Norovirus Dynamics in Wastewater Discharges and in the Recipient Drinking Water Source: Long-Term Monitoring and Hydrodynamic

Norovirus dynamics in wastewater discharges and in the recipient drinking water source: Long-term monitoring and hydrodynamic modelling

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Supporting Information. Details regarding the sampling programme, norovirus analyses, hydrodynamic modelling, measured microbial concentrations in wastewater, simulated microbial concentrations in source water.

Abstract

Norovirus (NoV) that enters drinking water sources with wastewater discharges is a common cause of waterborne outbreaks. The impact of wastewater treatment plants (WWTPs) on the river Göta älv (Sweden) was studied using monitoring and hydrodynamic modelling. The concentrations of NoV genogroups (GG) I and II in samples collected at WWTPs and drinking water intakes (source water) during one year were quantified using duplex real-time reverse-transcription PCR. The mean (standard deviation) NoV GGI and GGII genome concentrations were 6.2 (1.4) and 6.8 (1.8) in incoming wastewater, and 5.3 (1.4) and 5.9 (1.4) Log_{10} genome equivalents (g.e.) L^{-1} in treated wastewater, respectively. The reduction at the WWTPs varied between 0.4 and 1.1 Log_{10} units. In source water, the concentration ranged from below the detection limit to 3.8 Log_{10} g.e. L^{-1}. NoV GGII was detected in both wastewater and source water more frequently during the cold than the warm period of the year. The spread of NoV in the river was simulated using a three-dimensional hydrodynamic model. The modelling results indicated that the NoV GGI and GGII genome concentrations in source water may occasionally be up to 2.8 and 1.9 Log_{10} units higher, respectively, than the concentrations measured during the monitoring project.

Key words: Göta älv; *E. coli*; norovirus; somatic coliphages; sewage; real-time PCR; water quality.

1 Introduction

Human norovirus (NoV) is highly infectious.[1-2] NoV spreads rapidly through person-to-person contact, airborne droplets, and water and food.[1] Immunity after the disease is incomplete and short-lived,[3] thus repeated infections of the same agent are common. Stool from infected individuals contains 10^5 to 10^{12} NoV per gram.[4] Viruses are generally resistant to current methods of wastewater treatment and often remain infectious for a long time in the environment.[5] Thus, wastewater discharges to drinking water sources pose risks of waterborne disease outbreaks.[6-7]

NoV is one of the most common causes of nonbacterial waterborne outbreaks of gastroenteritis in all age groups.[8] One of the largest waterborne NoV gastroenteritis outbreaks in Sweden in recent years occurred in the municipality of Lilla Edet in 2008, with approximately 2400 cases. It was suspected that the cause of the outbreak was the contamination of the drinking water source – the river Göta älv.[9]

Traditionally, microbial water quality control is based on detection of faecal indicator microorganisms, e.g. *E. coli*, coliforms, and coliphages. However, there is a lack of correlation between the concentrations of faecal indicator bacteria and specific pathogens, such as NoV.[10-12] Yet, there are contradictory reports regarding the correlation between the concentrations of somatic coliphages and NoV in source water.[11, 13] Thus, traditional water quality monitoring may not fully predict the health risks.[14]

Little is known about the concentrations of NoV in source water, but this information is crucial for the assessment of health risks for drinking water consumers.[15] Molecular methods, like reverse transcription – quantitative polymerase chain reaction (RT-qPCR) with a high sensitivity for detection of NoV in water,[16] have the potential to provide this information.

Microbial water quality can also be studied by hydrodynamic models, which simulate the spread of faecal contamination in a water source. Hydrodynamic modelling can be used to describe the temporal and spatial variability of microbial concentrations,[17] to study the influence of different processes on the microbial water quality,[18] and to quantify the relative impact of different faecal sources.[19] In addition, this approach can be used to quantify the concentrations that are below the detection limits of analytical methods, yet still high enough to be relevant to consumer health.[20]

The aim of this study was to determine the seasonal dynamics of NoV in wastewater and in the recipient drinking water source – the river Göta älv in Sweden. For this purpose, the concentrations of NoV and faecal indicators *E. coli* and somatic coliphages were (i) measured during long-term monitoring, and (ii) simulated by means of hydrodynamic modelling.

2 Methods

2.1 Study area

Göta älv is a river that drains Lake Vänern into the strait Kattegat at the city of Gothenburg on the west coast of Sweden (Figure S1). The total catchment area of the river Göta älv is 50 233 km^2, which constitutes approximately 10 % of the area of Sweden. The part of the catchment area that is located downstream of Lake Vänern is approximately 3500 km^2. The length of the river between the outflow from Lake Vänern and the mouth of the river is 93 km. The vertical drop of the river is approximately 44 m. The water flow in the river Göta älv is regulated by several hydropower stations (Figure S1) and varies strongly; the mean and the maximum water flows are 550 and 1000 $m^3 s^{-1}$, respectively. The transport time between the outflow from Lake Vänern and the mouth of the river is between 1.5 and 5 days.

The river is used as a water source for the drinking water supply of 700 000 consumers in several municipalities, including Gothenburg with 500 000 consumers. Between Lake Vänern and the water intake for the city of Gothenburg (Figure S1) the river receives wastewater from approximately 100 000 persons. Approximately 95 % of this wastewater is treated at municipal wastewater treatment plants (WWTPs), while 5 % is treated by on-site sewer systems.

2.2 Sampling

Source water samples (n=58) were collected from five drinking water treatment plants (DWTPs) along the river: Skräcklan in Vänersborg, Överby in Trollhättan, Lilla Edet, Dösebacka in Kungälv, and Lärjeholm in Gothenburg (Figure S1). Wastewater samples (n=160) were collected from four WWTPs: Holmängen in Vänersborg, Arvidstorp in Trollhättan, Ellbo in Lilla Edet, and Ryaverket in Gothenburg (Figure S1).

Sampling was conducted during one year, from 8 June 2011 to 5 June 2012 (Table S1). Source water samples were collected as 10.5 L grab samples of the incoming water at the DWTPs. Wastewater

samples were collected at the WWTPs continuously for 24 hours using automatic flow rate controlled samplers; 1.5 L was used for further analyses. The 24 hour sampling was done to compensate for fluctuations in concentration. All samples were collected in sterile glass containers and kept at 4 °C until analysis. From each sample, 0.5 L was used for faecal indicator analyses.

2.3 Norovirus analyses

In this study, NoV genogroups (GG) I and II were studied. NoV genome concentrations were analysed at the Microbiology Laboratory, Medical Services, County Hospital Ryhov, Jönköping. The details regarding enrichment from source water and wastewater, extraction of RNA and reverse transcription, detection and quantification by TaqMan real-time PCR, and controls of the methodology are described in supporting information.

For source water, the limit of quantification (LOQ) was 3.8×10^{1} g.e. L^{-1}, and the limit of detection (LOD) was extrapolated to 1.1×10^{1} g.e. L^{-1}. For wastewater, the LOQ was 1.5×10^{4} g.e. L^{-1}, and the LOD was extrapolated to 4.5×10^{3} g.e. L^{-1}.

To control virus enrichment and RNA extraction steps, the samples were spiked with murine norovirus 1 solution (MNV-1). The recovery rate was estimated for each sample using quantitative real-time PCR for MNV-1. Inhibition control of the reverse transcription step was done with Alien Reference RNA VIC QRT-PCR Detection Kit. To rule out PCR inhibition and to optimise PCR performance, tick-borne encephalitis virus (TBEV) was used.

The process control MNV-1 showed that the virus recovery rate varied between 1.2 % and 23 % with a mean of 18 % for source water, and between 0.1 % and 31 % with a mean of 5 % for wastewater. All reported concentrations were corrected for the recovery rate. Inhibition control of the reverse transcription step showed no inhibition. The PCR inhibition control TBEV showed no inhibition.

2.4 Faecal indicator analyses

The concentrations of *E. coli* were analysed within four hours after sampling at ALcontrol AB, Linköping, Sweden, according to the Swedish standard SS 028167-2 (membrane filtration method). The concentrations of somatic coliphages were analysed on the sampling day at Lackarebäck laboratory, Gothenburg, Sweden, according to the standard ISO 10705-2 (plaque assay method).

2.5 Statistical analyses

The measured data were Log_{10} transformed and analysed using t-tests and correlation analyses. To analyse the seasonality of concentrations, the warm and cold periods were defined based on whether the air temperature was above or below +10 °C, respectively. The warm period included 13 sampling occasions (8 Jun 2011 – 12 Oct 2011 and 9 May 2012 – 5 Jun 2012); and the cold period included 14 sampling occasions (26 Oct 2011 – 25 Apr 2012). To compare the number of samples above the LOD during the warm and cold periods, Fischer's exact test was used. P-value <0.05 was considered statistically significant.

When concentrations were below the LOD, the value for LOD was used for calculations. When the NoV genome concentration was above the LOD, but below the LOQ, the geometric mean of the LOD and LOQ was used for calculations: 2.0×10^{1} g.e. L^{-1} for source water and 8.2×10^{3} g.e. L^{-1} for wastewater.

2.6 Hydrodynamic modelling

2.6.1 Model setup

To evaluate the impact of the WWTPs on the source water, the hydrodynamic conditions and the transport of NoV and faecal indicators in the river Göta älv were simulated. The details about the model setup and validation can be found in Sokolova et al.[21] and in supporting information.

The three-dimensional time-dependent hydrodynamic model MIKE 3 FM (MIKE Powered by DHI) was used. This model is based on the numerical solution of three-dimensional incompressible Reynolds

averaged Navier-Stokes equations invoking the assumptions of Boussinesq and of hydrostatic pressure. The model consists of continuity and momentum equations and is closed by a turbulent closure scheme. The water density was assumed to be homogenous (barotropic formulation). The modelling domain was approximated with prisms (triangles in horizontal plane) using a flexible mesh approach. The length of the triangles' sides varied from 20 to 90 m. In vertical direction, the river was divided into 10 layers with a thickness that could vary depending on the depth and water surface elevation in the river (sigma-layers).

The transport of NoV, *E. coli*, and somatic coliphages in the river Göta älv was simulated using the water quality model ECO Lab (MIKE Powered by DHI), which was coupled with the hydrodynamic model of the river. The water quality model used flow fields from the hydrodynamic model to calculate the microbial concentrations in the river. The processes accounted for in the model are: advection, dispersion, and decay (for faecal indicators only).

It was assumed that NoV does not decay in the river (same as by Sokolova et al.),[22] since NoV is highly resistant to environmental degradation in water.[7, 23-24] For example, Bae and Schwab (2008)[23] reported the nucleic acid decay of a NoV surrogate to be 0.08 ± 0.02 Log_{10} per day in surface water at 25 °C. Based on this, the decay in the river Göta älv can be estimated to be at most 0.40 ± 0.10 Log_{10}, given the transport time (< 5 days) and water temperature (< 25 °C). In addition, a sensitivity analysis was conducted by simulating the NoV decay in the same way as for somatic coliphages, since coliphages are considered a useful surrogate for behaviour of enteric viruses in water environment.[25]

The decay of faecal indicators was described according to Equation 1:[26-27]

$$\frac{dC}{dt} = -k_0 \cdot \theta_I^{\,Int} \cdot \theta_T^{\,(Temp-20)} \cdot C \qquad (1)$$

In Equation 1, C is the faecal indicator concentration; t is the time; k_0 (1/day) is the decay rate at 20 ˚C for a salinity of 0 ‰ and darkness; ϑ_I is the light coefficient; *Int* (kW/m^2) is the light intensity integrated over depth; ϑ_T is the temperature coefficient; *Temp* (˚C) is the water temperature.

The coefficients in Equation 1 for *E. coli* and somatic coliphages were determined based on the data from the microcosm trials performed in different seasons for the conditions of Lake Rådasjön in Sweden.[27] The coefficients k_0, ϑ_I and ϑ_T were set to 0.76, 1 and 1.04 for *E. coli* and to 0.25, 1 and 1.08 for somatic coliphages, respectively.[21]

The discharges of treated wastewater from eight WWTPs (Holmängen, Arvidstorp, Hjärtum, Ellbo, Nygård, Lödöse, Älvängen, and Diseröd) were considered in the model as contamination sources (Figure S1). The magnitude of these discharges was described using the mean values for the respective WWTPs (Table S3). The water flow in the tributaries was described using the mean values.

2.6.2 Scenarios

Several scenarios were formulated to represent the conditions in the river: different NoV loading caused by the seasonality of diseases; different water temperature, which may affect the microbial decay; different water flow, which affects contaminant transport in the river.

In case of NoV, due to the assumption of no decay, the water temperature was irrelevant and was not accounted for. On the other hand, the NoV genome concentration in wastewater is dependent on the prevalence of infection in the human population, and the prevalence of infection may be dependent on the season. Thus, the NoV genome concentrations in treated wastewater were described using the values calculated for the cold and warm periods by combining the measured data from all WWTPs for each period (Table S4; see also the definition of the periods in Section 2.5 Statistical analyses).

In case of the faecal indicators *E. coli* and somatic coliphages, scenarios for low and high water temperature were simulated to account for the temperature dependent decay. The concentrations in treated wastewater were described using the data measured at the respective WWTPs. For the WWTPs, for which measured data were not available, the values for concentrations were calculated by combining all the measured data from the other WWTPs (Table S4).

Four scenarios were formulated: low flow cold, low flow warm, high flow cold, and high flow warm. In case of NoV, these scenarios described the combination of low or high flow conditions with the NoV genome concentrations representative for the warm or cold periods. In case of faecal indicators, these scenarios described the combination of low or high flow conditions with warm or cold water in the river.

To account for the variability of microbial concentrations in discharges of treated wastewater, the scenarios were simulated using the median (baseline) and 95th percentile (worst case) values. The water temperature was specified using the mean values for winter and summer (data for 2002 – 2013): 2.4 and 17.7 °C respectively. The water flow for the low and high flow scenarios was specified as 200 and 850 m^3 s^{-1} respectively.

3 Results

3.1 Measured concentrations of norovirus and faecal indicators in wastewater

3.1.1 Incoming wastewater

The measured concentrations of NoV GGI and GGII genome in incoming wastewater at each sampling occasion are shown in Figure 1 (see also Table S5). For incoming wastewater, the mean (standard deviation) Log_{10} concentrations of NoV GGI and GGII genome for the combined data from the WWTPs in Vänersborg, Trollhättan, and Lilla Edet were 6.2 (1.4) and 6.8 (1.8) Log_{10} g.e. L^{-1}, respectively. For incoming wastewater, all NoV genome concentrations that were above the LOD, were also above the LOQ.

Combined data for incoming wastewater at the WWTPs in Lilla Edet and Trollhättan showed that NoV GGI was detected equally frequent during the warm (22 out of 26 samples) and the cold (25 out of 28 samples) periods (Fischer's exact test). For NoV GGII, the number of positive samples collected

during the warm (15 out of 26) and the cold (26 out of28) periods differed significantly (Fischer's exact test). As only few samples were obtained from Vänersborg during the warm period, this WWTP was omitted in the seasonality analysis. All samples from the Vänersborg WWTP (n=9; obtained during 26 Oct 2011 – 5 Jun 2012) were positive for NoV GGI and GGII.

E. coli and somatic coliphages were detected in all collected samples of incoming wastewater; the descriptive statistics are shown in Table S5. For incoming wastewater, no significant differences were observed between the warm and the cold periods for *E. coli* and somatic coliphages (independent samples t-test).

At the Trollhättan WWTP, the concentrations of NoV GGII genome and *E. coli* in incoming wastewater positively correlated (bivariate correlation analysis). No other correlations between the concentrations of NoV genome and of faecal indicators in incoming wastewater were observed.

3.1.2 Treated wastewater

The measured concentrations of NoV GGI and GGII genome in treated wastewater at each sampling occasion are shown in Figure 1 (see also Table S5). For treated wastewater, the mean (standard deviation) Log_{10} concentrations of NoV GGI and GGII genome in the combined data from the WWTPs in Vänersborg, Trollhättan, Lilla Edet, and Gothenburg were 5.3 (1.4) and 5.9 (1.4) Log_{10} g.e. L^{-1}, respectively. For treated wastewater, all NoV genome concentrations that were above the LOD, were also above the LOQ.

Combined data for treated wastewater at the WWTPs in Lilla Edet and Trollhättan showed that NoV GGI was detected equally frequent during the warm (16 out of 26 samples) and the cold (17 out of 28 samples) periods (Fischer's exact test). For NoV GGII, the number of positive samples collected during the warm (15 out of 26) and the cold (26 out of 28) periods differed significantly (Fischer's exact test). As only few samples were obtained from Vänersborg and Gothenburg during the warm period, these WWTPs were omitted in the seasonality analysis. All samples from the WWTPs in

Vänersborg (n=9; obtained during 26 Oct 2011 – 5 Jun 2012) and Gothenburg (n=7; obtained during 21 Dec 2011 – 5 Jun 2012) were positive for NoV GGI and GGII.

E. coli and somatic coliphages were detected in all collected samples of treated wastewater; the descriptive statistics are shown in Table S5. For treated wastewater, no significant differences were observed between the warm and the cold periods for *E. coli* and somatic coliphages (independent samples t-test).

In treated wastewater, no correlations between the concentrations of NoV genome and of faecal indicators were observed.

3.1.3 Reduction of the norovirus and faecal indicator concentrations at the wastewater treatment plants

The Log_{10} reduction at each WWTP was calculated as the mean of the difference between the Log_{10} concentrations in incoming and treated wastewater on each sampling occasion. At the Vänersborg WWTP, the Log_{10} reduction for NoV GGI and GGII was 0.4 and 0.7, respectively. At the Lilla Edet WWTP, the Log_{10} reduction for NoV GGI, GGII, and somatic coliphages was 0.8, 0.7, and 1.5, respectively. At the Trollhättan WWTP, the Log_{10} reduction for NoV GGI, GGII, *E. coli*, and somatic coliphages was 1.1, 1.0, 1.2, and 0.9, respectively.

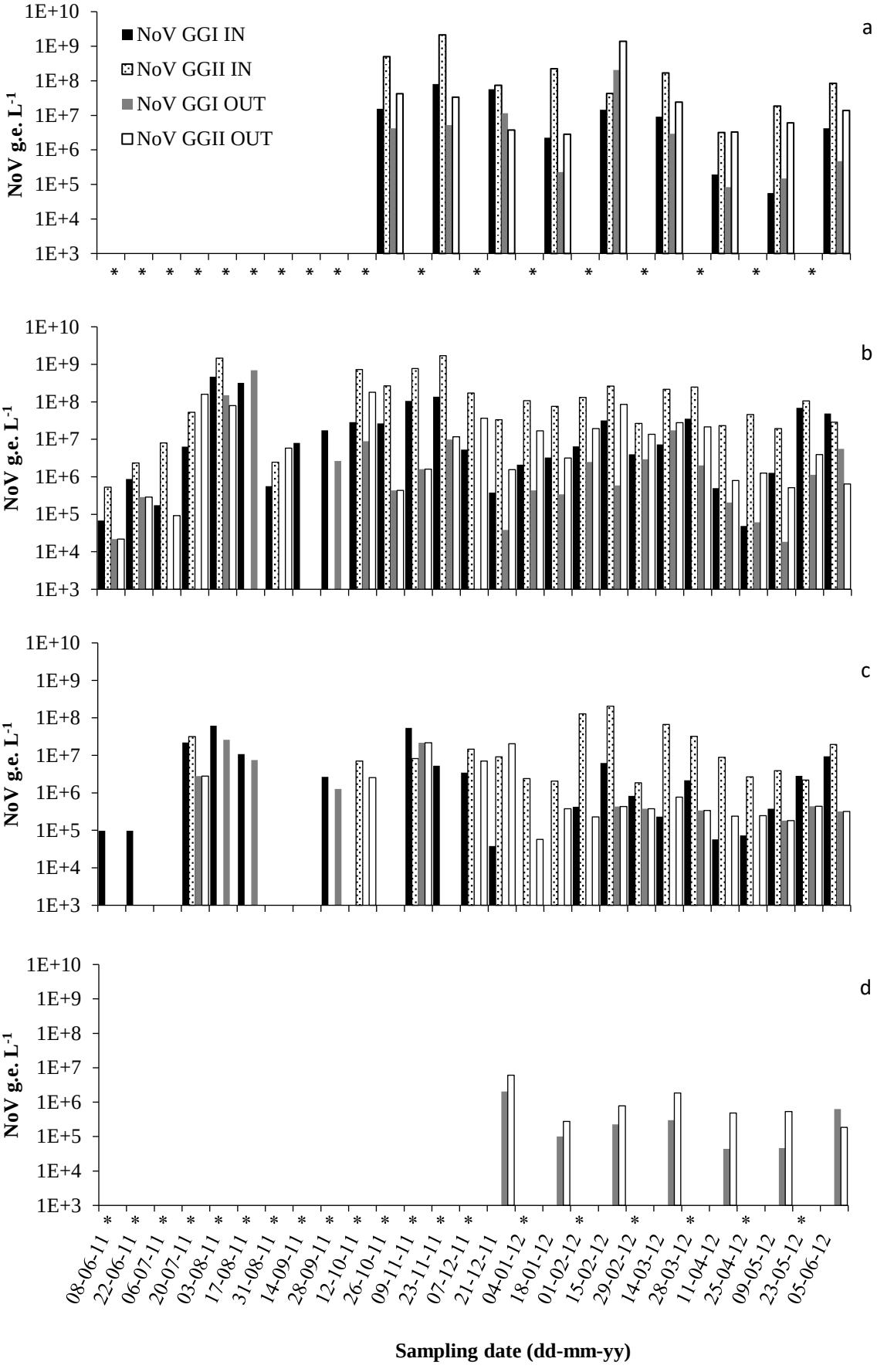
a
b
c
d
NoV GGI IN
NoV GGII IN
NoV GGI OUT
NoV GGII OUT
NoV g.e. L-1
1E+10
1E+9
1E+8
1E+7
1E+6
1E+5
1E+4
1E+3
08-06-11
22-06-11
06-07-11
20-07-11
03-08-11
17-08-11
31-08-11
14-09-11
28-09-11
12-10-11
26-10-11
09-11-11
23-11-11
07-12-11
21-12-11
04-01-12
18-01-12
01-02-12
15-02-12
29-02-12
14-03-12
28-03-12
11-04-12
25-04-12
09-05-12
23-05-12
05-06-12
Sampling date (dd-mm-yy)

Figure 1 Measured concentrations of norovirus genome in incoming (IN) and treated (OUT) wastewater at the wastewater treatment plants in Vänersborg (a), Trollhättan (b), Lilla Edet (c), and Gothenburg (d) (only treated wastewater). The star (*) indicates that no sample was collected. The absence of bars for the dates, on which samples were collected, indicates that the NoV genome concentration was below the limit of detection (4.5×10^3 g.e. L^{-1}).

3.2 Measured concentrations of norovirus and faecal indicators in source water

In source water samples from Vänersborg, Trollhättan, Lilla Edet, Kungälv, and Gothenburg DWTPs, the concentrations of NoV GGI and GGII genome were above the LOD in 7 out of 58 and in 18 out of 58 samples, respectively (Figure 2). The NoV genome concentrations were below the LOQ in all source water samples.

Combined data for source water at the Trollhättan, Lilla Edet, and Gothenburg DWTPs showed that NoV GGI was detected in 3 out of 42, while NoV GGII was detected in 13 out of 42 samples. For NoV GGI, the difference between the number of positive samples collected during the warm (0 out of 24) and the cold (3 out of 18) periods was not significant (Fischer's exact test). For NoV GGII, the number of positive samples collected during the warm (3 out of 24) and the cold (10 out of 18) periods differed significantly (Fischer's exact test). As only few samples were obtained from Vänersborg and Kungälv during the warm period, these DWTPs were omitted in the seasonality analysis.

For source water, no significant differences were observed between the warm and the cold periods for *E. coli* and somatic coliphages (independent samples t-test).

Since the NoV genome concentrations were below the LOQ in all source water samples, the correlations with faecal indicators could not be analysed.

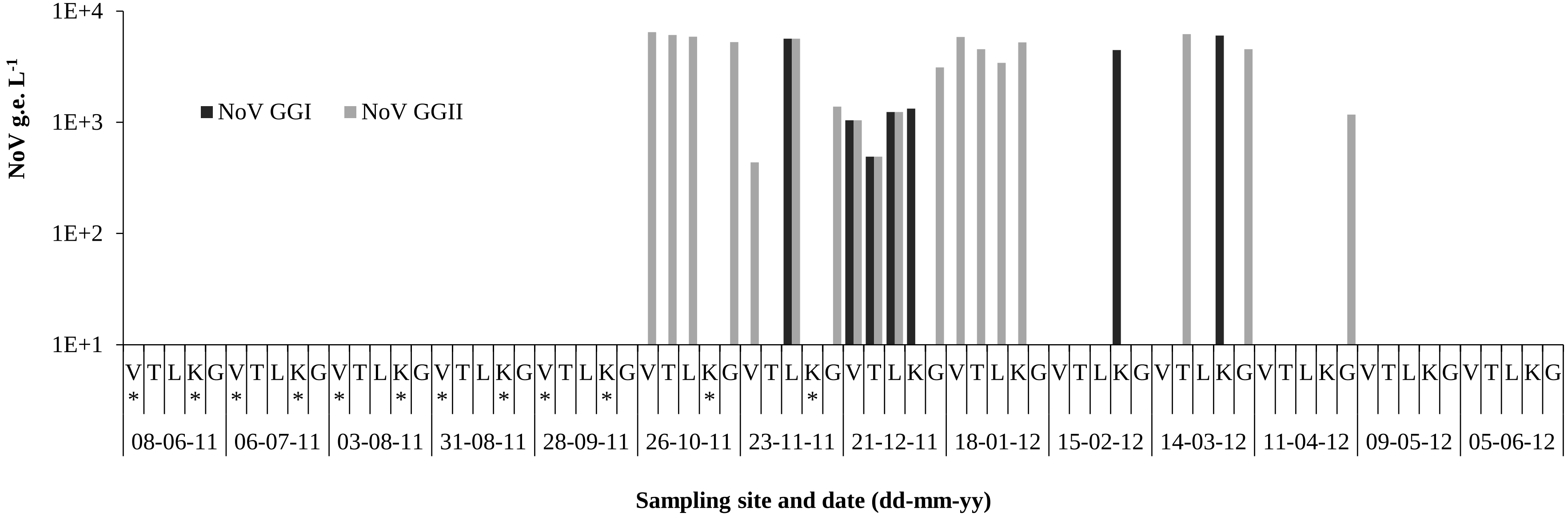

Figure 2 Measured concentrations of norovirus genome in source water at the water intakes in Vänersborg (V), Trollhättan (T), Lilla Edet (L), Kungälv (K), and Gothenburg (G). The star (*) indicates that no sample was collected. The absence of bars for the dates, on which samples were collected, indicates that the NoV genome concentration was below the limit of detection (1.1×10^1 g.e. L^{-1}). The NoV genome concentrations were below the limit of quantification in all source water samples.

3.3 Simulated concentrations of norovirus and faecal indicators in source water

For the baseline scenario (Figure S2), the modelling results for NoV GGI were similar under warm and cold conditions, while the modelling results for NoV GGII were higher under cold than under warm conditions. For the worst case scenario (Figure S2), the modelling results for both NoV GGI and GGII were higher under warm than under cold conditions. For both NoV GGI and GGII, the genome concentrations were higher under low flow conditions compared to high flow conditions (Figure S2).

The modelling results for *E. coli* and somatic coliphages (Figure S2) were higher under cold than under warm conditions, due to the temperature dependent decay. Under cold conditions, the modelling results were higher under low flow conditions, due to dilution in a smaller volume of water in comparison to high flow conditions. However, under warm conditions, the effect of dilution was counteracted by the effect of decay, since the latter was more pronounced under conditions of low flow because of longer transport time (Figure S2).

The comparison of the modelling results with the measured data (Table 1) was limited by the fact that in the model only one type of contamination source was considered, i.e. treated discharges from the WWTPs. In reality, other sources exist, e.g. emergency discharges from the WWTPs and the sewer system, and discharges from on-site sewer systems. Moreover, the comparison for NoV was also limited by the fact that only few concentrations were above the LOD, and all concentrations were below the LOQ.

The simulated NoV genome concentrations at the water intakes, for the baseline scenario, were either in agreement or lower than the measured concentrations (Table 1). However, for the worst case scenario, the simulated NoV genome concentrations at the water intakes were much higher (up to 2.8 and 1.9 Log_{10} units for NoV GGI and GGII, respectively) than the measured concentrations.

306 The simulated *E. coli* concentrations at the water intakes, for the baseline scenario, were in
307 agreement with the mean measured concentrations for Trollhättan and Lilla Edet, but lower (0.7
308 Log_{10} units) for Gothenburg (Table 1). For the worst case scenario, the simulated *E. coli*
309 concentrations at the water intakes were in agreement with the maximum measured concentrations
310 for Trollhättan and Lilla Edet, but lower (0.9 Log_{10} units) for Gothenburg (Table 1).

311 The simulated concentrations of somatic coliphages at the water intakes, for the baseline scenario,
312 were in agreement with the mean measured concentrations (Table 1). For the worst case scenario,
313 the simulated concentrations of somatic coliphages at the water intakes were in agreement with the
314 maximum measured concentrations for Lilla Edet and Gothenburg, but lower (0.7 Log_{10} units) for
315 Trollhättan and higher (1.1 Log_{10} units) for Kungälv (Table 1).

316 The sensitivity analysis showed that the maximum simulated NoV genome concentrations at the
317 water intakes were on average 0.15 and at most 0.40 Log_{10} units lower, when the NoV decay was
318 taken into account (Table S6).

319

Table 1 Measured and simulated concentrations (Log_{10} transformed) of norovirus (NoV) genome and faecal indicators in source water at the drinking water treatment plants in Trollhättan (T), Lilla Edet (L), Kungälv (K), and Gothenburg (G).

	Measured		Simulated	
Place	N [a]	Minimum – Maximum (Mean)[b]	Minimum – Maximum Baseline	Minimum – Maximum Worst case
NoV GGI, Log_{10} (g.e. L^{-1})				
T	1 (14)	<1.0 – 2.7	1.6 – 2.4	3.6 – 5.5
L	2 (14)	<1.0 – 3.8	2.1 – 2.9	4.1 – 6.0
K	3 (7)	<1.0 – 3.8	2.2 – 2.9	4.1 – 6.0
G	0 (14)	<1.0	2.2 – 2.9	4.1 – 6.0
NoV GGII, Log_{10} (g.e. L^{-1})				
T	4 (14)	<1.0 – 3.8	1.6 – 3.3	4.2 – 5.1
L	4 (14)	<1.0 – 3.8	2.1 – 3.7	4.7 – 5.6
K	1 (7)	<1.0 – 3.7	2.2 – 3.8	4.7 – 5.6
G	5 (14)	<1.0 – 3.7	2.2 – 3.8	4.7 – 5.6
E. coli, Log_{10} (CFU L^{-1})				
T	22 (25)	<1.0 – 3.0 (2.2)	1.8 – 2.4	2.5 – 3.1
L	25 (27)	<1.0 – 3.0 (2.4)	2.2 – 2.6	2.9 – 3.4
K	-	-	2.1 – 2.5	2.9 – 3.2
G	26 (26)	2.3 – 3.9 (3.0)	1.8 – 2.3	2.5 – 3.0
Somatic coliphages, Log_{10} (PFU L^{-1})				
T	9 (10)	<1.0 – 3.3 (1.9)	1.1 – 1.7	2.0 – 2.6
L	10 (10)	1.8 – 3.0 (2.2)	1.7 – 2.3	2.5 – 3.2
K	5 (5)	1.3 – 2.0 (1.7)	1.7 – 2.3	2.5 – 3.1
G	9 (9)	1.3 – 3.2 (2.3)	1.6 – 2.2	2.5 – 3.1

[a] number of samples above the LOD (total number of samples)

[b] When the concentration was below the LOD, the value for LOD was used. For NoV in source water, the LOD was extrapolated to 1.1×10^{1} g.e. L^{-1}, i.e. 1.0 Log_{10} units. The mean NoV genome concentrations were not calculated, due to a small number of concentrations above the LOD.

4 Discussion

In this article, the concentrations of NoV genome and faecal indicators at the WWTPs and in the recipient drinking water source – the river Göta älv were studied in a one-year monitoring project. The effect of treated wastewater discharges from these WWTPs on source water was studied using hydrodynamic modelling.

Our findings add to previous studies[28-29] on NoV genome concentrations in wastewater and recipient source water, and reduction of NoV by wastewater treatment. The detection of NoV in treated wastewater is in agreement with previous results.[5, 30] The reduction of NoV in the WWTPs was of the same magnitude as the reduction of faecal indicators – around one Log_{10} unit; this is also in agreement with previous results.[30] Our findings regarding the seasonal variation of NoV in source water are also in agreement with the previous observations from other European countries.[5, 31] The more frequent detection of GGII compared to GGI during the colder period is in agreement with previous reports[32] and is suggested to reflect the high prevalence of NoV GGII infections during the colder period of the year.[33]

A limitation of the NoV measurements is that the virus recovery in this study varied strongly; this is however a common problem for this type of studies.[34] An improvement of the virus enrichment methodology to increase the recovery rate and reproducibility of NoV detection is desired.[34] A potential inhibition[35] during cDNA synthesis and the PCR reaction was ruled out. Another limitation of the studies based on PCR is the referring to genome equivalents and not to infectious viruses.[36]

The modelling results for the faecal indicators were generally in agreement with the measured concentrations in source water (Table 1). In some cases, the simulated concentrations of faecal indicators were lower than the measured concentrations. This underestimation can be explained by the fact that only the influence of the regular discharges from the WWTPs was studied, not of other faecal sources. For example, on-site sewer systems may also contribute to the faecal load into the river because of often poor treatment. While the modelling results for NoV for the baseline scenario

were in agreement with the measured concentrations (Table 1), the modelling results for the worst case scenario indicated that the concentrations at the water intakes could be much higher than the measured NoV genome concentrations. The plausibility of the modelling results for NoV is supported by the modelling results for faecal indicators.

A limitation of this modelling approach is that it was assumed that microorganisms were not attached to particles. Particle – microorganism interactions as well as sedimentation and resuspension are complex and site-specific processes.[37-39] Due to the lack of data for the study area, these processes were not included in the model, in order not to increase uncertainty. The decay of NoV was neglected, since the transport time in the river is short, and NoV is highly resistant to environmental degradation in water.[7, 23-24] The validity of this approach was confirmed by the sensitivity analysis (Table S6).

Outbreaks related to drinking water contaminated with NoV[40-41] result in suffering for patients and high costs for the community.[40] Quantitative microbial risk assessment that is widely used to analyse and inform the management of the drinking water supply system,[42-43] requires the data on pathogen concentrations in source water. The monitoring project showed that measurements of the NoV genome concentrations in source water, while useful as input for risk assessment, may not provide the complete picture, due to a relatively low frequency of measurements, and many concentrations below the limits of quantification and detection. To address the limitations of monitoring and analytical methods, the measured data can be supplemented by the results of hydrodynamic modelling.[20, 22, 44] This study demonstrated that the modelling approach can be very useful to describe the NoV genome concentration in source water. The modelling results provided insights that the NoV genome concentrations at the water intakes may occasionally be much higher than the concentrations measured during the monitoring project. Moreover, the modelling approach emphasises the importance of knowing the contamination sources in the catchment; this is in

agreement with the recommendations by the World Health Organisation on mitigating the risks close to the contamination source.[25]

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Supporting Information available: details regarding the sampling programme, norovirus analyses, hydrodynamic modelling, measured microbial concentrations in wastewater, simulated microbial concentrations in source water. This information is available free of charge via the Internet at http://pubs.acs.org.

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