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Short- and long-term variations of norovirus concentrations in the Meuse river during a 2-year study period

Therese Westrell[a,b], *Peter Teunis*[c], *Harold van den Berg*[d], *Willemijn Lodder*[d], *Henk Ketelaars*[e], *Thor Axel Stenström*[a,b], *Ana Maria de Roda Husman*[d,*]

[a]*Department of Parasitology, Mycology and Water Microbiology, Swedish Institute for Infectious Disease Control (SMI), 171 82 Solna, Sweden*
[b]*Department of Water and Environmental Studies, Linköping University, 581 83 Linköping, Sweden*
[c]*Computerization and Methodological Consultancy Unit, National Institute of Public Health and the Environment (RIVM), 3720 BA Bilthoven, The Netherlands*
[d]*Microbiological Laboratory for Health Protection, National Institute of Public Health and the Environment (RIVM), 3720 BA Bilthoven, The Netherlands*
[e]*Waterwinningsbedrijf Brabantse Biesbosch (WBB), 4251 NN Werkendam, The Netherlands*

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ABSTRACT

Faecally impacted surface waters used for drinking water production may encompass risk for norovirus infections. To be able to assess a possible health risk, noroviruses should be quantified and fluctuations identified. In 2001, norovirus concentrations in the river Meuse displayed a seasonal distribution with high peaks during wintertime as determined by RT-PCR on serially diluted RNA. An intensified day-by-day sampling scheme in the winter of 2002/2003 revealed that the winter peak consisted of several peaks of varying duration and magnitude, possibly due to contamination events in the catchment. The highest estimated concentration was 1700 PCR-detectable units per litre (95% CI 250–8000), which if coinciding with failing treatment could lead to significant numbers in drinking water. Adaptive dynamic filtering was shown to adequately predict subsequent sample concentrations. If valid, such analyses could prove to be useful as early warning systems in risk management of water sources.

1. Introduction

Noroviruses (NoV; previously Norwalk-like viruses) are the most common cause of gastroenteritis in people of all age groups (Koopmans and Duizer, 2004). The viruses belonging to the *Caliciviridae* are highly infectious and cause numerous outbreaks annually. The occurrence of NoV outbreaks in the population shows a seasonal pattern, mainly occurring during wintertime (Mounts et al., 2000; Lopman et al., 2004). The relative impact of waterborne NoV outbreaks in relation to other routes of transmission needs to be resolved. NoV has, however, been reported more and more frequently as an essential aetiological agent of waterborne gastroenteritis. In some of the recent NoV outbreaks, the causal relationship between a contaminated water source and faecal samples of cases could clearly be established with molecular methods (Brown et al., 2001; Anderson et al., 2003; Nygård et al., 2003; Hoebe et al., 2004). Often treatment failure or inadequate removal was implied as the cause of waterborne disease. An infected individual can shed NoV both via vomits and faeces

*Corresponding author. Tel.: +31 30 274 4325; fax: +31 30 274 4434.
E-mail address: AM.de.Roda.Husman@rivm.nl (A.M. de Roda Husman).
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in high numbers. The highest shedding occurs during the symptomatic phase when vomits or faeces can contain 10^8 and 10^9 virus particles per mL, respectively, as estimated by electron microscopy (Hedlund, 2004). NoV shedding in faeces has also been confirmed from patients after the cessation of symptoms (Marshall et al., 2001; Rockx et al., 2002). The viruses have been detected in high levels in raw sewage (Lodder et al., 1999) as well as treated sewage (van den Berg et al., 2005). Once discharged into recipient waters, viruses can survive for long periods depending on temperature and solar irradiation (Allwood et al., 2003). NoV have been detected by RT-PCR both in fresh waters, such as rivers and lakes, and sea waters in different parts of the world (Katayama et al., 2002; Hörman et al., 2004; Lodder and de Roda Husman, 2005). To date numerous attempts to culture NoV have failed to yield replicating virions (Duizer et al., 2004) and therefore the infectivity of these viruses in environmental samples cannot be established. Human volunteer studies have shown that exposure to relatively low doses, based on NoV RT-PCR units, may lead to infection and illness (Lindesmith et al., 2003).

The aims of this study were to quantitatively describe NoV concentrations in surface water over a whole year and to perform adaptive dynamic filtering to study the occurrence of peaks and possibly their shapes/magnitude.

2. Materials and methods

2.1. Sampling

The water samples were taken from the river Meuse at the intake to the Biesbosch storage reservoirs that serve as the raw water supply for several waterworks in the southern and western parts of The Netherlands. The Meuse is a moderately polluted and highly eutrophicated river running from France, through Belgium and out to the North Sea on the coast of The Netherlands. Large volume water sampling (200–500 L) from the river was conducted monthly during year 2001. Between December 9 2002 and January 10 2003 an intensified sampling programme was carried out with weekly large volume water samples followed by small volume water samples (10 L) from each of the four consecutive days. Turbidity, expressed in formazine turbidity units, FTU, was determined using the Hanna 193703 microprocessor turbidity meter (range 0.00–1000 FTU).

3. Virus detection

3.1. Concentration of water samples

The large and small volume water samples were first concentrated by a conventional filter adsorption–elution method (Lodder and de Roda Husman, 2005). Large volume water samples were filtered and eluted at the point of sampling while 10-L samples were processed in the laboratory. Magnesium chloride was added to a water sample to a final concentration of 0.05 M (pH 3.8) to facilitate binding to a commercial cartridge filter (1.2 μm Nominal) (Millipore, Etten-Leur, The Netherlands). Viruses were eluted from the filter with a 3% beef extract (Difco, Amsterdam, The Netherlands)/Tris solution (pH 9.0). The typical retentate volume of the large volume water sample was approximately 1800 mL and for the 10-L water sample, this was approximately 650 mL. The resulting retentate was neutralised (to pH 7.2) and subjected to two-phase separation for molecular analysis or to culture methods.

3.1.1. Concentration and purification of virus from the eluate by two-phase separation

To 650-mL eluate, 1% (w/v) Dextran T40, 10% (w/v) PEG 6000, 0.2 M NaCl and 10 mM phosphate buffer (pH 7.2) were added and mixed for 1 h at 4 °C on a horizontal shaker. The suspension was then transferred to a separation funnel and left overnight at 4 °C. After separation, the bottom phase and the interphase were harvested. Further purification was done by spin-column gel chromatography using Sephadex G200 (ICN, Zoetermeer, The Netherlands), and by ultrafiltration in a Centricon 100 microconcentrator, 100,000 MW cut-off (Amicon, Dronten, The Netherlands). The average retentate volumes were 1–5 mL.

3.1.2. RNA extraction, RT-PCR, gel electrophoresis, Southern blotting and hybridisation

RNA was extracted from the retentate by an in-house silica beads method (Lodder and de Roda Husman, 2005) modified from the method originally described by Boom et al. (1990).

For NoV detection, we used a single round RT-PCR assay on tenfold serially diluted RNA extract (endpoint dilution) directed at the viral RNA polymerase gene (ORF1) (Vennema et al., 2002) as described previously (Lodder and de Roda Husman, 2005). NoV positive stool specimens obtained from patients with gastroenteritis were used as a positive control in the RNA extraction and the RT-PCR. For rotavirus detection, we performed a RT-PCR assay for generic molecular detection of rotaviruses amplifying the VP6 gene fragment as described earlier (Villena et al., 2003). The amplification products were analysed by electrophoresis in 2% agarose gels and visualised under UV illumination after staining with SYBR Gold nucleic acid gel stain (Molecular probes, Leiden, The Netherlands) followed by specific probe hybridisation (Lodder and de Roda Husman, 2005).

3.1.3. Cell culture of enteroviruses

Virus infectivity was determined by a monolayer plaque assay with Buffalo Green Monkey kidney (BGM) cells (Lodder and de Roda Husman, 2005). Culture medium was removed from the BGM monolayer after which the eluate (1.1 g per flask) and antibiotic mixture were added to the flasks. The positive control samples used in the cell culture method were virus isolates, coxsackievirus type B4, originally obtained from the 'Voorns kanaal' by BGM cell culture. The cultures were incubated at room temperature for 120 min and overlaid with supplemented Medium 199 agar as described before (Lodder and de Roda Husman, 2005). After 9 days of incubation at 37 °C, the cells were neutral red stained and 24 h later, the plaques were enumerated. The virus concentration, expressed as plaque-forming units per litre (pfu L^{-1}), in the original water sample was calculated from the test volume and the virus count.