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RESEARCH ARTICLE

Quantitative RT-PCR detection of human noroviruses and hepatitis A virus in fresh produce and surface water used for irrigation in the Mansoura and Giza regions, Egypt

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Abstract

Surface water used as an irrigation source can be a significant source of viral contamination of fresh produce. Enteric viruses such as hepatitis A virus (HAV) and human norovirus genogroup I (HNoV GI) and genogroup II (HNoV GII) can be transmitted to human via fresh produce when irrigated with contaminated water or when prepared by infected food handlers. In the current study, we investigated the presence of HAV, HNoV GI and GII in fresh produce and surface water used in cultivation of this produce using real-time PCR. Samples were collected from six different points in the Mansoura and Giza regions, Egypt. Our analysis showed that at least one virus was found in 41.6% (30/72) of surface water samples and 27% (13/48) of fresh produce samples. HAV (23/72) with a mean viral concentration = 4×10^6 genome copies/litre (GC/L) was the most frequently identified virus in surface water samples, followed by human norovirus genogroup II (HNoV GII) (15/72, with a mean concentration = 1.2×10^6 GC/L, and human noroviruses genogroup I (HNoV GI) (12/72, with a mean concentration = 1.4×10^4 GC/L). Additionally, HAV (10/48) with a mean concentration = 5.2×10^5 genome copies/gram (GC/g) was also the most frequently detected virus in the fresh produce samples, followed by HNoV GII (8/48, with a mean concentration = 1.7×10^4 GC/g); meanwhile, HNoV GI (6/48) was less detected virus with a mean concentration = 3×10^3 GC/g. This work suggests a wide prevalence of human enteric viruses in surface waters and fresh produce, which is of concern when the fresh produce is eaten raw. Thus, additional monitoring for viral pathogens in irrigation water and food is needed to increase the awareness of this issue to rise the control measures to reduce illness from contaminated food.

Keywords Fresh produce · Water · Hepatitis A virus · Noroviruses · Real-time PCR

Introduction

Water and foodborne illness are two of the most serious public health issues, resulting in a considerable number of deaths in people each year (WHOa 2005). Waterborne illness is a global problem which is responsible for more than 2.2 million deaths per year (WHO 2005; Bitton 2014). Children account for around 1.4 million of these deaths (WHO 2015). In Egypt, the presence of enteric viruses, such as hepatitis A and E viruses, norovirus, rotavirus, adenovirus, human noroviruses, and human astrovirus has recently been reported in river water used in irrigation purpose (Elmahdy et al. 2020; Shaheen et al. 2018, 2019, 2020; Shaheen and Elmahdy 2019a, b). This underlines that irrigation water can play a role in transferring of these viruses to fresh produce.

Hepatitis A virus (HAV) and human noroviruses (HNoV) are recognized as major public health concerns where they are responsible for the majority of acute hepatitis or non-bacterial gastroenteritis which are occasionally fatal (Vaughan et al. 2014; Koo et al. 2010). HAV belong to the *Picornaviridae* family while HNoV is classified in the *Caliciviridae*. Both viruses possess a single-stranded

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and positive-sense RNA genome as they are small non-enveloped viruses. HNoV which was classified into ten genogroups (HNoV GI-GX) is responsible for about 20% of all cases of acute gastroenteritis, worldwide (Chhabra et al. 2019; Ahmed et al. 2014), with over 213,000 deaths and 677 million cases reported annually (Pires et al. 2015). Significant rates of morbidity and mortality due to HNoV infection are reported in risk groups comprising elderly, children, and immunocompromised individuals (Tian et al. 2014; Payne et al. 2013; Siebenga et al. 2009; Harris et al. 2008; Hutson et al. 2004). Also, HAV is a major cause of acute viral hepatitis, with nearly 1.4 million cases per year globally (Jacobsen 2009; Yong and Son 2009), and a high hepatitis A endemicity in areas with low sanitation (Osuolale and Okoh 2015; Gupta and Ballani 2014; Yong and Son 2009). They are primarily transmitted via the vomit-oral and faecal-oral routes, including contact with infected person, contact with contaminated surfaces, or by ingestion of polluted water and food: particularly vegetables, soft fruits, and shellfish (Kotwal and Cannon 2014; Matthews et al. 2012; Nainan et al. 2006).

Contamination of groundwater or surface water with these viruses represents a potential risk of infection through food or drinking water (Moreira and Bondelind 2017; Mathijs et al. 2012). The contamination of fresh produce can occur during pre-harvesting, due to contact with contaminated irrigation water or during post-harvesting due to an infected worker through handling of the product or by contaminated equipment through the production process (Leon-Felix et al. 2010; Seymour and Appleton 2001; Bidawid et al. 2000a, b). Viral contamination of irrigation, ground, surface water, and soil has been investigated in previous studies (Gerba and Choi 2006; Lodder and de Roda Husman 2005; Santamaria and Toranzos 2003). The aim of this study was to investigate the presence of HAV and HNoV in fresh produce and surface water used for irrigation.

Material and methods

Study area

Dakahlia Governorate is located at the northeast of Nile Delta in Egypt. Mansoura (the capital of Dakahlia Governorate) is the most important cities in Dakahlia Governorate. Giza is located on the west bank of the Nile, 4.9 km southwest of central Cairo, and is a core city of the Greater Cairo Metropolis. The two regions have been chosen because of the fast rate of urbanization and very low studies on the simultaneous detection of these viruses in both fresh produce and irrigation water were made on them.

Sampling

A total of 72 surface water samples and 48 fresh produce (24 green onions and 24 lettuces) samples were collected from three sites at two regions (Giza and Mansoura) in Egypt. As shown in Fig. 1, three water samples were collected monthly from each region during the period March 2019 to February 2020. Also, 3 fresh produce samples from each kind were collected monthly from the same sites at each region according to the harvest season (November 2019 to February 2020).

Determination of virus recovery from fresh produce and water samples

In this study, we used murine norovirus (MNV-1) as a process control virus to investigate the virus recovery in all virus detection assays. In brief, autoclaved surface water samples (5 L) were inoculated with 200 µL of MNV-1 suspension (4.7×10^8 GC/mL) prior to concentration. Also, leaves of green and lettuce were chopped into small pieces then 50 g of each sample was weighed and sanitized in chlorinated water for 20 min. After sanitation, the leaves of each fresh produce were divided into two equal parts and each part was placed into a sterile bag under aseptic conditions. One portion of the fresh produce sample was inoculated with the same amount of virus prior to concentration, while the other portion was left as blank. The efficiency of MNV-1 recovery from inoculated samples was assessed using the protocols described below. Furthermore, a representative sample was taken from green onion and lettuce collected during this study and inoculated with human MNV-1 as sample process control virus (SPCV) (previously tested negative for MNV-1 by RT-qPCR).

Virus concentration

Virus concentration from surface water samples was performed by using a protocol described by Katayama et al. (2002) with slight modification. In brief, 4 L of collected water sample was mixed with 40 mL of 2.5 M $MgCl_2$ (5 mL of MgCl2/500 mL sample) then filtered through a negatively charged membrane (0.45 µm pore size and 142 mm diameter). Filter membrane was rinsed with 200 mL of 0.5 mM H_2SO_4 solution (pH 3.0). The membrane was removed and transferred to sterile glassware then eluted in 10 mL of 1 mM NaOH ($pH = 10.5$). After stirring for 10 min, the eluate was neutralized by adding 50 µL 1 mM Tris–EDTA buffer solution (pH 8.0) and 50 µL of 50 mM H_2SO_4 solution. After that, the neutralized eluate was ultra-filtrated by using a Centriprep Concentrator system then the filtrate was

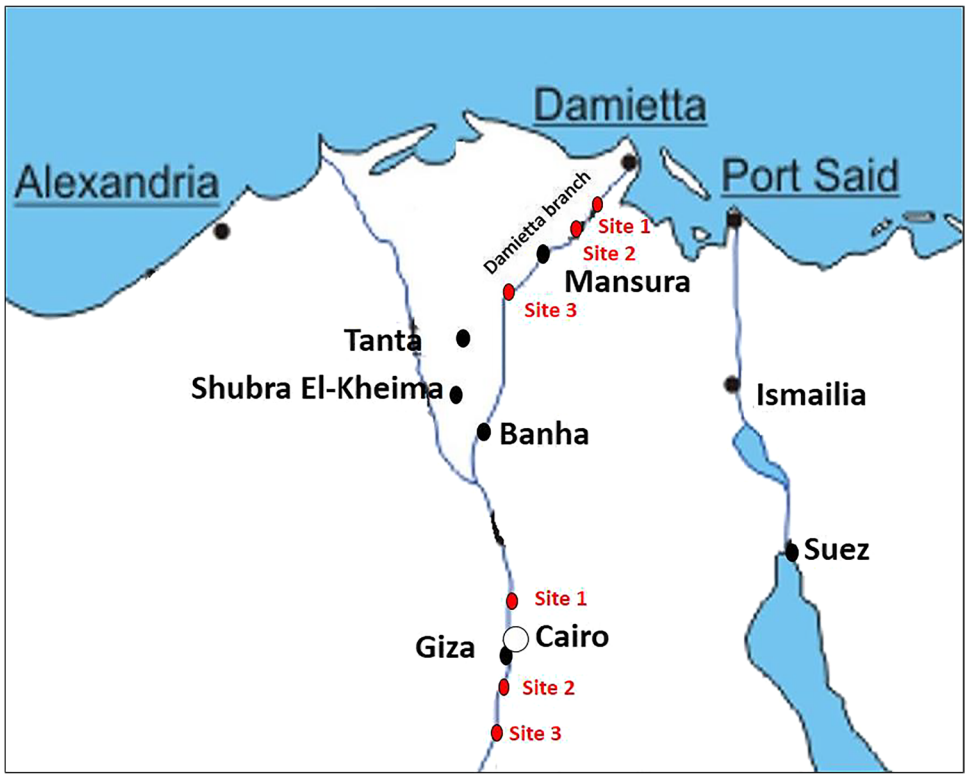

Fig. 1 The field of surface water and fresh produce collection at different sites in Mansoura and Giza

centrifuged at 1500 × *g* for 10 min at 4 °C. After removing the filtrate, the pellet was rinsed twice with 10 mL of double distilled water by the same procedure, and a final volume of 2 mL was obtained. The concentrated samples were stored at − 80 °C until the next analysis. On the other hand, virus elution from fresh produce samples was done according to ISO/TS 15,216–1 method (ISO 15216–1:2017 2017). Briefly, 25 g of green onion or lettuce in small pieces was mixed with 40 mL of Tris–glycine buffer (100 mM Tris–HCl, 50 mM glycine, and 1% beef extract, pH 9.5) in a sterile plastic bag. After 20 min at room temperature with constant rocking (approximately 70 oscillations/min) to elute the viruses from the surface of the fresh produce sample, the sample was distributed into clean centrifuge tubes. After centrifugation at 10,000 × *g* for 30 min at 4 °C, the vegetable matter was discarded and the eluate was transferred into clean centrifuge tubes then the pH was adjusted 7.2 ± 0.3 using 1.0 N HCl. For the PEG precipitation, 0.25 volume of 50% (w/v) polyethylene glycol 8000/1.5 M NaCl was added to the eluates and incubated on rocking at 120 rpm at 4 °C for 1 h. After additional centrifugation for 30 min at 10,000 × *g* at 4 °C, the resulting pellets were dissolved in 500 µL of 10 mM PBS then stored at − 20 °C until use.

RNA extraction and virus quantification

The final concentrates were used to extract viral RNA using a QIAamp MiniElute Virus Spin kit (Qiagen, Germany) according to the manufacturer's instructions. Phosphate buffer saline as a negative control for nucleic acid extraction was applied. For molecular detection of HAV, HNoV GI and GII, and MNV-1, the qPCR was carried out according to Jothikumar et al. (2005), Kageyama et al. (2003), and Lee et al. (2015), respectively, using one-step Rotor-Gene Probe RT-PCR kit. All amplifications were conducted in duplicate using Rotor-Gene system (QIAGEN, Germany). For each assay, PCR amplicon for each positive control was generated by cloning the amplicon into a plasmid (pGEM-T Easy Vector (Promega) for HAV strain HM175 and pCR2.1-TOPO vector (Promega) for HNoV GI, GII, and MNV-1), and concentrations of a purified plasmid DNA were calculated using a Nano Drop spectrophotometer (Thermo Fisher Scientific, USA). Standard curves were prepared by ten-fold serial dilutions of the positive control plasmids. Ultra-pure water was used as negative controls in each assay to ensure that there was no cross-contamination in the assay. To increase PCR efficiency, serial ten-fold dilutions were prepared to viral nucleic acids to dilute the inhibitors (if found). Viral nucleic acid was amplified in a 25 µL real-time PCR mixture containing 5 µL of the RNA extract, 12.5 µL RT-PCR Master Mix (Qiagen, Germany), 400 nM each primer, 250 nM of probe, and nuclease-free water to complete the volume reaction up to 25 µL. This mixture was transferred into a 48-well microplate then loaded into the Rotor-Gene system. Fluorescence data were measured at the end of annealing step. All primers and probes used in this study, as well as the thermal

Table 1 Primers and probes utilized for HAV and HNoV examined in this study

Virus	Primer sequence 5'-3'	Thermal cycling conditions	References
HAV	Fw: GGTAGGCTACGGGTGAAAC R: GCGGATATTGGTGAGTTGTT Probe: FAM-CTTAGGCTAATACTTCTATGAA GAGATGC-BHQ	48 °C for 30 min, followed by 95 °C for 15 min then 40 cycles of 95 °C for 10 s, 55 °C for 20 s, and 72 °C for 15 s	Jothikumar et al. (2005)
HNoV GI	Fw: CGYTGGATGCGNTTYCATGA Rv: CTTAGACGCCATCATCATTYAC Probe: FAM -AGATYGCGATCYCCTGTCCA-TAMRA	50 °C for 2 min, followed by 95 °C followed by 10 min, then 45 cycles of 94 °C for 20 s and 56 °C for 1 min	Kageyama et al. (2003)
HNoV GII	Fw: CARGARBCNATGTTYAGRTGGATGAG Rv: TCGACGCCATCTTCATTCACA Probe: FAM-TGGGAGGGCGATCGCAATCT-TAMRA		
MNV-1	Fw: ACGCTCAGCAGTCTTTGTGA Rv: CTGGCCTCAGAGCCATTG Probe: FAM-CGCTGCGCCATCACTCATCC-TAMRA	95 °C for 10 min then 40 cycles of at 95 °C for 15 s and 60 °C for 1 min	Lee et al. (2015)

cycling conditions of each virus, are shown in Table 1. The percentage of MNV-1 recovery rate from spiked water or fresh produce samples was calculated by using the following formula: amount of virus detected after spiking experiments/amount of viral inoculum × 100 (Hennechart-Collette et al. 2021).

Statistical analysis

Statistical analyses were performed using GraphPad Prism version 5.0 (USA) software. The critical *P* value for the test was set at < 0.05. The Pearson correlation was applied to evaluate the correlations between viral distributions and water/fresh produce samples. The mean viral load was compared between samples with a one-way analysis of variance. The detection limits of the qPCR were established according to the highest dilution where it was possible to virus quantification (the detection limits for HAV were 2.5×10^1 genome copies in 25 mg of fresh produce and in 10 L of water whereas the detection limit for both HNoV GI and HNoV GII was 2×10^1 genome copies in 25 mg of fresh produce and in 10 L of water).

Results

MNV-1 recovery assay

In the spiked surface water, green onion, and lettuce, the MNV-1 recoveries were $4.5 \times 10^8 \pm 3.7 \times 10^8$ GC/L (95.7%), $3.2 \times 10^8 \pm 2.4 \times 10^8$ GC/g (68%), and $2.7 \times 10^8 \pm 2.1 \times 10^8$ GC/g (57.4%), respectively.

Detection of HAV and HNoV in surface water samples

Of the 72 water samples, 23 samples (31.9%) were positive for HAV, 12 (16.7%) for HNoV GI, and 15 (20.8%) for HNoV GII. The viral load of HAV ranged from 1.2×10^2 to 8.3×10^7 GC/L, with a mean viral load of 4.3×10^6 GC/L. The viral load of HNoV GI ranged from 2.6×10^2 to 7.3×10^4 GC/L, with a mean viral load of 1.4×10^4 GC/L. The HNoV GII concentrations ranged from 8.1×10^2 to 7.8×10^6 GC/L, with a mean viral load of 1.2×10^6 GC/L. The most positive samples for HAV and HNoV were found in the samples collected from Mansoura region than those collected from Cairo region (Table 2). This variation was statistically significant ($P \leq 0.01$).

Detection of HAV and HNoV in fresh produce samples

The prevalence of foodborne HAV and HNoV is given in Table 3. Of the 24 green onion samples, 9 samples were found to be contaminated with at least one virus. HAV, HNoV GI, and HNoV GII were detected in 6/24 (25%), 3/24 (12.5%), and 5/24 (20.8%), respectively. The HAV load ranged from 1.2×10^2 to 1.9×10^5 GC/g, with a mean viral load of 4.5×10^4 GC/g. The HNoV GI concentration ranged from 7.7×10^2 to 7.2×10^3 GC/g with a mean viral load of 2.9×10^3 GC/g. The HNoV GII load ranged from 2.2×10^2 to 7.2×10^3 GC/g with a mean viral load of 3.1×10^3 GC/g. Of the 24 lettuce samples, 7 samples were found to contain at least one of the viruses. HAV, HNoV GI, and HNoV GII were detected in 4/24 (16.6%), 3 (12.5%), and 3 (12.5%), respectively. The HAV load ranged from 4.9×10^3 to

Table 2 Quantitative detection of HAV and HNoV (GI and GII) in irrigation water samples used for the production of fresh produce

Month	Region	HAV (GC/L)[a]			HNoV GI (GC/L)[b]			HNoV GII (GC/L)[c]		
		S1	S2	S3	S1	S2	S3	S1	S2	S3
March	Man	-	6.70E+05	-	-	-	-	-	-	-
	Giza	-	-	8.53E+04	-	-	-	-	-	-
April	Man	6.25E+05	-	-	-	-	2.56E+02	1.98E+06	-	-
	Giza	-	-	-	-	-	-	-	-	-
May	Man	-	-	8.23E05	-	-	-	-	-	5.27E+06
	Giza	-	-	-	UD	-	-	-	-	-
June	Man	8.62E+04	3.27E+04	-	-	7.25E+03	-	5.81E+04	9.34E+05	-
	Giza	-	-	5.84E+03	-	-	5.12E+03	-	-	7.82E+06
July	Man	-	-	-	8.26E+02	-	-	7.62E+04	-	-
	Giza	4.25E+02	-	-	-	-	-	-	-	-
Aug	Man	1.24E+02	-	-	-	-	-	4.17E+03	-	-
	Giza	-	-	2.11E+03	-	7.20E+02	3.84E+04	-	-	7.25E+05
Sept	Man	-	4.90E+05	-	-	-	-	-	UD	-
	Giza	5.61E+05	-	4.35E+04	5.24E+03	-	7.28E+04	5.87E+04	-	-
Oct	Man	-	-	-	-	-	2.41E+03	-	-	4.57E+05
	Giza	-	1.84E+04	-	-	-	-	-	2.81E+04	-
Nov	Man	8.34E+07	-	-	-	-	-	-	-	8.10E+02
	Giza		-	5.91E+06	-	-	2.71E+04	-	-	-
Dec	Man	4.68E+05	UD	-	4.87E+03	-	-	-	-	-
	Giza	-	-	7.30E+04	-	-	-	7.24E+03	-	-
Jan	Man	3.28E+03	3.75E+05	-	-	5.64E+02	-	-	7.58E+03	-
	Giza	-	-	-	-	-	-	-	-	-
Feb	Man	-	6.72E+04	-	-	-	-	-	-	-
	Giza	UD	2.57E+02	4.19E+05	-	-	-	-	-	5.72E+04

- refers to negative samples; UD refers to a positive sample with viral concentration under the detection limit and considered as a negative sample. [a]The R^2 and PCR efficiency values for HAV were 0.955 and 96.0%, respectively. [b]The R^2 and PCR efficiency values for NoV GI were 0.934 and 94.7%, respectively. [c]The R^2 and PCR efficiency values for NoVGII were 0.955 and 97.2%, respectively

4.8×10^6 GC/g, with a mean viral load of 1.2×10^6 GC/g. The HNoV GI load ranged from 2.4×10^2 to 8.2×10^3 GC/g, with a mean viral load of 3.1×10^3 GC/g. The HNoV GII load ranged from 7.2×10^3 to 2.9×10^4 GC/g, with a mean viral load of 4×10^4 GC/g.

Distribution of single and multiple viral agents in the positive water samples

As shown in Table 4, a single viral agent was found in 46.6% (14/30) of the positive samples, with HAV (9/72) being the most frequently found virus in samples contained only one viral agent. HNoV GI was found in 3 (10%) samples as a single viral agent whereas 2 (6.6%) samples contained only HNoV GII. Contamination with two viruses was detected in 40% (12/30) of positive samples: 6.6% (2/30) of positive samples contained both HNoV GI and GII, 10% (3/30) contained both HNoV GI and HAV, and 23.3% (7/30) contained both HNoV GII and HAV. The three viral agents were detected in 4 (13.3%) of positive samples.

Distribution of single and multiple viral agents in the positive fresh produce samples

In the green onion samples, single viral agent was detected in 25% (6/24) of positive samples. HAV, HNoV GI, and HNoV GII were detected in 50% (3/6), 16.6% (1/6), and 33.3% (2/6) as a single viral agent in the positive samples, respectively. Only one positive sample contained two viral agents (HAV + HNoV GII) while two samples contained the three viral agents (HAV + HNoV GI + HNoV GII). In the lettuce samples, contamination with single viral agent was found in 20.8% (5/24) of the positive samples. HAV (3/5) was the most frequently found alone in these samples, followed by HNoV GI (1/5) and by HNoV GII (1/5). Contamination with two viruses was detected only in one positive sample that contained both HNoV GI and HNoV GII. Also, combination of the three viral was detected in 14.3% (1/7) of the positive samples (Tables 5, and 6).

Table 3 Quantification detection of HAV and HNoV (GI and GII) in fresh produce samples

Month	Region	HAV (GC/g)		HNoV GI (GC/g)		HNoV GII (GC/g)	
		G. Onion[a]	Lettuce[b]	G. Onion[a]	Lettuce[b]	G. Onion[a]	Lettuce[b]
NoV	S1	-	7.20E+04 M	-	-	-	-
	S2	-	-	-	UD	2.24E+02 G	-
	S3	1.92E+05 M	UD	-	-	-	-
Dec	S1	7.16E+03 M	-	-	2.40E+02 M	7.25E+03 M	-
	S2	6.51E+04 G	-	7.24E+03 G	-	2.85E+03 G	8.24E+04 G
	S3	-	-	-	-	-	-
Jan	S1	1.13E+02 G	4.80E+06 M	-	9.21E+02 M	UD	7.23E+03 M
	S2	-	-	6.25E+02 M	-	-	-
	S3	3.68E+03 M	-	7.69E+02 M	-	4.68E+03 M	-
Feb	S1	UD	4.91E+04 G	-	-	7.67E+02 G	-
	S2	8.23E+02 G	9.57E+04 M	-	-	-	-
	S3	-	-	-	8.23E+03 M	-	2.94E+04 M

M refers to sample collected from Mansoura; G refers to sample collected from Giza;—refers to negative samples; UD refers to a positive sample with viral concentration under the detection limit and considered as a negative sample. [a]The R^2 and PCR efficiency values for HAV were 0.948 and 95.7%, respectively. The R^2 and PCR efficiency values for NoV GI were 0.937 and 96.9%, respectively. The R^2 and PCR efficiency values for NoVGII were 0.921 and 95.5%, respectively. [b]The R^2 and PCR efficiency values for HAV were 0.922 and 94.3%, respectively. The R^2 and PCR efficiency values for NoV GI were 0.948 and 94.8%, respectively. The R^2 and PCR efficiency values for NoVGII were 0.942 and 97.2%, respectively

Table 4 Profiles of viruses detected in the surface irrigation water ($n=72$)

	Detection of viruses	No of positive samples
Single	HAV	9
	HNoV GI	3
	HNoV GII	2
Double	HNoV GI + GII	2
	HAV + HNoV GI	3
	HAV + HNoV GII	7
Multiple	HAV + HNoV GI + GII	4
Total of positive samples (percentage)		30 (41.6%)

Discussion

Enteric viruses can be transferred to fresh produce by various routes during growth, harvest, packaging, transportation, and preparation of foods. Several viral outbreaks associated with fresh produce consumption have been identified or suspected to have originated from direct contact with virus-contaminated irrigation water (Shaheen et al. 2019; Prez et al. 2018; Gerba et al. 2006). Human HNoV and HAV are major etiological contributors to foodborne illness outbreaks. A systematic review published in 2018 stated that HNoV and HAV caused 48.7% and 46.1% of all viral outbreaks linked to fresh produce

Table 5 Profiles of viruses detected in the green onion ($n=24$)

	Detection of viruses	No. of positive samples
Single	HAV	3
	HNoV GI	1
	HNoV GII	2
Double	HNoV GI + GII	0
	HAV + HNoV GI	0
	HAV + HNoV GII	1
Multiple	HAV + HNoV GI + GII	2
Total of positive samples (percentage)		9 (37.5%)

Table 6 Profiles of viruses detected in the lettuce ($n=24$)

	Detection of viruses	No. of positive samples
Single	HAV	3
	HNoV GI	1
	HNoV GII	1
Double	HNoV GI + GII	1
	HAV + HNoV GI	0
	HAV + HNoV GII	0
Multiple	HAV + HNoV GI + GII	1
Total of positive samples (percentage)		7 (29.1%)

consumption, respectively, worldwide (Chatziprodromidou et al. 2018). Unfortunately, in Egypt, there is currently no official reporting system focus on foodborne viral illness outbreaks, and there is no active surveillance system for foodborne viral infections. Hence, information on the involvement of Egyptian foods in enteric illness outbreaks is limited (Shaheen et al. 2019; Zaher et al. 2008).

There are several previous studies from Egypt reported the detection of enteric viruses in surface water used as an irrigation source (Shaheen et al. 2020, 2018; Shaheen and Elmahdy 2019a, b). However, very low studies focused on the simultaneous detection of these viruses in both fresh produce and irrigation water (Shaheen et al. 2019). Indeed, monitoring these viruses in both the fresh produce and irrigation water can provide a useful tool to reduce the risk of foodborne disease linked to the occurrence of these viruses. In this context, the aim of this study was to investigate the presence of HAV and HNoV in surface waters and fresh produce irrigated by this water and collected from two regions (Giza and Mansoura) in Egypt.

Overall, in both matrices ($n = 120$), HAV showed a relatively higher prevalence rate (27.5%, 33/120 samples). HNoV was detected in 25% (30/120) of both matrices where HNoV GII (19.2%, 23/120 samples) was higher than HNoV GI (15%, 18/120 samples). In contrast, the detection rate of HNoV GI was higher than HNoV GII genogroup identified both in irrigation water and vegetable samples (Emilse et al. 2021). In a recent study from Egypt, HAV was detected in 11/32(34.4%), 6/32 (18.7%), 8/32 (25%), 9/32 (28%), and 10/32 (31%) of surface water, leek, green onion, watercress, and lettuce samples, respectively (Shaheen et al. 2019). Khan et al. (2014) detected HAV in all cultivated vegetables due to exposure to faecally contaminated irrigation water. In addition, several HAV outbreaks associated with green onion consumption at several restaurants were documented in 2003 in Monaca, Pennsylvania; in 2000 in Kentucky and Florida; in 2003 in Georgia, and North Carolina; and in 1999 in Ohio (Amon et al. 2005; Wheeler et al. 2005; Datta et al. 2001; Dentinger et al. 2001). Indeed, Bidawid et al. (2000a, b) reported that 9.2% of contaminated lettuce by infectious virus particles comes from contaminated hands. In the farm and during the growing stage, food can become contaminated by contact with sewage, contaminated fertilizers, or the use of contaminated irrigation water (Directorate 2002).

In Egyptian study, Shaheen et al. (2018) detected HNoV GI in 10/32 (31%), 11/32 (34.4%), 13/32 (40.6%), and 9/32 (28%) of lettuce, watercress, green onion, and leek samples, respectively, while the virus was detected in 10/32 (31.2%) of surface water used as an irrigation source. A series of outbreaks of HNoV GI and HNoV GII gastroenteritis related to the consumption of lettuce between 18 and 20 January 2010 were documented to Danish authorities (Ethelberg et al. 2010). In Canada and Malaysia, HNoV GI was found in 3.33%, 25%, and 13.33% of red onions, strawberries, and green onions, respectively (Brassard et al. 2012; Hidayah et al. 2011). Various prevalence rates of HNoV in leafy greens were found in several countries: 2.9% in Italy (Purpari et al. 2019), 12.4–50% in France (Loutreul et al. 2014; Baert et al. 2011), 5.3% in UK (Cook et al. 2019), and 33.3% in Belgium (Baert et al. 2011).

In this study, HAV detection in both matrices was higher than HNoV prevalence. This finding is similar to our previous study conducted on the same two regions (Shaheen et al. 2019), suggesting that surface irrigation water could be the source of fresh produce contamination. Since irrigation water can transmit the viral contamination for fresh produce, thus primary products must be produced only in regions where an appropriate water quality is used for irrigation purposes. If the quality control of irrigation water is not controlled, prior consumption, it is suggested to immerse the fresh produce with drinking water containing sodium hypochlorite (15–20 ppm free chlorine levels) for $^{>}$ 2 min to reduce viral loads on the vegetable surfaces (Bosch et al. 2018).

This is the first report of HNoV GII detection in fresh produce in Egypt. However, this study has some limitations to not investigate the viral genotypes in the positive samples. Difficulties in HAV and HNoVs sequencing were due to the small amount of PCR product obtained from the RT-qPCR and cannot be used for that purpose. This fact has also been reported in other studies (Felix-Valenzuela et al. 2012; Baert et al. 2011). A major limitation in this study is that RT – qPCR technique used in the detection of viruses cannot differentiate between the infections and non-infectious virus particles in the positive samples. Molecular assays may pick up both non-infectious and infectious viral particles. In this way, detection of viral nucleic acid in a sample does not necessarily mean the presence of infectious virus particles in that sample (Hamza et al. 2009). Thus, detection of enteric viruses by the molecular technique cannot confirm the role of fresh produce as transmitting routes of infectious enteric viruses.

An additional limitation in this study is the absence of clinical data. However, previous Egyptian studies have reported the detection of HAV and HNoV among the population in both regions. Zaki and El Kheir (2017) identified HNoV (GI and GII) in 30% of diarrheal specimens collected from Mansura City. A recent study from Mansura region, HNoV was also detected in 70.5% of diarrheal samples with HNoV GII as the prevalent genotype (Zaki et al. 2019). This finding agrees with our study that HNoV GII is the most prevalent genotype. In Cairo region, HNoV (GI and GII) and HAV were also identified in samples collected from patients with severe diarrhea or hepatitis-related symptoms (Kamel et al. 2009, 2010, 2011). The HNoV genotypes as well as HAV detected in the clinical samples are also detected in

fresh produce, suggesting that viral contamination of the fresh produce could be originated from contact with contaminated irrigation water.

In the current study, the viral load of HAV was higher than HNoV in the surface water and fresh produce samples. Comparatively, HAV had high variability in the viral loads, where some fresh produce samples had as low as 1.1×10^2 GC/g up to 8.3×10^7 GC/mL detected in one of the surface water samples tested. In surface water samples, most of the positive samples had viral concentrations between 10^4 and 10^5 GC/L while between 10^3 and 10^4 GC/g in fresh produce samples. High variability in the viral loads was also observed for HNoV where some fresh produce samples had as low as 2.2×10^2 GC/g which increased to 7.8×10^6 GC/mL detected in one of the surface water samples tested. Most of the positive surface water samples had HNoV concentrations between 10^3 and 10^4 GC/L. However, the HNoV in fresh produce samples had low variability with viral loads ranged from 2.2×10^2 to 8.2×10^4 GC/g. The low prevalence of HAV and HNoV with low concentrations in fresh produce than surface water may be due to their direct exposure on fresh produce surfaces to the ultraviolet radiation emitted from the sun.

The low viral concentrations found in the positive fresh produce and surface samples are by far higher than the infective dose required to induce disease by most of the enteric viruses, ranging between 10 and 100 viral particles for human rotavirus and HAV and even less for human HNoV (Yezli and Otter 2011). Thus, this low infective dose represents a potential risk for a viral outbreak if these fresh produce reach the consumers. In comparison, another study from Mexico found that the mean viral loads of HAV ranged from 2.8×10^2 to 2.4×10^3 GC/g while HNoV loads ranged from 2.1×10^2 to 1.3×10^3 GC/g (Felix-Valenzuela et al. 2012), which is lower than viral concentrations detected in this study. All precautions were applied in this study to prevent cross-contamination in the RT-qPCR reactions, and no amplification was found in negative controls.

In conclusion, this study shows the presence of human enteric viruses in surface water and fresh produce samples collected from different sites in Egypt. In addition, the results obtained in this work highlight the importance of viral surveillance programme for fresh produce and irrigation source to prevent cross contamination between the water environment and fresh produce.

Author contribution MNFS was the contributor in the plane design, performing RNA extraction and real time PCR, and data analysis as he was a major contributor in writing the manuscript. EME was the contributor in the plane design, performing RNA extraction and real time PCR, and data analysis. LHIM was the contributor in the sample collection, virus concentration, and in performing RNA extraction and real time PCR. IAH was the contributor in the plane design and data analysis as she revised the manuscript. ERSS was the contributor in the plane design, performing real time test, and data analysis as she revised the manuscript.

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Declarations

Ethics approval and consent to participate Not applicable.

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Competing interests Not applicable.

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