Int.J.Curr.Microbiol.App.Sci (2018) *7*(12): 3664-3679

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 7 Number 12 (2018)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2018.712.415

Characterization of Some Enteroviruses in Irrigating Water of Rural and Urban Areas in Egypt and in Clinical Samples with the Assessment of Ethanolic Extract of *Nigella sativa* in Response to these Viruses

Waled Morsy El-Senousy[1], Faten S. Bayoumi[2*], Sara A. Mokhtar[3] and Rana K. Abd El-Tawab[3]

[1]Department of Water Pollution Research, Environmental Research Division and Food Borne Viruses Group, Centre of Excellence for Advanced Science, National Research Centre, Giza, Egypt
[2]Department of Microbiology and Immunology, Faculty of Pharmacy, MSA, Department of Immunogenetics, Human Genetics and genome research Division, National Research Centre, Giza, Egypt
[3]College of Pharmacy, MSA University, Egypt

**Corresponding author*

Keywords

Noroviruses, adenoviruses, Rota viruses irrigating water, Real time PCR, *Nigella sativa*, RT-PCR, Cell Culture RT-PCR

Article Info

Accepted:
28 November 2018
Available Online:
10 December 2018

A B S T R A C T

This study aimed to investigate the frequency of enteroviruses in irrigation water of Egyptian rural and urban areas and also studying the effect of ethanolic extract of *Nigella sativa* on these viruses *in vitro*. 23 samples were collected from irrigation water sites which irrigate freshly eaten crops on El-Giza and El-Menofya Governorates from March 2017 to May 2017; fourteen samples from five rural sites in El-Menofya which no sanitation systems serve these regions and nine samples from three urban sites in El-Giza served by sanitation systems were investigated. From these samples, Adenoviruses were the highly detected (39.1%) followed by rota viruses (26.1%) and then noroviruses (8.7%). Presence of sanitation systems may affect the frequency of enteric viruses in irrigation water. Rotaviruses were the most frequent in the diarrheal samples collected from the same areas of irrigation water samples followed by adenoviruses and finally noroviruses. Considerable reduction of genome copies and infectious units of both rotavirus Wa strain and adenovirus type 7 was observed by treatment with ethanolic extract of Nigella Sativa *in vitro*.

Introduction

Water is playing a powerful role in the transmission of different types of viruses. Fecal oral route is the main cause of transmitting enteric viruses to people or which called waterborne viral diseases. Patients suffering from gastroenteritis or hepatitis may excrete from 105 to 1011 virus particles per gram of stool, Monsalvo (2016). Usually these viruses have been found in groundwater, sewage, rivers, irrigating systems and even in treated drinking water and they are recognized as major public health hazards.

It was found that these viruses had not been removed or inactivated by procedures taken for treatment water. Part of the problems that we facing with viruses in treated water and raw wastewater lies in the distribution systems and the failure to ensure the complete removal of viral pathogens consequently, viruses become environmental pollutants and the solid-associated viruses in wastewater effluents are discharged into aquatic environments and accumulate in the sediments where they persist longer than in the water column (Kovac *et al.,* 2009). As a matter of fact, sediments act as a reservoir from which viruses are re-suspended in the water column by several natural or artificial phenomena (Hassard *et al.,* 2016).

The fate of microbial enteric pathogens may take many potential routes in the water environment. Humans are exposed to enteric viruses through various routes: shellfish grown in contaminated waters, food crops grown in land irrigated with wastewater and/or fertilized with sewage, sewage-polluted recreational waters and contaminated drinking water. Usually, waterborne infections are acquired through the ingestion of contaminated water or shellfish, Pepper *et al.,* (2006).

Rotavirus is a member of family reoviridea composed of segmented double strained RNA and triple layer capsid. Group A rotavirus is responsible for 90% of clinical disease of rotavirus Acip *et al.*, (2009). The rotavirus is considered as the common cause of gastroenteritis in children less than 5 years old around the world, in 2013, WHO estimated the deaths caused by rotavirus infection in children less than 5 years annually the result was 215.000 children and more than 2 million children hospitalized with dehydration. After application of rotavirus vaccine (RotaTeq and Rotarix) in industrial and low resource countries the hospitalization decreased by 90% (Acip *et al.,* 2009). If the infection occurs even one time in life will give 85% immunity toward severe gastroenteritis, 75% immunity toward any rotavirus gastroenteritis and 35% immunity toward any infection of rotavirus. (Bernstein 2009). In Europe, many countries record different rates of rotavirus infection and hospitalized cases. The rate of hospitalized children aged less than 5 years is changing in Denmark from 3.8/1000 per year to 12.8 in Ireland. In 2004, the rotavirus turn to modifiable disease in Ireland, so from this time the rising in notification of rotavirus disease become sustained especially in children aged less than 4 years (September, 2016).

Human adenovirus (HAdV) is a double stranded DNA, non-enveloped virus that belong to family Adenoviridea with 7 species from A to G, 70 distinct genotypes and 50 serotypes that numbered from 1 to 50 There is many different symptoms of adenovirus infection according to the site of replication of each species for example upper or lower respiratory tract human adenovirus infection caused by species B, C and E, eye conjunctiva caused by species B and D and gastrointestinal tract infection occur by F and G species. Occurrence of human Adenovirus outbreaks usually in closed population such as military instillation and hospitals. In Egypt: Eighty-eight adenovirus (Ad) isolates and associated clinical data were collected from walk-in patients with influenza-like illness in Egypt during routine influenza surveillance from 1999 through 2002, Metzgar *et al.,* (2005). Respiratory Adenovirus distributions are geographically variable, and serotype prevalence has not been previously characterized in this region (Metzgar, 2005).

Norovirus is a positive non-enveloped single stranded RNA virus that belongs to family Caliciviridea Norovirus divided into 5 genotypes from GI to GV and the strains that

can infect human are in GI, GII and GIV (Richards *et al.,* 2004; Scipioni *et al.,* 2008). Norovirus cause gastroenteritis in children and adults. It can be transmitted from person to another person through fecal oral route or droplets of vomits which are stable in environment. Low dose of viral particles (≥18) can cause infection. It has the ability to stay alive in high and low temperature and has a resistance against many chemical disinfectants Andrew *et al.,* (2012). It is common in winter so it called winter vomiting disease. Symptoms of infection are nausea, vomiting, headache, diarrhea, dizziness and stomach pain. Norovirus consider the most common cause of viral gastroenteritis and major cause of outbreaks in closed populations such as vacation settings, schools, nursing homes and hospitals (Franck, 2014).

In Cairo, Egypt, rotavirus (Group A) is the most frequent RNA enteric viruses in raw sewage and Nile water and also the most resistant RNA enteric viruses to treatment processes in water and wastewater treatment followed by HAV, astrovirus and enterovirus and finally, noroviruses respectively (Villena *et al.,* 2003; El-Senousy *et al.,* 2004; El-Senousy *et al.,* 2007; Pinto *et al.,* 2007; El-Senousy *et al.,* 2013a; El-Senousy *et al.,* 2013c). In Delta, Egypt, rotavirus has high prevalence in water and wastewater (El-Senousy and El-Mahdy 2009; El -Senousy *et al.,* 2013c) while norovirus has low prevalence in Egyptian naturally contaminated irrigation water and fresh produce (El-Senousy *et al.,* 2013b). Improvement of sensitivity, specificity, and validation of methods for detection and quantification of norovirus in Egyptian irrigation water and fresh produce was done (El-Senousy *et al.,* 2013b) and the differentiation between infectious and non-infectious astroviruses in Egyptian sewage and water samples was also done (El-Senousy *et al.,* 2007). In the study of El-Senousy and Co-workers (2013a) high prevalence of human rotavirus and adenovirus in sewage and water samples before and after treatment steps and also in clinical samples was reported. Adenovirus

Material and Methods

Samples collection

23 samples were collected from irrigation water sites used to irrigate freshly eaten crops on sites 1, 2, and 3 and in El-Giza Governorate and El-Salamlek, Aboud, Koshk Galila, Bawabat, and Asroma sites in El-Menofya Governorates from March 2017 to May 2017. Fourteen samples from five sites in El-Menofya which no sanitation systems serve these regions and nine samples from three sites in El-Giza served by sanitation systems were investigated. Ten liters from each had a little higher frequent than rotavirus and it was detected all the year while rotavirus ha its peak in autumn and winter. El-Senousy and Abou El-Ela (2017) suggested high efficiency of small pilot wastewater treatment plant in viral removal from Egyptian raw sewage. Another study was reported for efficient microbial removal in Egyptian sewage (Tawfik *et al.,* 2012). A lot of trials to discover antiviral drug (using natural products or synthetic compounds) or vaccine depending on the frequent Egyptian strain were reported (Abdo *et al.,* 2012; Ateya *et al.,* 2016; Bassyouni *et al.,* 2012; El-Baz *et al.,* 2013; El-Senousy *et al.,* 2013c; Essawy *et al.,* 2011; Hamdy and El-Senousy, 2013; Hamdy *et al.,* 2013; Matloub *et al.,* 2015; Matloub *et al.,* 2017; Rashad *et al.,* 2015). *Nigella sativa* Linn (Ranunculaceae) is popularly known as black cumin with a wide spectrum of pharmacological activities including anti-inflammatory, antibacterial, antifungal and antihelmenthic (Dwarampudi *et al.,* 2012).

The objective of this study was to investigate rotaviruses, adenoviruses, and noroviruses in

the irrigation water from different parts in rural and urban Egyptian regions. Also, to study the effect of *Nigella sativa* on rotaviruses and adenoviruses depending on the mechanism of blocking the host receptors *in vitro*. Sample were collected and transferred to the laboratory to be concentrated in the same day. Also, diarrheal samples from children 0-5 years were collected from governmental hospitals serve the region where the environmental samples collected (100 samples from El-Giza Governorate and 100 samples from El-Menofya Governorate) in the same time of environmental samples collection.

Concentration of irrigation water samples

Samples were concentrated by filtration through negatively charged nitrocellulose membranes (0.45 µm pore size and 142 mm diameter) after addition of AlCl3 to a final concentration of 0.5 mM and acidification to pH 3.5.

The viruses adsorbed to the membrane were eluted with 75 ml of 0.05 M glycine buffer, pH 9.5 containing 3% beef extract (Lab-Limco powder, Oxoid; Smith and Gerba, 1982; Rose *et al.,* 1984). All samples were reconcentrated using an organic flocculation method (Katzenelson *et al.,* 1976).

Briefly, the eluate was acidified to pH 3.5 using HCl (5N) and centrifuged at 3000 rpm for 15 minutes, the supernatant was discarded and the pellet was dissolved in 1 ml and 5 ml of Na2HPO4 (0.14N, pH 9).

Concentration of Clinical Samples

Approximately 0.1 g of stool samples was weighed, diluted 1:10 in nuclease- free H2 0 and vortexes for 30 sec. Samples were clarified by centrifugation at 7.000 rpm for 10 min at room temperature. Viral RNA was extracted from 140 µl of the supernatant.

Viral nucleic acid extraction

Viral RNA was extracted from 140 µl of the supernatant using BIOZOL Total RNA Extraction reagent (BIOFLUX—Japan) and according to the manufacturer's instructions and to a 30 µl final volume.

RT-PCR of a Fragment of the VP6-Coding Gene of Rotaviruses Group A

The primers used for RT-PCR were the forward VP6-F 5-GATGGATCNACTACAT AGT-3 and the reverse VP6-R 5-GTCCGGTTCATAGGTCCTGG -3 primers (0.5 µm for each), and according to Iturriza-Gomara and co-workers (2002) using 100 U of M-MLV reverse transcriptase enzyme (Promega—USA) in a total volume of 10 µl and 1.5 U of Taq DNA polymerase (Biobasic—Canada) in a total volume of 50 µl. Nested PCR amplification of the target rotavirus VP6 fragment was performed using the forward primer,

VP6-NF 5-GCTAGTTTAAGGGATACA-3, and the reverse primer, VP6-NR 5-TCTATAGCCCGTTAATC-3 (1 µm for each), and according to Gallimore and co-workers (2006) to amplify 155 bp fragment. PCR products (10 µl) were analyzed by electrophoresis on 3 % agarose gels (Panreac-Spain).

Quantification of Rotavirus Group A Genome Copies Using Real-Time RT-PCR Method

Real-time TaqMan RT-PCR was performed for positive samples in the previous RT-PCR screening. Real-time PCR was done using rotavirus @Ceeram Tools™ Food and Environmental kit and according to manufacturer's instructions using Rotavirus - Q Standard (Ceeram Tools).

Cell Culture RT-PCR (CC-RT-PCR) for Quantification of Infectious Rotavirus Particles

Rotavirus CC-RT-PCR assay was performed according to Abad and co-workers (1997); El-Senousy and co-workers (2007); and Ghazy and co-workers (2008). The assay was performed on suspensions of infected MA104 cells. Primers VP6-F and VP6-R were used. The RT-PCR method was the same as described previously. The detection limit in this tissue culture assay using 100 µl of inoculum is 1X101 CC -RT-PCR units/ml, where CC-RT-PCR unit is the reciprocal endpoint dilution detectable by CC-RT-PCR.

RT-PCR for the Detection of Norovirus

Viral RNA of the capsid gene was amplified using the RT-PCR method according to kageyama and co-workers (2003) and kojima and co-workers (2002).

Ten microliters from the nested- PCR were analyzed by 2% agarose gel electrophoresis and visualized by ethidium bromide staining. The lengths of the products from the nested-PCR were 330 bp for norovirus GGI and 344 bp for norovirus GGII.

Molecular assays for human norovirus genome copies quantification

A standardized one-step real-time TaqMan RT-PCR using previously described primers and probes was employed for the detection of human noroviruses GGI (da Silva *et al.,* 2007; Svraka *et al.,* 2007) and GGII (Kageyama *et al.,* 2003; Loisy *et al.,* 2005) in 5 µl of nucleic acid extracts.

Quantification through a real-time procedure following the same conditions as for noroviruses with previously described primers and probes (Pintó *et al.,* 2009) was done.

Extraction of DNA

It was done as described previously by Kapperud and co-workers (1993) and modified by Estrada *et al.,* (2007). Fifty µl of sample concentrate were added to 50 µl of 1X PCR buffer containing 0.2mg of Proteinase K/ml. After being incubated at 37°C for 1h, the suspension was boiled for 10 min and then centrifuged at 12500 rpm for 5 min at 4°C. The supernatant was used for performing the PCR.

Detection of Adenoviruses Using Nested PCR

It was done according to Puig and co-workers (1994) using the specific primers hex AA 1885, hex AA 1913 for the first round PCR and nehex AA 1893 and nehex AA 1905 for the second round PCR for detection of human adenovirus and were selected from the DNA sequence of the open reading frame of hexon gene. PCR products (10 µl) were analyzed by electrophoresis on 3% agarose gels (Panreac-Spain).

Real-Time PCR for quantification of adenoviruses

Real-time TaqMan PCR was performed for positive samples in the previous PCR screening. Real-time PCR was done using adenovirus @ceeram Tools™ Food and Environmental kit and according to manufacturer's instructions using adenovirus - Q Standard (Ceeram Tools) and using a real-time PCR thermal cycler (Rotor-Gene Q, Qiagen).

Cell Culture-PCR (CC-PCR) technique for quantification of adenovirus infectious units

It was done according to Esawy and co-workers (2011) and Abdo and co-workers (2012) Adenovirus cell culture-PCR (CC-

PCR) assay was performed on suspensions of the infected Hep-2 cell line. Set of primers, hex AA 1885and hex AA 1913 was used. The detection limit in this tissue culture assay using 100 µl of inoculum is 1x101 CC- PCR units/ml (u/ml), An adenovirus CC-PCR unit is defined as the reciprocal endpoint dilution detectable by CC- PCR.

Plant materials

Nigella sativa seeds were collected from Egyptian market in Cairo in July 2017.

Extraction

The *Nigella sativa* seeds were washed with water to remove dust particles, Shade dried and extracted by boiling 500 g of the seed powder (twice) in 3000 mL of 95% Ethanol for 30 min at 70°C according to Dwarampudi and co-workers (2012).

Cytotoxicity test

It was done according to Simoes, *et al.,* (1999) and Walum, Strenberg, and Jenssen (1990). Briefly, decontamination of samples was done by adding 24 _µL of 100× of antibiotic–antimycotic mixture to 1 ML of the sample.

Then, bi-fold dilutions were done to 100 _µL of original dissolved samples and 100 _µL of each dilutions were inoculated in MA104 and Hep-2 cell lines (obtained from the Holding Company for Biological Products and Vaccines VACSERA, Egypt) previously cultured in 96 multi well plates (Greiner-Bio one, Germany) to estimate the non-toxic dose of the tested samples.

Cytotoxicity assay was done using cell morphology evaluation by inverted light microscope and cell viability test applying trypan blue dye exclusion method.

Cell morphology evaluation by inverted light microscopy

MA104, and Hep-2 cell cultures (2×10^5 cells/mL) were prepared separately in 96-well tissue culture plates (Greiner-Bio one, Germany). After 24 h incubation at 37 ◦C in a humidified 5% (v/v) CO2 atmosphere cell monolayers were confluent, the medium was removed from each well and replenished with 100 _µL of bi-fold dilutions of different samples tested prepared in DMEM (GIBCO BRL). For cell controls 100 _µL of DMEM without samples was added. All cultures were incubated at 37 ◦C in a humidified 5% (v/v) CO_2 atmosphere for 72 h. Cell morphology was observed daily for microscopically detectable morphological alterations, such as loss of confluence, cell rounding and shrinking, and cytoplasm granulation and vacuolization. Morphological changes were scored (Simoes *et al.,* 1999).

Cell viability assay

It was done by trypan blue dye exclusion method (Walum *et al.,* 1990). MA104, and Hep-2 cell cultures (2×10^5 cells/mL) were grown in 12-well tissue culture plates (Greiner-Bio one, Germany). After 24 h incubation, the same assay described above for tested samples cytotoxicity was followed by applying 100 µL of tested samples dilutions (bifold dilutions) per well. After 72 h the medium was removed, cells were trypsinized and an equal volume of 0.4%

Results and Discussion

Detection of rotaviruses, noroviruses, and adenoviruses in irrigation water samples

Adenoviruses were detected in 9/23 (39.1%) of the samples, rotaviruses were detected in 6/23 (26.1%) of the samples while (w/v). Trypan blue dye aqueous solution was added

to cell suspension. Viable cells were counted under the phase contrast microscope.

Determination of rotavirus Wa strain, and adenovirus type 7 Titers Using plaque assay

The mixture was incubated for 1/2 hr in 37°C. The non-toxic doses of the ethanolic extract of *Nigella sativa* was inoculated directly in MA-104 and Hep-2 cell lines and after 1 hr incubation in 37°C addition of maintenance media was performed. After 24 hours incubation, the inoculation of (100µl) 10 fold dilutions of 100µl of different doses of rotavirus Wa strain, and adenovirus type7 ($1X10^5$, $1X10^6$, $1X10^7$) was carried out separately into MA104, and Hep-2 cell lines in 12 multi well- plates.

The infectivities of the rotavirus stocks were activated with 10 µg/ml trypsin for 30 min at 37°C. After 1 hr of incubation for adsorption at 37°C in a 5% CO_2-water vapor atmosphere without constant rocking. The plates were rocked intermittently to keep the cells from drying. After adsorption, 1 mL of 2X media (Dulbecco's Modified Eagle Medium, Gibco-BRL (DMEM) plus 1ml 1% agarose was added to each well, 0.5 µg/ml was added to the media-agarose mixture in the case of rotavirus Wa strain and the plates were incubated at 37°C in a 5% CO2-water vapor atmosphere. After the appropriate incubation period, the cells were stained with 0.4% crystal violet after formaline fixation, and the number of plaques counted.

The viral titers were then calculated, and expressed as plaque-forming units per milliliter (pfu/mL) (Schmidtke *et al.,* 1998). Noroviruses were detected in 2/23 (8.7%) of the samples. Adenoviruses were detected in 7/14 (50%) in Menofya sites and 2/9 (22.2%) in El-Giza sites. Rotaviruses were detected in 5/14 (35.7%) in Menofya sites and 1/9 (11.1%) in El-Giza sites. Noroviruses were detected in 2/14 (14.3%) in Menofya sites and 0/9 (0%) in El-Giza sites (Table 1).

Quantification of genome copies of the investigated viruses in the positive irrigation water samples indicated that rotavirus genome copies ranged from 6 X 10^2 to 6 X 10^3 genome copies/litre, adenovirus genome copies ranged from 2 X 10^4 to 9 X 10^4 genome copies/litre, and noroviruses genome copies ranged from 1 X 10^2 to 2 X 10^2 genome copies/litre (Table 2). No difference was observed between rural and urban regions.

Quantification of the infectious units of the investigated viruses in the positive irrigation water samples indicated that rotavirus infectious units 8X10 to $9X10^3$ infectious units/litre, adenovirus infectious units were $1X10^2$ infectious units/litre in all investigated samples.

Some samples showed no infectivity for rotavirus, although the viral genome was detected in these samples (Table 3). No difference was observed between rural and urban regions.

The frequencies of rotaviruses, adenoviruses, and noroviruses were 35%, 22%, and 8% respectively in clinical specimens of El-Menofya, while they were 27%, 19%, and 4% respectively in clinical specimens of El-Giza (Table 4).

The non-toxic doses of ethanolic extract of *Nigella sativa* were 130 µg/ml and 120 µg/ml in MA-104 and Hep-2 cell lines respectively.

The reductions of rotavirus Wa strain and adenovirus type 7 were 33.3% and 26.7% respectively when inoculated in MA-104 and Hep-2 cell lines previously treated with the non-toxic doses of ethanolic extract of *Nigella sativa* (Tables 5 and 6).

Table.1 Detection of rotaviruses, noroviruses, and adenoviruses in irrigation Water samples using PCR

	Samples	rotaviruses	noroviruses	adenoviruses
El-Salamlek	14/3/2017	+	-	+
Aboud	14/3/2017	-	-	-
Koshk Galila	14/3/2017	+	+	+
Giza site 1	14/3/2017	-	-	-
Giza site 2	14/3/2017	+	-	+
Giza site 3	14/3/2017	-	-	-
El-Salamlek	22/3/2017	-	-	-
Aboud	22/3/2017	-	-	-
Koshk Galila	22/3/2017	+	-	+
Giza site 1	22/3/2017	-	-	-
Giza site 2	22/3/2017	-	-	+
Giza site 3	4/2017	-	-	-
El-Salamlek	4/2017	-	-	-
Aboud	4/2017	+	-	+
Koshk Galila	4/2017	-	-	+
Giza site 1	4/2017	-	-	-
Giza site 2	4/2017	-	-	-
Giza site 3	4/2017	-	-	-
El-Salamlek	5/2017	-	-	-
Aboud	5/2017	-	-	-
Koshk Galila	5/2017	-	-	-
Bawabat	5/2017	-	-	+
Asroma	5/2017	+	+	+

Table.2 Quantification of rotaviruses, noroviruses and adenoviruses genome copies/litre in irrigation water samples

	Samples	**rotaviruses**	**noroviruses**	**adenoviruses**
El-Salamlek	14/3/2017	$6X10^3$	-	**$8X10^4$**
Aboud	14/3/2017	-	-	-
Koshk Galila	14/3/2017	$4X10^3$	$2X10^2$	**$5X10^4$**
Giza site 1	14/3/2017	-	-	-
Giza site 2	14/3/2017	$1X10^3$	-	**$2X10^4$**
Giza site 3	14/3/2017	-	-	-
El-Salamlek	22/3/2017	-	-	-
Aboud	22/3/2017	-	-	-
Koshk Galila	22/3/2017	$3X10^3$	-	**$9X10^4$**
Giza site 1	22/3/2017	-	-	-
Giza site 2	22/3/2017	-	-	**$8X10^4$**
Giza site 3	4/2017	-	-	-
El-Salamlek	4/2017	-	-	-
Aboud	4/2017	$1X10^3$	-	**$9X10^4$**
Koshk Galila	4/2017	-	-	**$2X10^4$**
Giza site 1	4/2017	-	-	-
Giza site 2	4/2017	-	-	-
Giza site 3	4/2017	-	-	-
El-Salamlek	5/2017	-	-	-
Aboud	5/2017	-	-	-
Koshk Galila	5/2017	-	-	-
Bawabat	5/2017	-	-	**$2X10^4$**
Asroma	5/2017	$6X10^2$	$1X10^2$	**$9X10^4$**

Table.3 Quantification of rotaviruses, noroviruses and adenoviruses infectious units/litre in irrigation water samples

Samples		rotaviruses	adenoviruses
El-Salamlek	14/3/2017	$9X10^3$	**$1X10^2$**
Aboud	14/3/2017	-	-
Koshk Galila	14/3/2017	8X10	**$1X10^2$**
Giza site 1	14/3/2017	-	-
Giza site 2	14/3/2017	9X10	**$1X10^2$**
Giza site 3	14/3/2017	-	-
El-Salamlek	22/3/2017	-	-
Aboud	22/3/2017	-	-
Koshk Galila	22/3/2017	9X10	**$1X10^2$**
Giza site 1	22/3/2017	-	-
Giza site 2	22/3/2017	-	**$1X10^2$**
Giza site 3	4/2017	-	-
El-Salamlek	4/2017	-	-
Aboud	4/2017	0	**$1X10^2$**
Koshk Galila	4/2017	-	**$1X10^2$**
Giza site 1	4/2017	-	-
Giza site 2	4/2017	-	-
Giza site 3	4/2017	-	-
El-Salamlek	5/2017	-	-
Aboud	5/2017	-	-
Koshk Galila	5/2017	-	-
Bawabat	5/2017	-	**$1X10^2$**
Asroma	5/2017	0	**$1X10^2$**

Table.4 Detection of rotaviruses, noroviruses, and adenoviruses in clinical samples using PCR

Type of virus	Frequency and percentage of viral frequency in clinical specimens of El-Menofya	Frequency and percentage of viral frequency in clinical specimens of El-Giza
Rotaviruses	35/100 (35%)	27/100 (27%)
Adenoviruses	22/100 (22%)	19/100 (19%)
Noroviruses	8/100 (8%)	4/100 (4%)

Table.5 Antiviral activity of non-toxic doses of tested materials against rotavirus Wa strain

Tested materials	Initial Viral titre	Final viral titre	% of reduction	Mean % of reduction
Ethanolic extract of *Nigella sativa*	$1X10^5$	$6X10^4$	40%	33.3%
	$1X10^6$	$7X10^5$	30%	
	$1x10^7$	$7x10^6$	30%	

Table.6 Antiviral activity of non-toxic doses of tested materials against adenovirus type 7

Tested materials	Initial Viral titre	Final viral titre	% of reduction	Mean % of reduction
Ethanolic extract of *Nigella sativa*	$1X10^5$	7X104	30%	26.7%
	$1X10^6$	7X105	30%	
	$1x10^7$	8x106	20%	

About 3.4 million people especially children die every year as a result of diseases related to water. Absence of treatment in most sources of used water led for producing human enteric viruses. There are more than 100 viral species that are the main cause of infection to human. Among the many viruses discharged are such pathogens as rotavirus, norovirus, and adenovirus. The significance of these viruses in human range between gastrointestinal, bronchitis and conjunctivitis. Most of these agents transmitted via fecal oral rout or person to person contact. Irrigation water is one of the main sources of enteric viruses transmission to human and animals especially when irrigates freshly eaten crops.

Our results indicated that of enteric viruses investigated in irrigation water; adenoviruses were the most frequent followed by rotaviruses, and finally noroviruses. These results are comparable with the pattern detected in ground water used for irrigation in Giza Governorate (El-Senousy *et al.*, 2013d). This pattern was observed in samples collected from either Menofya or Giza sites. On the other hand, the frequency of viruses in irrigation water samples collected from Menofya is higher than the viral frequency in Giza irrigation water samples. This pattern is clear for all the studied viruses. This higher frequency of adenoviruses, rotaviruses, and noroviruses in Menofya irrigation water samples than Giza irrigation water samples may return to the absence of wastewater treatment systems in the sampling region of Menofya. On the other hand, there are wastewater treatment systems serve the sampling regions of Giza. The presence of viruses in irrigation water in our study may represent a health hazard; however this irrigation water irrigates freshly eaten crops in all sampling regions in both Governorates. The higher frequency of gastroenteritis causing viruses in the diarrheal specimens collected from El-Menofya than the

specimens collected from El-Giza may confirm this conclusion.

Qualitative analysis is not enough to ensure the health hazard, so quantitative analysis for both genome copies and infectious units was used. Again, the number of genome copies of adenoviruses was higher than rotaviruses. Noroviruses were less than adenoviruses and rotaviruses in the number of genome copies. The number of noroviruses quantified in our study is closely related to the number quantified in irrigation water samples collected previously from Egyptian Delta region (El-Senousy *et al.*, 2013b). El-Senousy *et al.*, (2007) confirmed the relationship between the number of genome copies and infectious units. Our article gives a new evidence for the confirmation of this direct relationship. The number of infectious units of noroviruses could not be quantified because till now, there is no cell line suitable for propagation of noroviruses. Quantitatively, there was no difference between the number of genome copies of all the studied viruses in either regions served with wastewater treatment systems or regions suffer from absence of these systems. The same point was observed for the number of infectious units for both rotaviruses and adenoviruses.

The frequency pattern in irrigation water was different from the pattern observed in diarrheal samples which rotavirus was the most frequent virus. Rotavirus is the first viral causative agent for gastroenteritis worldwide followed by adenovirus. On the contrary, adenovirus is the most frequent virus in the environmental samples. It may return to the higher stability of adenovirus against the environmental conditions like temperature and pH. It gives higher survival rate for adenovirus (DNA genome) than rotavirus (RNA genome). Also, adenovirus has not a peak of seasonal variation, while rotavirus has a peak in autumn and winter. It gives a chance to adenovirus to be detected in the environmental samples all the year.

The reductions of rotavirus Wa strain and adenovirus type 7 were 33.3% and 26.7% respectively when inoculated in MA-104 and Hep-2 cell lines previously treated with the non -toxic doses of ethanolic extract of *Nigella sativa*. The pre-inoculation of the extracts may lead to block the receptors specific for both viruses in the two specific cell lines (host systems). This mechanism differs from the other mechanisms which depend on the direct action on the virus or stop the replication cycle. Although, the percentage of reduction is not so high (less than 50%). it may be promising according to the used mechanism (blocking the receptors specific for viruses). Using of the natural products in regular way or as additives to some daily used food may lead to some protection against gastroenteritis causing viruses.

References

Abad, F. X., Pinto´, R. M., Villena, C., Gajardo, R., and Bosch, A. (1997). Astrovirus survival in drinking water. Applied and Environment Microbiology, 63, 3119–3122.

Abdo M.H., M.E. Goher, M.F. Sayed (2012): Environmental evaluation of Ismailia Canal water and sediment, Egypt. J. Egypt. Acad. Soc. Environ. Dev., 13 (2), pp. 61-78

Acip, P., Rutledge, T.F. and Boyd, M.F., 2009. Morbidity and Mortality Weekly Report Prevention of Rotavirus Gastroenteritis Among Infants and Children Recommendations of the Advisory Committee, Pp. 58.

Andrew M.Q. King, Michael J. Adams, Eric B. Carstens, and Elliot J. Lefkowitz

Family – Caliciviridae.2012, Pages 977–986

Ateya A., Al-Gendy A.A., Kotob S., Hafez, A. (2016): Chemical constituents, antioxidant, antimicrobial and anti-inflammatory activities of *Erysimum corinthium* boiss. (Brassicaceae). International Journal of Pharmacognosy and Phytochemical Research 2016; 8(10); 1601-1609

Bassyouni FA, Saleh TS, El Hefnawi M, Abd El-Moez SI, El-Senousy WM, Abdel-Rehim M. Synthesis, Pharmacological Activity Evaluation and Molecular Modeling of New Polynuclear Heterocyclic Compounds Containing Benzimidazole derivatives. Archive of pharmacal Research ARPR. 2012; 35: 2063-2075

Bernstein, D.I., 2009. Rotavirus overview. The Pediatric infectious disease journal, 28 (3 Suppl), pp. S50-3. Available at: http://content.wkhealth.com/linkback/openurl?sid=WKPTLP:landingpage&an=00006454-200903001-00002%5Cnhttp://www.ncbi.nlm.nih.gov/pubmed/19252423.

Da Silva, A., Le Saux, J., Parnaudeau, S., Pommepuy, M., Elimelech, M. and Le Guyader, F. (2007). Evaluation of Removal of Noroviruses during Wastewater Treatment, Using Real-Time Reverse Transcription-PCR: Different Behaviors of Genogroups I and II. Applied and Environmental Microbiology, 73(24), pp.7891-7897

Dwarampudi L.P., Dhanabal SP, Shanmugam R, Muruganantham N (2012): Antipsoriatic activity and Cytotoxicity of ethanolic extract of *Psoralia corylifolia* seeds. Hygeia. J.D. Med. Vol.4 (2), Oct. 2012, 41-48

El-Senousy W.M., Sidkey N.M, Abu Senna A S.M.N. Abed N.N. Hasan S.F. (2013): Revalence of Rotaviruses and Noroviruses in ground water of some rural areas in El-Giza governorate, Egypt. The New Egyptian Journal of Medicine Vol.:48; No.: 3 Supplement 1st March 2013

El-Baz, F.K., El-Senousy, W.M., El-Sayed, A.B., Kamel, M.M., Amal, A. Mohamed. In vitro antiviral and antimicrobial activities of algal extract of *Spirulina platensis*. J App Pharm Sci, 2013; 3 (12): 052-056

El-Senousy WM [1], Abou-Elela SI Assessment and Evaluation of an Integrated Hybrid Anaerobic-Aerobic Sewage Treatment System for the Removal of Enteric Viruses. Food Environ Virol. 2017 Sep; 9(3): 287-303

El-Senousy WM, Guix S, Abid I, Pintó RM, Bosch A. Removal of Astrovirus from Water and Sewage Treatment Plants, Evaluated by a Competitive Reverse Transcription-PCR. Appl. Environ. Microbiol. 2007; 73: 164-16

El-Senousy, W. M. and El-Mahdy, E. M. (2009). Detection and genotyping of rotaviruses in water treatment plants of El-Dakahlia Governorate. Egypt. J. Biotechnol., Vol. 31: pp. (25-34

El-Senousy, W. M., Barakat, A. B. Ghanem, H. E. and Kamel, M. A. (2013a). Molecular Epidemiology of Human Adenoviruses and Rotaviruses as Candidate Viral Indicators in the Egyptian Sewage and Water Samples. World Appl. Sci. J., Vol. 27: pp. (1235-1247).

El-Senousy, W. M., Costafreda, M. I., Pintó, R. M. and Bosch, A. (2013b). Method validation for norovirus detection in naturally contaminated irrigation water and fresh produce. Int. J. Food Microbiol., Vol. 167: pp. (74-79).

El-Senousy, W. M., Pintó, R. M. and Bosch, A. (2004). Epidemiology of human enteric viruses in the Cairo water environment. The 1st International Conference of Environmental Research

Division on Sustainable Development Environmental Challenges Facing Egypt. National Research Centre, Cairo, Egypt

El-Senousy, W., Costafreda, M., Pintó, R. and Bosch, A. (2013). Method validation for norovirus detection in naturally contaminated irrigation water and fresh produce. International Journal of Food Microbiology, 167(1), pp.74-79.

Esawya A.M., Eman F. Ahmeda, Wafaa A. Helmya, Nahla M. Mansourb, Waled M. El-Senousyc, Mounir M. El-Safty Production of levansucrase from novel honey *Bacillus subtilis* isolates capable of producing antiviral levans. Carbohydrate Polymers 86 (2011) 823–830

Estrada, B., Maeland, A.D., Gisselbrecht, S.S., Bloor, J.W., Brown, N.H., Michelson, A.M. (2007). The MARVEL domain protein, Singles Bar, is required for progression past the pre-fusion complex stage of myoblast fusion. Dev. Biol. 307(2): 328--339.

Gallimore, C. I., Taylor, C., Gennery, A. R., Cant, A. J., Galloway, A., Iturriza-Gomara, M. and Gray, J. J. (2006). Environmental Monitoring for Gastroenteric Viruses in a Pediatric Primary Immunodeficiency Unit. J. Clinic. Microbiol., Vol. 44: pp. (395–399)

Ghazy MM, El-Senousy WM, Abdel-Aatty AM, Hegazy B, Kamel M. Performance evaluation of a waste stabilization pond in a rural area in Egypt. American Journal of Environmental Sciences. 2008; 4: 316-326.

Hamdy NA, El-Senousy WM, Fakhr IMI. Enaminone as Building Blocks in Organic Chemistry: a Novel Route to Polyfunctionally 2-substituted 5, 6, 7, 8-tetrahydronaphthalenes and their Antiviral Evaluation. Journal of Heterocyclic Chemistry. 2013; 50: 337-343.

Hamdy NA, El-Senousy WM. Synthesis andf antiviral evaluation of some novel pyrazoles and pyrazolo [3,4-d] pyridazines bearing 5,6,7,8-tetrahydronaphthalene. Acta Poloniae Pharmaceutical Drug Research. 2013; 70: 99-110

Hassard F., Gwyther L.C., Farkas K., Andrews A., Jones V., Cox B., Brett H., Jones L.D. McDonald E.J., and Shelagh K. Malham K.S, (2016): Abundance and Distribution of Enteric Bacteria and Viruses in Coastal and Estuarine Sediments—a Review. Front Microbiol. 2016; 7: 1692. Published online 2016 Nov 1. doi: 10.3389/fmicb.2016.01692. PMCID: PMC5088438

Iturriza-Go´mara M , Wong C, Blome S, Desselberger U, Gray J. Mo-lecular characterization of VP6 genes of human rotavirus isolates: correlation of genogroups with subgroups and evidence of independent segregation. J Virol 2002; 76: 6596–6601.

Kageyama T, Kojima S, Shinohara M, Uchida K, Fukushi S, Hoshino FB, Takeda N, Katayama K. Broadly reactive and highly sensitive assay for Norwalk-like viruses based on real-time quantitative reverse transcription-PCR. J Clin Microbiol. 2003 Apr; 41(4):1548-57.

Kapperud, G., Vardnund, T., Skjerve, E., Hornes, E., and Michaelsen, T. E. (1993). Detection of pathogenic Yersinia enterocolitica infoods and water by immunomagnetic separation, nested poly-merase chain reaction and colorimetric detection of amplified DNA. Applied and Environment Microbiology, 59, 2938–2944

Katzenelson E.B., Badri Fattal · Tova Hostovesky (1976): Organic (4 Flocculation: an Efficient Second-Step Concentration Method for the Detection

of Viruses in Tap Water. Applied and Environmental Microbiology, (32): 638-9.

Kojima S, Kageyama T, Fukushi S, Hoshino FB, Shinohara M, Uchida K, Natori K, Takeda N, Katayama KGenogroup-specific PCR primers for detection of Norwalk-like viruses. J Virol Methods. 2002 Feb; 100(1-2):107-14.

Kovac K, Gutiérrez-AguirreMijovski JZ, Schultz AC, Raspor P.I, Banjac M, Peterka M, Poljsak-Prijatelj M, Ravnikar M, A novel method for concentrating hepatitis A virus and caliciviruses from bottled water. J. Virol Methods. 2009 Dec; 162(1-2): 272-5

Loisy, F., Atmar, R.L., Guillon, P., Le Cann, P., Pommepuy, M. and Le Guyager, F.S., 2005. Real-time RT-PCR for norovirus screening in shellfish. Journal of Virological Methods 123:1–7.

Matloub1 A.A., El-Senousy W.M., Abo El-Khair Badawy El-Sayed3, Hanan Farok Alyin vitro assessment of anti-HCV, antioxidant, cytotoxic and hypolipidemic activities of glycoprotein isolated from *Spirulina platensis*. Asian Pac J Trop Dis 2017; 7(11): 676-682

Matloub1 A.A., Elsouda S.S., El-Senousy W.M (2015): In vitro Antiviral, Cytotoxic, Antioxidant and Hypolipidemic Activites of Polysaccharide Isolated from Marine Algae. International Journal of Pharmacognosy and Phytochemical Research; 7(5); 1099-1111

Metzgar, D., Osuna, M. and Yingst, S. (2005). PCR Analysis of Egyptian Respiratory Adenovirus Isolates, Including Identification of Species, Serotypes, and Coinfections. J Clin Microbiol. 2005 Nov; 43(11): 5743-52.

Monsalvo M.V. (2016) Wastewater and Public Health: Bacterial and Pharmaceutical Exposures. Apple academic press. Inc

Pepper L. Ian, Charles P. Gerba, Mark L. Brusseau.2006 Environmental and Pollution Science. 2nd edition. Elsevier

Pintó, R.M., Costafreda, M.I. and Bosch, A., 2009. Risk Assessment in Shellfish-Borne Outbreaks of Hepatitis A. Applied and Environmental Microbiology 75: 7350– 7355.

Puig, A. and Gilbert, H. F. (1994) Protein disulfide isomerase exhibits chaperone and anti-chaperone activity in the oxidative refolding of lysozyme. J. Bid. Chem. 269, 7764-7771

Rashad, M.M., A.E. Mahmoud, M.M. Ali, M.U. Nooman and A.S. Al-Kashef, 2015. Antioxidant and anticancer agents produced from pineapple waste by solid state fermentation. Int. J. Toxicol. Pharmacol. Res., 7: 287-296.

Richards, G.P., Watson, M.A., Fankhauser, R.L., Monroe, S.S., 2004.Genogroup I and II noroviruses detected in stool samples by real-time reverse transcription-PCR using highly degenerate universal primers. Applied and Environmental Microbiology 70, 7179– 7184.

Rose JB, Gerba CP (1991 of risk assessment) Use for development of microbial standards. Wat Sci Tech 24:29–33

Rose, J. B., Singh, S. N., Gerba, C. P. and Kelley, L. M. (1984). Comparison of microporous filters for concentration of viruses from wastewater. Appl. Environ. Microbiol., Vol. 47: pp. (989-992)

Schmidtke M, Knorre C, Blei L, Stelzner A, Birch-Hirschfeld E.Penetration and Antiviral Activity of Coxsackievirus B3 (Cvb3)- Specific Phosphorothioate Oligodeoxynucleotides (Ps-Odn). Nucleosides and Nucleotides. 1998; 17: 1557-1566.

Scipioni, A., Mauroy, A., Vinje, J., and Thiry, E., "Review of Animal Noroviruses" (2008). Public Health Resources. 86

September, U., 2016. Rotavirus 19., (September), pp.1–9.

Simões CMO, Amoros M, Girre L. Mechanism of antiviral activity of triterpenoid saponins, Phytoth Res. 1999; 21: 317-325

Smith, E.M., and C.P. Gerba. 1982. Development of a method for the detection of human rotavirus in water and sewage. Appl. Environ. Microbiol. 43:1440-1450.

Svraka S, Duizer E, Vennema H, de Bruin E, van der Veer B, Dorresteijn B, Koopmans M. Etiological role of viruses in outbreaks of acute gastroenteritis in The Netherlands from 1994 through 2005. J Clin Microbiol. 2007 May; 45(5):1389-94.

Tawfik A.B., Reto Stöckli,Allen Goldstein, Shelley Pressley, Allison L. Steiner Quantifying the contribution of environmental factors to isoprene Flux interannual variability / Atmospheric Environment 54 (2012) 216-224.

Villena, C., El-Senousy, W. M., Abad, F. X., Pintó, R. M. and Bosch, A. (2003). Group A rotavirus in sewage samples from Barcelona and Cairo: Emergence of unusual genotypes. Appl. Environ. Microbiol., Vol. 69: pp. (3919–3923).

Walum E, Strenberg K, Jenssen D.1990. Understanding Cell Toxicology: Principles and Pratice. Ellis Howood, New York. 97-111

How to cite this article:

Waled Morsy El-Senousy, Faten S. Bayoumi, Sara A. Mokhtar and Rana K. Abd El-Tawab. 2018. Characterization of Some Enteroviruses in Irrigating Water of Rural and Urban Areas in Egypt and in Clinical Samples with the Assessment of Ethanolic Extract of *Nigella sativa* in Response to These Viruses. *Int.J.Curr.Microbiol.App.Sci.* 7(12): 3664-3679.
doi: https://doi.org/10.20546/ijcmas.2018.712.415