Environmental monitoring of astrovirus and norovirus in the Rosetta branch of the River Nile and the El-Rahawy drain, Egypt

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ABSTRACT

Sewage discharge is considered to be the primary source of viral contamination in aquatic environments. This study was conducted to evaluate the impact of El-Rahawy wastewater on the water quality of the Rosetta branch of the River Nile (Rosetta River Nile) through detection of astrovirus (AstV) and norovirus (NoV) in the water and sediments of both sites. For this purpose, we collected 72 wastewater and 12 sediment samples from El-Rahawy drain, and 12 river water and 12 sediment samples from Rosetta River Nile before and after mixing with El-Rahawy wastewater between April 2017 and March 2018. AstVs and NoVs were identified in wastewater (40.2% versus 25%), El-Rahawy sediment (41.6% versus 20.8%), river water after mixing with wastewater (25% versus 16.6%), river water before mixing with wastewater (8.3% versus 0%), river sediment after mixing with wastewater (16.6% versus 8.3%), and no viruses were found in river sediments before mixing with wastewater. AstV genogroup B and NoV genogroup GI were the most frequently detected genotypes in the analyzed samples, with a peak incidence in the winter months. Increasing detection rates of both viruses in El-Rahawy drain samples and river water taken from the Rosetta branch after receiving El-Rahawy wastewater reflect the impact of this drain on the water quality of this stretch of the River Nile.

Key words | astrovirus, El-Rahawy, norovirus, Rosetta branch of the River Nile, wastewater

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INTRODUCTION

Gastroenteritis is the second leading cause of mortality worldwide, causing approximately 1.3 million preventable deaths in children under 5-years old annually, mainly in developing countries (WHO 2009). Based on this alarming data, 88% of these deaths are linked to unsafe water, poor hygiene, and inadequate sanitation. Improvements in access to adequate sanitation and safe water can help in reducing this elevated number of deaths due to diarrhea illness (Black *et al.* 2003).

The most common causes of viral diarrhea are human astroviruses (AstVs) and noroviruses (NoVs), globally (Jeong *et al.* 2011; Lopman *et al.* 2011). They are nonenveloped viruses and possess a single-stranded and positive-sense RNA. Based on nucleotide and amino acid sequence analysis information, human AstVs are classified into two genogroups: AstV-A (AstV-1 to AstV-5 and AstV-8 genotypes) and AstV-B (AstV-6 and AstV-7 genotypes) (Hata *et al.* 2015); and NoVs are classified into seven different genogroups (NoV GI – NoV GVI), of which NoV GI, NoV GII, and NoV GIV infect humans (Vinjé 2015).

AstVs and NoVs show the ability to infect people of all ages; causing a wide variety of symptoms, such as abdominal pain, diarrhea, dehydration, nausea, and vomiting (Gallimore *et al.* 2004; Bhattacharya *et al.* 2006). A large amount of viral particles is released in the feces of infected persons (Atmar *et al.* 2008), which are finally distributed through the wastewater network (Aw & Gin 2010). These viruses are generally not removed by wastewater treatment plants (WWTPs), and therefore they can be discharged into rivers at noticeable levels (Prevost *et al.* 2015).

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The Rosetta branch of the River Nile in Egypt (Rosetta River Nile) serves as a major source of potable water, however it receives large volumes of agricultural, industrial, and domestic wastewater after or prior to treatment from the El-Rahawy drain, daily. Several environmental studies have been performed to address the impact of this wastewater on the water quality of Rosetta River Nile (Azzam *et al.* 2014; Mostafa 2015; Mostafa & Peters 2016). In this study, we determined the occurrence of NoV and AstV in wastewater in the El-Rahawy drain as well as in the water of Rosetta River Nile to evaluate the impact of this wastewater on the water quality of this stretch of the River Nile.

MATERIALS AND METHODS

Study area

The River Nile in Egypt is divided into two branches (Rosetta and Damietta branches) in the delta region. The Rosetta River Nile is the largest freshwater stream in the delta region and it serves as a major source of potable water in Egypt. The El-Rahawy drain receives daily about 1.90 million m^3 of agricultural drainage and wastewater from the Abu-Rawash and Zenin WWTPs, then discharges directly into Rosetta River Nile.

Sample collection

During the period from April 2017 to March 2018, eight water samples were collected monthly from eight sites (S1-S8) in the study area. Six sites (S1-S6) were located along the El-Rahawy drain and two sites (S7,S8) were located on the Rosetta River Nile. At the same time, four sediment samples were collected monthly from four sites (S3,S4,S7, S8). The location of these sites is presented in Figure 1.

Viral concentration methods

Viral concentrations in the wastewater and river water samples were performed by using the protocol described by Katayama *et al.* (2002). For the sediment samples, viral concentrations were carried out according to a protocol previously described by EPA (1992), with minor modifications as described by Schlindwein *et al.* (2010), and for the concentration of virus, the PEG 6000 precipitation method was performed as described by Lewis & Metcalf (1988).

Extraction of viral nucleic acids

Nucleic acids were extracted from 140 μL of the eluate to obtain a final volume of 60 μL, using the QIAamp Viral

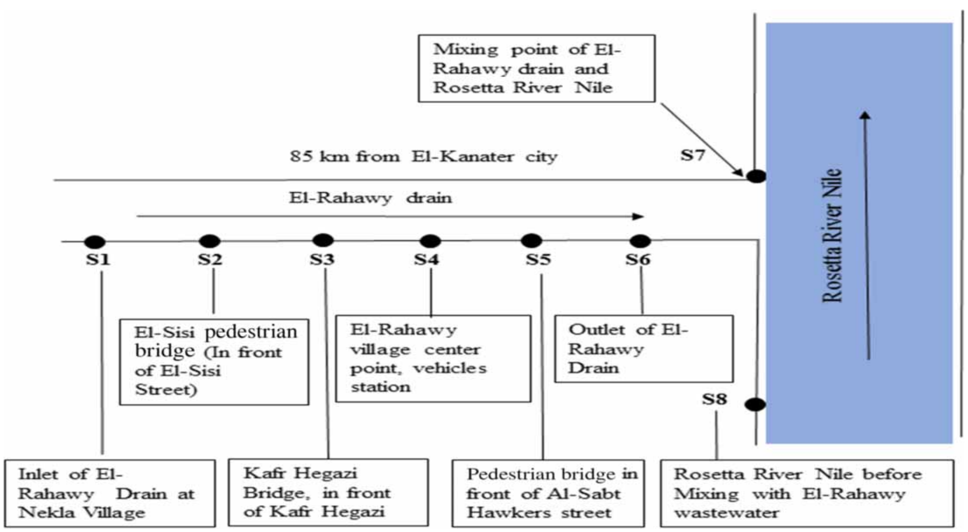

Figure 1 | Map of the sampling sites along the El-Rahawy drain and Rosetta River Nile.

RNA (Qiagen, Inc., Valencia, CA) according to the manufacturer's instructions.

Virus detection by semi-nested RT-PCR

The semi-nested RT-PCR was performed for NoV detection using COG1F/G1SKR and G1SKF primers for GI, and COG2F/G2SKR and G2SKF for GII as described by Kojima *et al.* (2002) and Kageyama *et al.* (2003). The expected size of the semi-nested RT-PCR products was 330 bp and 344 bp for NoV GI and NoV GII, respectively. Also, the semi-nested RT-PCR was conducted for AstV detection using A1/A2 and A2 internal for AstV-A and A1bis/A2bis and A2 internal as described by Guix *et al.* (2002) and El-Senousy *et al.* (2007). The expected size of the semi-nested RT-PCR products was 192–237 bp and 167 bp for AstV-A and AstV-B, respectively.

RESULTS AND DISCUSSION

Human activity influences the occurrence and prevalence of human astrovirus and norovirus in environmental waters (Aw *et al.* 2009; El-Senousy *et al.* 2014), and outbreaks of disease caused by these viruses have caused serious socioeconomic and health impacts (Grabow 2007). Although the presence of human astrovirus and norovirus in environmental waters has been widely reported (Lodder & de Roda Husman 2005; Pérez-Sautu *et al.* 2012; Hellmér *et al.* 2014), AstV and NoV monitoring in the Egyptian environment is relatively limited (El-Senousy *et al.* 2007, 2014; Kamel *et al.* 2009). In the current study, we investigated the presence of AstV and NoV in the wastewater of the El-Rahawy drain and in the water of the Rosetta River Nile which receives this wastewater. To the best of our knowledge, this is the first study in Egypt providing data on the presence of AstV and NoV in the El-Rahawy drain and the Rosetta branch of the River Nile. Up to now, there has only been one study from Egypt describing the detection of enterovirus in the wastewater of the El-Rahawy drain and the river water of the Rosetta branch of the Nile (Azzam *et al.* 2014).

In this study, we determined the presence of AstVs and NoVs in 72 wastewater samples taken from six sites (S1-S6) and 12 sediment samples taken from two sites (S3,S4) along the El-Rahawy drain as well as 24 river water and 12 sediment samples taken from two sites (S7,S8) on the Rosetta River Nile. Out of 72 wastewater samples taken, 40 (55.5%) were tested positive for at least one virus. A single virus and both viruses were identified in 33/40 (82.5%) and 7/40 (17.5%) samples, respectively (Table 1).

The highest detection rates for both viruses were found at site 6 (El-Rahawy drain outlet), and the lowest detection rates were found at site 1 (El-Rahawy drain inlet) (Table 1). This finding can be explained by the fact that other drains including industrial, agricultural and domestic wastewater which hasn't been treated, are discharged into the El-Rahawy drain along its length leading to microbial load increases, particularly at the discharge point (S6).

Astroviruses were detected in 29/72 (40.2%) of wastewater samples taken from six sites (S1-S6) along the El-Rahawy drain, 3/12 (25%) of river water samples taken from site 7 on the Rosetta branch, and 1/12 (8.3%) of river water samples taken from site 8 on the Rosetta branch (Table 1). These findings are lower than those reported in a study performed by El-Senousy *et al.* (2014) who detected AstV in 76.3% and 33.3% of analyzed wastewater and river water samples in Egypt, respectively. A higher frequency for AstV in urban and rural river waters was observed in a study from Kenya, where AstV was detected in 41.4% of analyzed water samples (Kiulia *et al.* 2010). Also, a higher detection rate for AstV in sewage was reported in studies conducted in Singapore and Kenya; however, our detection rate for AstV is much higher than those reported in sewage treatment plants in China (Aw & Gin 2010; Kiulia *et al.* 2010; He *et al.* 2011). This fluctuation of the detection rates may be due to differences in concentration and detection methods and geographical regions.

The distribution of AstV genogroups in the positive samples was as follows: AstV-A was detected in 11/29 (38%) of wastewater samples taken from sites 1–6 along the El-Rahawy drain and 1/3 (33.3%) of river water samples taken from site 7 on the Rosetta branch, whereas AstV-A was not found at site 8 on the Rosetta branch (Table 1).

On the other hand, AstV-B was detected in 18/29 (62%) of wastewater samples, 2/3 (66.6%) of river water samples taken from site 7, and 1/1 (100%) of river water samples taken from site 8 on the Rosetta branch (Table 1). This result is similar to other previous studies from Egypt reporting that AstV-B is commonly detected in environmental waters (El-Senousy *et al.* 2007, 2014).

Table 1 | Number of wastewater (S1-S6) and river water (S7, S8) samples positive for astrovirus (genogroups A and B) and norovirus (GI and GII)

	Astrovirus								Norovirus							
Month	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**
Apr. 2017	–	–	–		–	AstV-A		–	–	–	–	–	NoV GI	NoV GI	–	–
May 2017	–	AstV-A	–	–	AstV-B	–	–	–	–	NoV GII	–	NoV GI		–	NoV GII	–
Jun. 2017	–	–	AstV-B	–	–	–	–	–	–	–	–	–	–	–	–	–
Jul. 2017	–	–	–	–	–	AstV-B	–	–	–	–	–	–	NoV GII	–	–	–
Aug. 2017	–	–	–	AstV-B	–	–	–	–	–	–	NoV GI	–		–	–	–
Sep. 2017	–	–	AstV-A	–	–	AstV-B	–	–	–	–		NoV GII	–	–	–	–
Oct. 2017	AstV-A	–	AstV-A	AstV-B	AstV-A	AstV-B	–	AstV-B	–	–	–	–	–	NoV GI	–	–
Nov. 2017	–	AstV-B	–	–	–	AstV-B	AstV-A	–	–	–	–		–	–	–	–
Dec. 2017	–	AstV-B	AstV-B	AstV-A	AstV-B	AstV-B	–	–	NoV GI	–	–	NoV GII	–	NoV GII	–	
Jan. 2018	–	AstV-A	–	AstV-B	AstV-A	–	AstV-B	–	–	–	NoV GII		–	–	NoV GI	–
Feb. 2018	–	–	AstV-B	–	AstV-A	AstV-A	AstV-B	–	–	NoV GI	–	NoV GI	NoV GI	NoV GI	–	–
Mar. 2018	AstV-B	–	–	AstV-B	AstV-B	–	–	–	–		NoV GI	–	–	NoV GII	–	–
No. of positive samples	2	4	5	5	6	7	3	1	1	2	3	4	3	5	2	0

As shown in Table 1, noroviruses were detected in 18/72 (25%) of wastewater samples taken from six sites (S1-S6) along the El-Rahawy drain, 2/12 (16.6%) of river water samples taken from site 7 on the Rosetta branch, but NoVs were not detected in river water samples taken from site 8 on the Rosetta branch. Similar results were documented in a study from Brazil, in which NoV was detected in 18.8% of surface water samples (Vieira *et al.* 2012). The current results are higher than that reported in other studies from Egypt by Kamel *et al.* (2010) and El-Senousy *et al.* (2014), which detected NoVs in 18% and 6.9% of wastewater and river water samples, respectively. Also, the frequency of NoV detected in this study is higher than that reported by He *et al.* (2011), who detected NoV in 3.1% of sewage samples collected from three sewage treatment plants. In studies from Italy and Singapore, NoV was identified in nearly 100% of analyzed wastewater samples (Aw & Gin 2010; La Rosa *et al.* 2010). However, NoV detection rates in this study are lower than those observed in river water and urban sewage in Kenya (Kiulia *et al.* 2010) and wastewater directly discharged into the Uruguay River (Victoria *et al.* 2014). It is important to note that PCR methods used in the previously mentioned studies for NoV detection were different and therefore the comparison between the positivity rates is not linear.

The distribution of NoV genogroups in the positive samples was observed as follows: NoV GI was detected in 10/18 (55.5%) of wastewater samples taken from sites 1–6 along the El-Rahawy drain and 1/2 (50%) of river water samples taken from site 7 on the Rosetta branch whereas NoV GII was only identified in 8/18 (44.4%) of wastewater samples taken from sites 1–6 along the El-Rahawy drain and 1/2 (50%) of river water samples taken from site 7 on the Rosetta branch (Table 1). This finding is consistent with other studies from Egypt showing that NoV GI is commonly found in the environment (El-Senousy *et al.* 2007, 2014; Kamel *et al.* 2010). Kamel *et al.* (2010) explained the high incidence of NoV GI compared to NoV GII in the Egyptian environment by suggesting that NoV GI may be more warm water resistant than NoV GII.

In agreement with the data obtained from water and wastewater analysis, AstV was detected at higher rates than norovirus in sediment samples. Also, Ast-B and NoV GI genogroups were more frequently found in sediment samples than AstV-A and NoV GII genogroups. Of 24

Table 2 | Number of El-Rahawy sediment (S3-S4) and river sediment (S7, S8) samples positive for astrovirus (genogroups A and B) and norovirus (GI and GII)

	AstV				**NoV**			
Month	**S3**	**S4**	**S7**	**S8**	**S3**	**S4**	**S7**	**S8**
Apr. 2017	AstV-B	–	–		NoV GI	–	–	–
May 2017	–	–	–	–	–	–	–	–
Jun. 2017	–	–	–	–	–	–	–	–
Jul. 2017	AstV-A	–	–	–	–	–	–	–
Aug. 2017	–	AstV-B	–	–	–	NoV GII	–	–
Sep. 2017	–	AstV-B	–	–	–		–	–
Oct. 2017	AstV-B	–	AstV-A		–	–	–	–
Nov. 2017	–	–	–	–	–	–	–	–
Dec. 2017	AstV-A	–	–	–	NoV GII		NoV GI	
Jan. 2018	–	AstV-A	AstV-B	–	–	–	–	–
Feb. 2018	AstV-B	–	–	–	–	NoV GI	–	–
Mar. 2018	AstV-B	AstV-B	–	–	–	NoV GI	–	–
No. of positive samples	6	4	2	0	2	3	1	0

sediment samples taken from two sites (S3,S4) along the El-Rahawy drain, 11 were tested positive for at least one virus. AstV, NoV, and both viruses were detected in 6/11 (54.5%), 1/11 (9%), and 4/11 (36.4%), respectively (Table 2).

As shown in Table 2, these positive samples were related to AstV-A (4/10, 40%), Ast-B (6/10, 60%), NoV GI (3/5, 60%), and NoV GII (2/5, 40%). On the other hand, three out of 24 river sediment samples taken from two sites (S7,S8) were tested positive for virus. The three positive samples were related to AstV-A 1/3 (33.3%), AstV-B 1/3 (33.3%), and NoV G1 1/3 (33.3%) and no viruses were detected in all sediment samples collected from site S8 on the Rosetta branch (Table 2). The higher frequency of AstV in El-Rahawy sediment compared with its wastewater might be due to a higher level of viral concentration in sewage sediment than wastewater (Prado *et al.* 2014). However, NoV occurrence was higher in wastewater than in sediment. The mechanisms of virus adsorption to sediment are not fully understood and may vary depending on the virus type (da Silva *et al.* 2007) and further investigations are required for this issue.

Overall, detection and genotyping of human AstV and NoV over one year were conducted successfully in this study. AstV was higher than NoV in both wastewater and water samples. The low incidence of NoV may be explained by the fact that some viruses may be shed at a low level in the feces of infected people, or that some viral particles are unstable in environmental waters (Myrmel *et al.* 2006). Moreover, AstV genogroup B and NoV genogroup I accounted for the larger portion in wastewater and river water samples, suggesting that these genotypes are the main prevalent genotypes in the Egyptian environment. This finding is consistent with other studies (La Rosa *et al.* 2010; Zhu *et al.* 2018). In contrast, our result is not similar to studies conducted in Singapore and South Korea (Aw & Gin 2010; Kim *et al.* 2016). This variation may be attributed to the difference in the number of samples and geographical areas. A possible correlation between water and wastewater genotypes may be found in this study. Furthermore, the increased incidence rates of both viruses in river water and sediment samples taken from site 7 compared with site 8 on the Rosetta River Nile may lead to the conclusion that the El-Rahawy drain is a major source of water quality degradation of the Rosetta branch.

Although this study covered only one year's surveillance of these viruses, the detection peaks of AstV and NoV in the water and sediment samples of the Rosetta branch were similar to those in the wastewater and sediment samples of the El-Rahawy drain where our study showed a high positive ratio of AstV during winter/autumn and NoV during winter/spring seasons (Tables 1 and 2).

It is commonly accepted that AstV and NoV-related illnesses tend to occur in winter (Wyn-Jones *et al.* 2011; Zhou *et al.* 2014). Previous studies noted that environmental NoVs were more commonly detected in winter/spring (Kitajima *et al.* 2010; Kim *et al.* 2016). This phenomenon may be the result of increasing viral stability at lower temperatures (Myrmel *et al.* 2006).

A long-term monitoring of the circulation of these viruses in the local population is highly recommended to control and/or prevent virus infection. The surveillance period and genotypes obtained in this study are limited due to the absence of clinical data. However, it has been reported that Ast-B and NoV GI were the most circulated AstV and NoV genotypes among Egyptian children with acute diarrhea (El-Senousy *et al.* 2014), thus a possible relationship between the environmental and clinical genotypes may be found indicating the impact of environmental contamination on public health.

CONCLUSION

This study proves the occurrence of AstV and NoV in river water and wastewater samples collected from the Rosetta River Nile and the El-Rahawy drain in Egypt, highlighting the harmful effects of this drain on the water quality of the Rosetta River Nile. Also, this study highlights the significance of environmental and clinical monitoring of AstV and NoV to better understand their epidemiology and environmental circulation as well as to decrease the impact of the movement of these viruses in the population and the risk of acute viral gastroenteritis for the local population.

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