively	Quantatively (pfu/ml)	Qualitatively	Quantatively (pfu/ml)
Sh*	+	$4.5X10^6$	+	$1.0X10^4$	-	-	-	-
Sh**	+	$2.6X10^9$	+	$2.3X10^5$	-	-	-	-
Me*	+	$5X10^3$	+	$3X10^2$	-	-	-	-
Me**	-	-	-	-	-	-		
Ba*	+	$3.6X10^7$	+	$9.5X10^7$	+	$3.7X10^3$	-	-
Ba**	+	$8.0X10^9$	+	$1.4X10^8$	+	$2.1X10^3$	-	-

* Inlet raw water sample; ** Outlet treated water sample; + = Lysis; - = No lysis; Sh= Shibin El-Kom;

Ba= El-Bagour and Me= Menof.

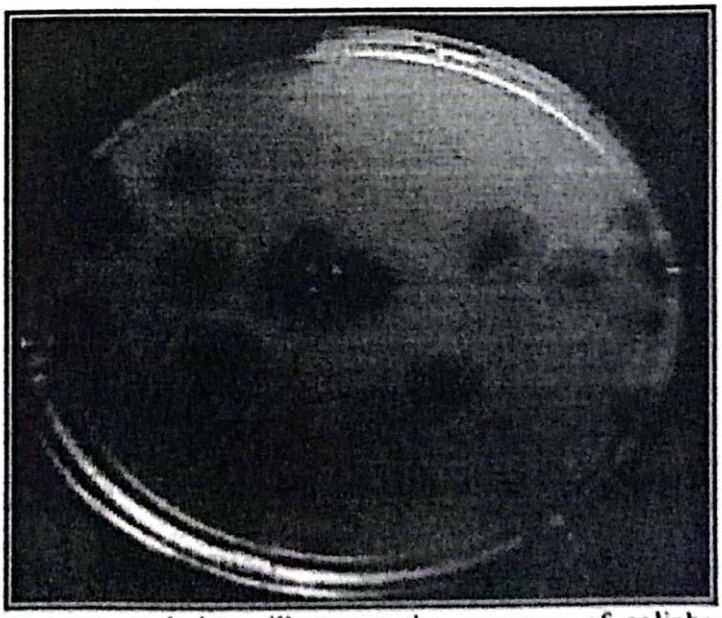

Figure 1. Plaque assay technique illustrates the presence of coliphages of different morphological plaques lysis of bacterial lawn by Bact-eriophage can be clearly seen.

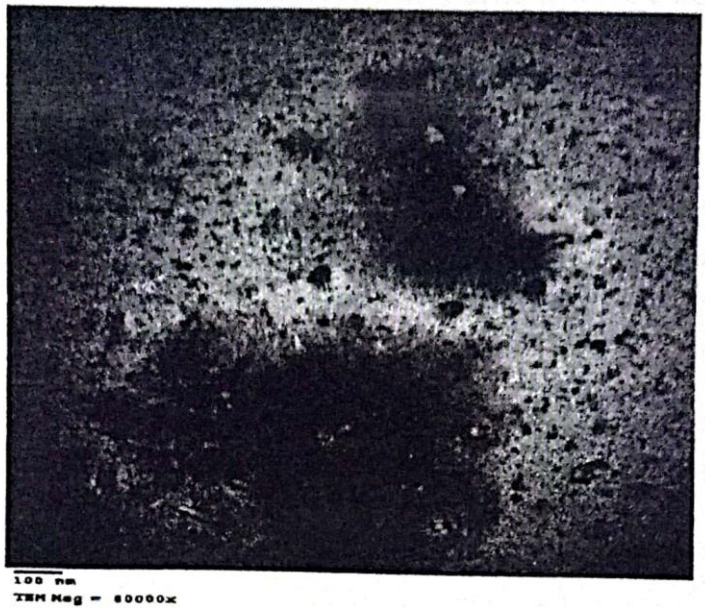

Figure 2. Micrograph of coliphage isolated by ultrafiltration of water samples showing phages with isometric heads and contractile tails.

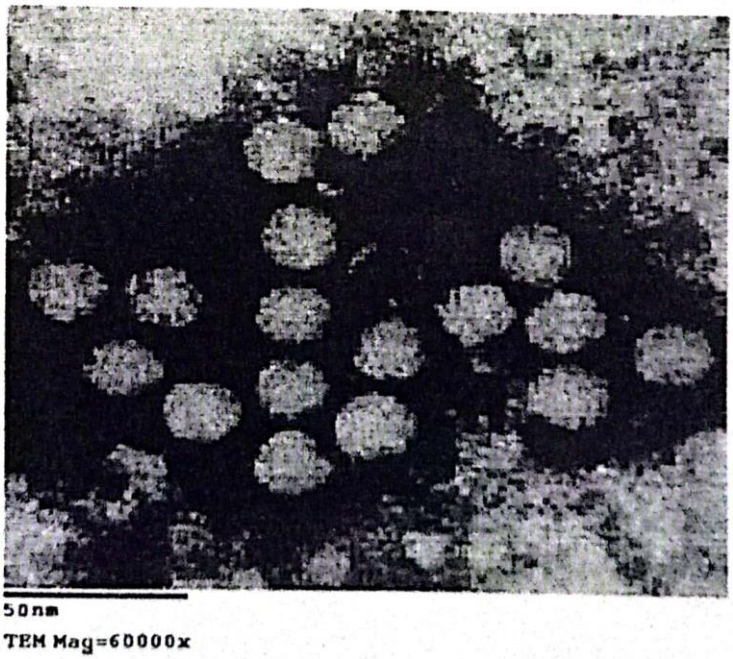

Figure (3): Electron micrograph of viruses stained with uranyl acetate which were found in raw water samples.

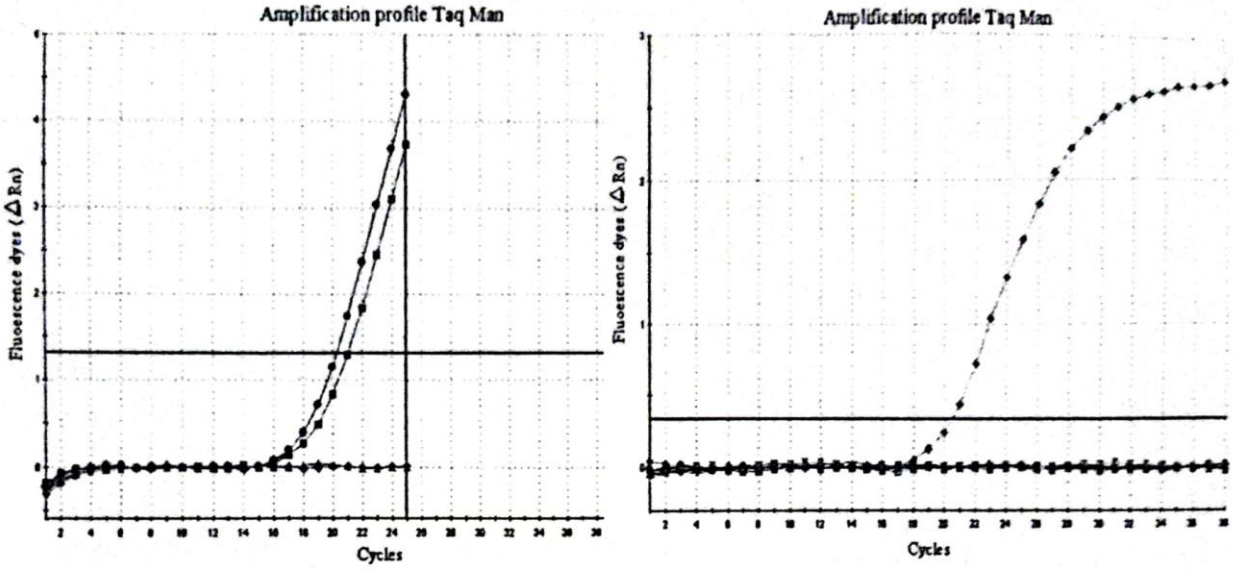

Figure 4. Amplification profiles of enteric and H5N1viruses obtained by real-time-RT-PCR with specific primers pairs in both of raw and chlorinated water samples.

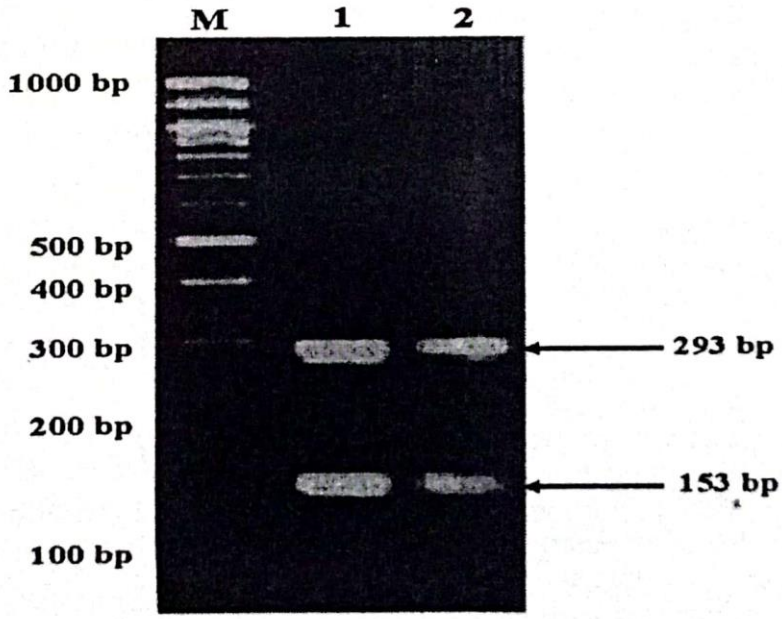

Figure 5. Agarose gel electrophoresis 2% showing the PCR products amplified using specific primer of enteroviruses from raw water samples.
M= marker weight DNA marker (1000 bp DNA ladder), both of Lane 1 and Lane 2 the PCR products (~ 153-bp and 293-bp).

DISCUSSION

In these investigation phages specific for *Escherichia coli* strain HB_1D_1 (coliphage), were isolated from sewage polluted natural water and drinking water with concentration ranged from 10^2 and 10^9, after enrichment and designated as C1 and C2 phages. Several authors have been also isolated coliphages from drinking water **(Brezina and Baldini, 2008)**, natural water **(El-Arabi, 2003)**. The isolated coliphages (C1 and C2 phages) produced a circular, clear plaque of about 3mm and 5mm in diameter.

Bacteriophage stock lysate specific for *E.coli* was prepared from circular plaques of 3mm and 5mm in diameter by the single plaque isolation method. The bacteriophages were propagated to obtain large amounts of the bacteriophage suspension by the liquid propagation method. Finally, the propagated bacteriophage stock lysates were purified by polyethylene glycol sedimentation to obtain purified, concentrated bacteriophage stock lysates. Authors, generally isolate the coliphages form both of surface and drinking water by the single plaque isolation method **(Othman, 1997 and El-Arabi, 2003)**.

Both of C1 and C2 phage particles have an isometric head with a long contractile tail and a short tail, these results are in agreement with **Yoon *et al.* (2001) and El-Arabi (2003)**.

Drinking water supplies associated with a wide spectrum of viral infections **(Grabow, 1996)**. Results of this study indicate the occurrence of enteroviruses in inlet water of both El-Bagour (Ba*) and Shibin El-kom (Sh*). This may be due to fecal contamination of El-Rayah El-Menofy water before feeding the station with raw water intake. These results were in agreement with **El-Esnawy *et al.* (2004) and Ali *et al.* (2004)** they mentioned that enteroviruses were detected in raw water and tap water in Egypt.

It was found that enteroviruses were detected in inlet water in summer season but not detected in other seasons. Chlorination; coagulation and sedimentation processes appear to be the most efficient steps in reducing the number of microorganisms in water. It was observed that the operation of sedimentation process,

the release of accumulated viruses in the sediments to the effluent, the site selection for intake and insufficient skill in operation of treatment process are the main problems in water treatment stations.

Currently, the standard methods in this study for the detection of enteroviruses and H5N1 in water samples involve RT-PCR and rt-RT-PCR able to identify all human viruses **(Schmidt *et al.*, 1978)**. Real-time-RT-PCR is a rapidly and sensitive method for the routine monitoring of enteroviruses and H5N1 in water samples because it is faster and simpler (qualitative and quantitative) than conventional RT-PCR and cell culture methods **(Brown *et al.*, 2007)**.

Kopecka *et al.* (1993) suggest that free viral RNA is rapidly broken down in wastewater, the divergence between these two parameters can explained either by the presence of viral capsids that contain the genome but have not or lost their infectious character, or by the presence of infectious enteroviruses that do not provoke a cytopathogenic effect on Vero cells. Therefore, the presence of genomes cannot be considered to be an indicator of more or less recent viral contamination.

In Egypt life attenuated poliovirus Sabin strains are used. Therefore the presence of the isolates in sewage would reflect that these viruses excreted from humans after polio virus (PV) immunization **(Wood *et al.*, 2000)**. Many authors **(Rutjes *et al.*, 2006; Brown *et al.*, 2007 and Lee *et al.*, 2008)** concentrated enteroviruses and H5N1 from both of surface and drinking water using ultrafiltration process and detected human viruses by RT-PCR and rt-RT-PCR and isolated poliovirus from raw and treated water samples.

The results can be achieved with 2 % agarose gel electrophoresis and visualized by ethidium bromide staining, thus rendered the technique straightforward and rapid. This protocol allows the results to be obtained within many hours, especially when we used these primers sets together with the RT-PCR and rt-RT-PCR systems. These primer pairs were chosen so that they can give rise to amplicons of different sizes (~153-bp and 293-bp for enteroviruses and 320-bp and 615-bp for H5N1) which could be easily resolved from each

other by gel electrophoresis. The positive PCR products with any primer set produced bright clearly visible bands of corrected size without non-specific reaction and these agree with **El-Esnawy *et al.* (2004)**.

While, the negative PCR product did not produce any DNA band or non-specific reaction. Since a set of amplicon with 4 bands of corrected sizes (~153-bp and 293-bp for enteroviruses and 320-bp and 615-bp for H5N1) will be expected if water samples contain both of enteroviruses and H5N1.

The results of this study showed that the avian influenza viruses were not detectable in all water samples (inlet and outlet) using both of RT-PCR and rt-RT-PCR. Persistence of H5N1 in water was reported by **Brown *et al.* (2007)** they mentioned that pH of water ranging from 7.4 to 8.2, temperature from 4°C to 17°C, and salinity from 0 to 20,000 ppm at levels normally encountered in nature can impact the ability of AI viruses to remain infective in water.

Previous laboratory studies using distilled water model showed that increasing temperature has a negative effect on the persistence of AI viruses in water (**Brown *et al.*, 2007**). None of these previous laboratory trials evaluated the persistence of H5N1 below 17°C or above 28°C. **Halvorson *et al.* (1983)** isolated AI viruses from 10 of 169 water samples collected over the two year study. Samples were collected after the water temperature fell below 12°C.

On the light of previously virological analyses of water samples, it was clear that both of El-Bagour and Shibin El-kom sites were suffering from viral contamination especially in both of summer and spring seasons.

In conclusion, the changes in *E.coli* number are correlated with phage in two sites namely El-Bagour and Shibin El-Kom. Bacteriophages can be used as potential indicators for viral contamination, but it is doubtful that they could substance for such traditional indicator bacteria as *E.coli* and monitoring of microbial quality of water is a must to control the spreading of pathogens transmitted by contaminated water and still represents an environmental health hazards in Egypt. Prevention of the Nile River

contamination will enhance the efficiency of drinking water treatment facilities for pathogenic removal. Therefore, all governmental and non-governmental organizations should be involved for effective implementation of a large scale sanitation program.

REFERENCES

Abdel-Aziz, G.S. (2005). Study on the water quality of the Nile River with relation to the environmental condition at El-Kanater El-Khyria region. M.Sc. Thesis. Fac. Sci (Girls) Al-Azhar Univ. Cairo, Egypt.

Accolas, J.P. and Spillmann, H. (1979). The morphology of six bacteriophages of *Streptococcus thermophilus*. *J. Appl. Bacteriol.* 47: 135-144.

Ali, M.A.; Al-Herrawy, A.Z. and El-Hawarry, S.E. (2004). Detection of enteric viruses, Giardia and Cryptosporidium in two different types of drinking water treatment facilities. *Dept. of Water Pollution Researches, National Research Centre, Dokki, Cairo, Egypt. Water Research* 38: 3931-3939.

Brezina, S.D. and Baldini, A.C. (2008). Detection of somatic coliphages as indicators of fecal contamination in estuarine waters. Departmenta de Biologia, Bioquimica y Farmacia, Universidad Nacional del sur (8000) Bahia Blanca, Argentina.

Brown, J.D.; Swayne, D.E.; Cooper, R.J.; Burns, R.E. and Stallknecht, D.E. (2007). Persistence of H5 and H7 avian influenza viruses in water. *Avian Dis.* 51:285-289.

Chapman, N. M.; Tracy, S.; Gauntt, C.J. and Fortmueller, U. (1990). Molecular detection and identification of enteroviruses using enzymatic amplification and nucleic acid hybridization, *J. Clin. Microbiol.* 28, 843-850.

El-Adly, A.A.; El-Senousy, M.W.; Samhan, A.F. and Mohamed, B.M. (2008). Photothermal Efficieny of Gold Nanorods in Controlling Microorganisms in Water. Water Pollution Research Dept., National Research Centre, Cairo, Egypt. *J. Appl. Sci. Res.* 4(12): 1811-1816.

El-Arabi, T.F. (2003). Study of characters of some microbial viruses isolated from Egyptian soil and water. M.Sc. In Agricultural Viruses, Faculty of Agriculture. Ain Shams University, Egypt.

El-Esnawy, N.A.; Kamel, A.H.; Abdo, S.N. and Ali, M.A. (2004). RT-PCR-based incidence of hepatitis A, hepatitis E and entero-viruses in three drinking water treatment stations. *Virology Lab., Water Pollution Research Dept., Environment Research Division, National Research Centre, Cairo-Dokki, Egypt. J. Biol. Pharm. Sci.* 2: 83-89.

Elewa, A.A.; Shehata, M.B.; Mohamed, L.F.; Badr, M.H. and Abdel Aziz, G. S. (2009). Water Quality Characteristics of the Nile River at Delta Barrage with Special Reference to Rosetta Branch. National Institute of Oceanography and Fisheries, Cairo, Egypt, 3(1):1-6.

Fong, T. and Lipp, E.K. (2005). Enteric Viruses of humans and animals in aquatic Environments: Health Risks, Detection, and Potential Water Quality Assessment Tools. *Microbiology and Molecular Biology Reviews* 69(2):357-371.

Grabow, W.O.K. (1996). Waterborne diseases: update on water quality assessment and control. *Water S.A.* 22: 193-202-268.

Halvorson, D.; Karunakaran, D.; Senne, D.; Kelleher, C.; Baily, C.; Abraham, A.; Hinshaw, V. and Newman, J. (1983). Epizootiology of avian influenza simultaneous monitoring of sentinel ducks and turkeys in Minnesota. *Avian Dis.* 27:77-85.

Katzenelson, E.; Fattal, B. and Hostovsky, T. (1976). Organic flocculation: an efficient second-step concentration method for the detection for viruses in tap water. *Appl. Environ. Microbial.* 59: 1213-1219.

Kfir, R.; Hilner, C.; du Preez, M. and Bateman, B. (1995). Studies evaluating the applicability of utilizing the same concentration techniques for the detection of protozoan parasites and viruses in water. *Wat. Sci. Techol.* pp: 417-423.

Kopecka, H.; Dubrou, S.; Prevot, J.; Marechal, J. and Lopez, P. (1993). Detection of naturally occurring enteroviruses in water by RT-PCR and hybridization. *Appl. Environ. Microbiol.* 59:1213-1219.

Lee, G.; Lee, C.; Park, C. and Jeong, S. (2008). Detection and molecular characterization of enteroviruses in Korean surface water by using integrated cell culture multiplex RT-PCR. *Water Analysis and Research center, Biomedical and Environmental Sciences* 21:425-431.

Othman, B.A. (1997). Isolation of lambdoid bacteriophage β_4 EC from sewage polluted drinking water. Proceedings of the 9th Conference of Microbiology, Cairo, pp: 25-27.

Rutjes, S.A.; Berg, H.L.; Lodder, W.J. and Husman, M.R. (2006). Real-time-RT-PCR detection of noroviruses in surface water by use of a broadly reactive nucleic acid sequence-based on amplification assay. *National Institute for Public Health and the Environment, Centre for Infectious Disease control. Appl. Environ. Microbiol.* 72(8):5349-5358.

Sadeghi, G. M.; Nourani, M.; Peyda, M. and Eslam, A. (2007). Microbiology quality assessment of rural drinking water supplies in Iran, *Dept. of Environmental Health and Public Health, School of Public Health, Zanjan University of Medical Science Zanjan, Iran, Journal of Agriculture & Social Sciences* 1(3):31-33.

Schmidt, N.J.; Ho, H.H.; Riggs, J.L. and Lennette, E.H. (1978). Sensitivity of various cell culture systems for isolation of viruses from wastewater and fecal samples. *Appl. Environ. Microbiol.* 36: 480-486.

Shieh, Y. S. C.; Wait, D.; Tai, L. and Sobsey, M. D. (1996). Method to remove inhibitors in sewage and other fecal wastes for entero-viruses detection by the PCR. *J. Virol. Methods* 54: 51-66.

Watanabe, K; Takesue, S.; Jin-Nai, K. and Yoshikawa, T. (1970). Bacteriophage Active Against the Lactic acid Beverage. Producing Bacterium *Lactobacillus casei. Appl. Microbiol.* 20:409-415.

WHO (World Health Organization): (1993). Guideline of Drinking Water Quality. (2nd edition) Vol.1, World Health Organization, Geneva.

Wood, D.J.; Sutter, R.W. and Dowdle, W.R. (2000). Stopping poliovirus vaccination after eradication: issues and challenges. Bull. WHO 78:347-357.

Wright, K. E. (1995). Typing and subtyping of influenza viruses in clinical samples by PCR. *Journal of Clinical Microbiology* 33: 1180-1184.

Yoon, S.S.; Kim, J.W.; Breidt, F. and Fleming, H.P. (2001). Characterization of a lytic *Lactobacillus plantarum* bacteriophage and molecular cloning of lysin gene in *Escherichia coli*. *Int. J. Food Microbiol*. 65:63-74.