Presence of Enteric Viruses in Source Waters for Drinking Water Production in the Netherlands

W. J. Lodder,* H. H. J. L. van den Berg, S. A. Rutjes, and A. M. de Roda Husman

Laboratory for Zoonoses and Environmental Microbiology, Centre for Infectious Disease Control Netherlands, National Institute for Public Health and the Environment, P.O. Box 1, NL-3720 BA Bilthoven, Netherlands

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The quality of drinking water in the Netherlands has to comply with the Dutch Drinking Water Directive: less than one infection in 10,000 persons per year may occur due to consumption of unboiled drinking water. Since virus concentrations in drinking waters may be below the detection limit but entail a public health risk, the infection risk from drinking water consumption requires the assessment of the virus concentrations in source waters and of the removal efficiency of treatment processes. In this study, samples of source waters were taken during 4 years of regular sampling (1999 to 2002), and enteroviruses, reoviruses, somatic phages, and F-specific phages were detected in 75% (range, 0.0033 to 5.2 PFU/liter), 83% (0.0030 to 5.9 PFU/liter), 100% (1.1 to 114,156 PFU/liter), and 97% (0.12 to 14,403 PFU/liter), respectively, of 75 tested source water samples originating from 10 locations for drinking water production. By endpoint dilution reverse transcription-PCR (RT-PCR), 45% of the tested source water samples were positive for norovirus RNA (0.22 to 177 PCR-detectable units [PDU]/liter), and 48% were positive for rotavirus RNA (0.65 to 2,249 PDU/liter). Multiple viruses were regularly detected in the source water samples. A significant correlation between the concentrations of the two phages and those of the enteroviruses could be demonstrated. The virus concentrations varied greatly between 10 tested locations, and a seasonal effect was observed. Peak concentrations of pathogenic viruses occur in source waters used for drinking water production. If seasonal and short-term fluctuations coincide with less efficient or failing treatment, an unacceptable public health risk from exposure to this drinking water may occur.

Surface waters are continuously contaminated with human-pathogenic viruses originating from sewage and other fecal waste sources (22). For instance, raw urban sewage may be discharged into surface water during heavy rainfall. Because enteric viruses are excreted in high concentrations by infected individuals (1, 21, 29), raw sewage can contain high concentrations of these viruses. Treated sewage is discharged into surface water, and previous studies have shown that depending on the applied treatment processes, treated sewage may still contain a high concentration of viruses (3, 8, 22, 23, 32, 43).

In the Netherlands, surface water is used as source water for the production of drinking water but is also used for recreational purposes and for shellfish cultivation for human consumption. Because of this possible exposure, surface water can be a source of pathogenic viruses to humans. Pathogenic enteric viruses include noroviruses, rotaviruses, hepatitis A and E viruses, and enteroviruses, which can pass asymptptomatically or lead to mild illness, e.g., gastroenteritis, or more severe illness, such as hepatitis, encephalitis, and meningitis (10). Although the exact role of reoviruses as human pathogens remains unclear and they mainly cause mild and asymptomatic illness, more severe illness, like meningitis, has been reported (17, 42). Numerous waterborne outbreaks have previously been described, particularly caused by noroviruses and rotaviruses (13, 15, 16, 25, 26, 33, 46).

Virus detection in environmental samples can be done either with cell culture methods or with molecular techniques (50). Because some viruses are difficult or even impossible to cultivate, molecular techniques are useful tools for detection. Even though these techniques do not discriminate between viable and nonviable viruses, these techniques are useful for monitoring the presence of these viruses in environmental samples. To use this information to estimate a possible public health risk following exposure to this water, a risk assessment should be performed to estimate the infection risk when the RNA of these viruses is detected in environmental samples.

In the Netherlands, surface water, besides groundwater, is used as source water for the production of drinking water. The Dutch Drinking Water Directive (2) describes that it is obligatory to establish the drinking water quality by quantitative microbial risk assessment (QMRA) for index pathogens. To comply with this legislation, the annual infection risk should be lower than one infected person in every 10,000 persons due to consumption of unboiled tap water. The necessary data to assess this risk are acquired by measuring the index pathogens in raw source water. The concentrations in the finished drinking water are subsequently estimated using the reduction by the treatment steps applied. Because the applied treatment processes differ between the locations where (surface) waters are used for the production of drinking water, this estimation should be done for each location separately. For the estimation of the concentration of infectious pathogenic viruses in the source water, the index pathogen is enterovirus. Furthermore, because of their characteristics, each group of microorganisms

* Corresponding author. Mailing address: Laboratory for Zoonoses and Environmental Microbiology, Centre for Infectious Disease Control Netherlands, National Institute for Public Health and the Environment, P.O. Box 1, NL-3720 BA Bilthoven, Netherlands. Phone: 31.30.274.3928. Fax: 31.30.274.4434. E-mail: willemijn.lodder@rivm.nl.

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(i.e., parasites, bacteria, and viruses) behaves differently in these treatment processes and therefore should be looked at separately.

After the enteric viruses are discharged into water, they can survive for prolonged periods in the aquatic environment (38), and the survival of these viruses depends on different factors, such as UV, temperature, and pH (11, 12). Because there are many different viruses belonging to the enteric virus group that have many different characteristics, the survival rate is also influenced by the virus type. Although these viruses are continuously discharged into the environment, the concentrations of viruses present in surface water are nevertheless generally low and, therefore, the analysis of large volumes of water is necessary.

Here, we studied the quality of Dutch source waters for drinking water production at 10 locations with respect to enteric viruses, because the source water quality, in combination with the applied treatment processes, has an impact on the drinking water quality. Samples were taken from 1999 to 2002, and the sampling period was different for each location. Besides the determination of enteroviruses as the viral index pathogen, the potentially human pathogenic noroviruses, reoviruses, and rotaviruses were also determined. Bacteriophages were determined because they have been suggested previously as useful indicators. The enteroviruses, reoviruses, and bacteriophages were detected by (cell) culture, and norovirus and rotavirus RNA was detected by reverse transcription-PCR (RT-PCR). To monitor the presence of inhibitory factors, which particularly influences the RT-PCR, an internal control RNA was used, to avoid false-negative results.

MATERIALS AND METHODS

Viruses. The positive-control samples used in the cell culture method were reovirus type 3 and coxsackie B4 virus (22). The positive control used for the detection of F-specific bacteriophages was MS2 (ATCC 15597-B1), and the positive control used for the detection of somatic coliphages was qX174 (ATCC 13706-B1). A norovirus-positive stool specimen obtained from a patient with gastroenteritis was used as a positive control (GGII.1; Hu/NV/6649/1994) in the RNA extraction and the RT-PCR. Rotavirus WA, kindly obtained from the Laboratory for Infectious Diseases and Perinatal Screening, National Institute for Public Health and the Environment, was used as a positive control for the RNA extraction and the RT-PCR.

Sampling and concentration. Large volumes of surface water for drinking water production, 200 to 600 liters, were collected from 10 locations in the Netherlands (Fig. 1). The locations were either the intake areas for the drinking water companies or upstream of a source water intake area. Each of the three source water intake locations at Andelse Maas, Lateraalkanaal Heel, and Bergse Maas receive water from the Maas River, which is largely contaminated with human and zoonotic pathogens from upstream sewage treatment plants, sewage overflows, and runoff (27). Similarly, the Rijn River contributes directly to the source water locations at Lobith, Lekkanaal, and Amsterdam-Rijnkanaal, and the Usel River feeds the intake locations at Usselmeer and Twentekanaal. The Drechtsche Aa is a river catchment in the north of the Netherlands with multiple upstream sewage treatment plants. Sampling at these locations was repeated 1 to 12 times in the period from February 1999 to December 2002. To obtain more information about the water characteristics, several physical parameters were measured, i.e., temperature, pH, and turbidity.

Water was concentrated by a conventional filter adsorption-elution method (22, 35). The viruses were eluted from the filter with a beef extract solution with a high pH (9.0), and the resulting eluate was directly neutralized with a concentrated acetic acid buffer (pH 5.0) to pH 7.2. The total retentate volume was approximately 1.8 liters. Two-thirds (±1.200 ml) of the eluate was further concentrated with an ultrafiltration method and was subsequently analyzed by cell culture for the detection of cultivable viruses and bacteriophages, and the other one-third (±600 ml) of the resulting eluate was subjected further to the two-phase separation method.

The eluate was further purified and concentrated using a two-phase separation method, and the RNA was subsequently extracted from the retentate volume with the modified extraction method of Boom et al. (6), as described previously (22, 23).

Cell culture. The determination of the concentration of infectious viruses was done by performing a monolayer plaque assay (22). Briefly, concentrate and an antibiotic mixture were added to a monolayer of Buffalo green monkey kidney (BGM) cells. After 2 h of incubation, whereby the viruses were allowed to adsorb to the cells, an agar overlay was added. After 9 days of incubation at 37°C, the cells were stained with a neutral red solution. After 24 h, the plaques were enumerated and the virus concentration in the original water sample was calculated from the tested volume and the plaque counts.

Enumeration of bacteriophages. The detection of F-specific bacteriophages was done using the host strain WG49, and the detection of somatic coliphages was done using the host strain WG5, according to ISO 10705–1 (2001) and ISO 10705–2 (2001), respectively. In the presence of bacteriophages, plaques could be enumerated after an overnight exposure of the concentrate to the host bacterial strains WG49 and WG5 (14). The bacteriophage concentration in the original water sample was calculated from the tested volume and the plaque counts.

RT-PCR. For norovirus detection, we used the viral RNA polymerase gene as the target for amplification with an RT-PCR method, as previously described (44), using the primer pair JV12Y/JV13i. To monitor to what extent the RT-PCR was inhibited, an internal control (IC) RNA was included in the RT step. The IC RNA was synthesized by the addition of complementary sequences of both primers JV12Y and JV13i, to part of the &-globin gene, with a total length of 254 nucleotides. After subsequent cloning downstream of a T7 RNA-polysmerase promoter, transcription was performed (36). After RT-PCR, the IC product can be easily distinguished from the norovirus product (329 nucleotides) by gel electrophoresis.

To determine which IC RNA concentration was the most optimal concentration to add to the RT step, a titration series of the RNA was tested. The concentration of the IC RNA which did not interfere with the amplification of the target RNA and gave a clear band after gel electrophoresis was chosen. The confirmation of the specificity of the RT-PCR products was done by hybridization with a mixture of probes (47). As described previously (45), the VP6 gene was used as the target for amplification for the generic molecular detection of rotaviruses, using the primer pair VP6-3 and VP6-4. The rotavirus RT-PCR performance was also monitored by including an IC in the RT step. The rotavirus IC RNA was synthesized as described previously for the norovirus IC RNA by adding complementary sequences of the primers VP6-3 and VP6-4 instead of the JV primers, with a total length of 254 nucleotides. A VP6 probe was used to confirm the specificity of the detected RT-PCR products. To semiquantitatively determine the norovirus and rotavirus concentrations in the samples, the RNA was diluted at 10-fold intervals.

Statistical methods. The numbers of virus particles present in water were compared by culture or RT-PCR on 10-fold serially diluted RNA (endpoint dilution). Virus concentrations in the undiluted samples were estimated as the most probable numbers by using the number of PFU or the presence or absence of virus genomes in the 10-fold RNA dilutions under the assumption that negative samples do not contain virus or viral RNA. Application of the Poisson distribution was justified by the assumption that the infectious virus particles or viral RNA was dispersed randomly in the sample. The maximum likelihood method was used to estimate the number of virus particles in the undiluted sample (30). A negative binomial model gives the best fit for the distribution of virus particles in the original and diluted samples.

RESULTS

Viruses were determined in surface water samples at 10 locations upstream of source water intake points for drinking water production; the locations are widespread over the Netherlands (Fig. 1). During 1999 to 2002, a total of 75 samples taken throughout this 4-year period from these 10 locations were tested. The mean virus concentrations per location are presented in Fig. 1, and the mean, median, minimum, and maximum values are presented in Table 1.

Detection of viruses by culture methods. In total, 75 samples taken throughout the 4 years, from 10 locations, were tested...
for viruses by (cell) culture. In 75% and 83% of all the samples tested, enteroviruses and reoviruses could be detected, respectively. During 1999 to 2002, the percentages of positive samples varied for both enteroviruses and reoviruses between 30 to 100% per location. The mean (Fig. 1A) and median enterovirus concentrations per location are shown in Table 1 and varied between 0.0052 and 2.4 PFU/liter and between 0 and 2.4 PFU/liter, respectively. Also, the mean (Fig. 1A) and median reovirus concentrations per location are shown in Table 1 and varied between 0.013 and 1.3 PFU/liter and 0 and 0.8 PFU/liter, respectively. The enterovirus concentrations in the positive samples ranged from 0.0033 to 5.2 PFU/liter, and those for reoviruses ranged from 0.0030 to 5.9 PFU/liter (Table 1).

Somatic coliphages were found in all of the tested water samples, and F-specific phages were found in 73 of 75 (97%) tested samples. The mean somatic and F-specific bacteriophage concentrations in the samples varied between 105 and 1.7 × 10^5 PFU/liter and between 2.0 and 4.3 × 10^5 PFU/liter, respectively, and are shown per location in Fig. 1A and Table 1. The median somatic and F-specific bacteriophage concentrations in the samples varied between 0.0033 to 5.2 PFU/liter and between 0 and 1.3 PFU/liter, and those for reoviruses ranged from 0.0030 to 5.9 PFU/liter (Table 1).

Overall, the concentration of viruses found, using culture methods, was somewhat higher in the colder months of the year than the concentrations found in the warmer months of the year (summer). This trend is seen for the locations in which both periods were sampled and appears to be independent of the sampling year (data not shown).

Detection of viruses by RT-PCR. Only four of the 10 samples taken at the Twentekanaal were analyzed for the detection of viruses by RT-PCR. Therefore, out of the 75 water samples, 69 were tested for the presence of norovirus and rotavirus RNA. Tenfold serially diluted RNA samples were analyzed by conventional RT-PCR (45, 47). In 45% and 48% of these samples, norovirus and rotavirus RNA could be detected, respectively.
TABLE 1. Median and mean concentrations, and minimum and maximum values, of somatic coliphages, F-specific phages, enteroviruses, reoviruses, noroviruses, and rotaviruses per sampling location, taken from 1999 to 2002

<table>
<thead>
<tr>
<th>Location</th>
<th>Period</th>
<th>n</th>
<th>Type of value</th>
<th>Concn of:</th>
<th>Somatic coliphages (PFU/liter)</th>
<th>F-specific phages (PFU/liter)</th>
<th>Enteroviruses (PFU/liter)</th>
<th>Reoviruses (PFU/liter)</th>
<th>Noroviruses (PDU/liter)</th>
<th>Rotaviruses (PDU/liter)</th>
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<td>Andelse Maas</td>
<td>2002</td>
<td>4</td>
<td>Median</td>
<td>1,114</td>
<td>8.0</td>
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<td>0</td>
<td>170</td>
<td>170</td>
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<td>Mean</td>
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<td>$6.2 \times 10^{-3}$</td>
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<td>132-217</td>
<td>132-217</td>
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<td>Mean</td>
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<td>219-522</td>
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<td>0-2.5 $\times 10^{-3}$</td>
<td>0-1.7</td>
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<td>Median</td>
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<td>936</td>
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<td>Mean</td>
<td>2.0 $\times 10^{4}$</td>
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<td>1.3</td>
<td>0.33</td>
<td>1.4</td>
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<td>Drentsche Aa</td>
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<td>Mean</td>
<td>105</td>
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<td>7.3 $\times 10^{-3}$</td>
<td>4.4</td>
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<td>Range</td>
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<td>3.8 $\times 10^{-3}$-0.20</td>
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<td>Lateraalkanaal Heel</td>
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<td>Mean</td>
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<td>0.78</td>
<td>26</td>
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<td>Range</td>
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<td>0.62-3.5</td>
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<td>Mean</td>
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<td>Range</td>
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<td>0.18-1.2 $\times 10^{4}$</td>
<td>0-0.46</td>
<td>30 $\times 10^{-3}$-5.9</td>
<td>0-23</td>
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<td>Twentekanaal</td>
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<td>Median</td>
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<td>Mean</td>
<td>964</td>
<td>61</td>
<td>$6.8 \times 10^{-3}$</td>
<td>0.16</td>
<td>0.75</td>
<td>2.2</td>
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<td>Range</td>
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<td>0.41-227</td>
<td>$0.42 \times 10^{-2}$</td>
<td>0-1.1</td>
<td>0-2.7</td>
<td>0-4.2</td>
<td>0-4.2</td>
</tr>
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</table>

* a n, number of samples tested.
* b One value determined.
* c n = 4 for the norovirus and rotavirus analysis.
The numbers of norovirus and rotavirus RNA detected in 69 tested samples ranged from 0 to 171 PCR-detectable units (PDU)/liter and from 0 to 2.2 × 10^3 PDU/liter, respectively. The mean norovirus and rotavirus concentrations found per location are shown in Table 1 and in Fig. 1B. The mean norovirus concentrations varied between 0 and 26 PDU/liter, and the mean rotavirus concentrations varied between 0.88 and 375 PDU/liter (Table 1).

An internal control (IC) RNA, for both norovirus and rotavirus, was included in the RT step to monitor for inhibition of the RT-PCR. No IC RNA was detected when undiluted RNA was analyzed in 61 of the 69 (88%) samples analyzed by norovirus RT-PCR, whereas in 28 of the 69 (41%) 10-times-diluted RNA samples, no IC RNA was detected. In the rotavirus RT-PCR, no IC RNA was detected in 65 (94%) and 48 (70%) of 69 samples when undiluted and 10-times-diluted samples were tested, respectively. In 21 (30%) of 69 samples for norovirus and 30 (43%) of 69 samples for rotavirus, where no IC and no virus-specific RNA could be detected, norovirus and rotavirus RNA could be detected in the 100- and 1,000-times-diluted RNA samples and therefore still produced a positive result. Nevertheless, in 34 (49%) samples, it remained unclear whether the samples were truly negative for the presence of either norovirus or rotavirus RNA or whether the samples were false negative due to inhibitory factors.

In the samples originating from nine of the 10 locations, at least one norovirus RNA could be detected, but in the samples taken at one location (Andelse Maas), no norovirus RNA could be detected (four samples tested). However, in all of the four samples taken at this location, rotavirus RNA was detected (15 to 182 PDU/liter). Overall, no correlation could be seen between the numbers of norovirus-positive samples and the rotavirus-positive samples. Phages could be detected in most samples, and overall a very strong correlation (P < 0.00005) between the concentrations of the somatic coliphages and the F-specific phages detected in these samples was found. Besides a correlation between the presence of these two phages and the enteroviruses (P < 0.0005), no other significant correlation between the tested viruses was seen.

Although samples from the different locations were taken over a period of 4 years and also taken in different months, a difference could be observed in virus concentrations between locations (Table 1 and Fig. 1). Virus concentrations found in the samples taken from the Lateraalkanaal Heel and the Beatrickkanaal were overall higher than those found in the samples taken at the other locations, with the lowest concentrations found in the Drentsche Aa. One of the five Drentsche Aa samples was taken after a heavy rainfall event that caused a sewage overflow at an upstream sewage treatment plant. In this sample, but not in the other four Drentsche Aa samples, norovirus RNA was detected.

Physical parameters. Because the characteristics of the surface water samples could influence the inactivation of the different viruses, the pH, temperature, and turbidity of the water samples were also measured for 93%, 77%, and 50% of the samples, respectively. Comparison of the available physical parameters with the virus concentrations in these samples showed a moderate correlation (P < 0.005) between the pH of the water and the norovirus concentrations (higher norovirus concentrations were found at higher pHs), but no other significant correlations were found.

**DISCUSSION**

Surface water used as source water for drinking water production may contain high concentrations of human pathogenic viruses, possibly affecting the drinking water quality. The human pathogenic noroviruses and rotaviruses were detected in 45% and 48% of the samples, respectively, by performing different concentration and detection methods, i.e., molecular methods, on the water samples. Infectious enteroviruses and reoviruses, which may impact public health, could be detected in approximately 80% of the tested samples. Source water quality is best identified by analysis of pathogenic viruses, but it has been suggested that phages can also be used (24). We found somatic coliphages and F-specific phages in 100% and 97% of the samples, respectively, which is not indicative of the presence of human pathogenic viruses. Moreover, in the two samples where no F-specific phages could be detected, enteroviruses were present, and in one sample rotavirus and norovirus RNA was also detected. A significant correlation was observed between phages and enteroviruses but not between phages and any of the other viruses and not between the other viruses. These results do not support a role for phages as indicators of source water quality. However, bacteriophages may be useful for determining the recovery of the applied methods and for treatment efficiencies (40).

The water quality, with respect to the virus contamination of the tested organisms, differed between the 10 source water locations (Table 1 and Fig. 1). For instance, overall, the concentrations of the viruses were low in samples taken from the Drentsche Aa, whereas the concentrations were generally higher in samples taken from the Lateraalkanaal Heel and the Beatrickkanaal. At various locations, samples were taken over several years, but regardless of the sampling year, the average virus concentrations found at a specific location in the same season were similar. Nevertheless, as can be seen in Table 1, up to a 5 log_{10} difference throughout the sampling period could be observed, suggesting that peak values regularly occur. At a particular location, e.g., Beatrickkanaal, large fluctuations in virus concentrations could be observed from one sampling date to the next compared with other locations (data not shown). It is important that for each location where surface water is used as source water for the production of drinking water, the possible sources of viral contamination are identified as suggested in the WHO water safety plan (4). When the source of contamination at an intake point for drinking water production is identified, using either the water safety plan or molecular source tracking, intervention measures can then be targeted to reduce viral loads in the surface water. Alternatively, source water locations may be relocated if possible.

The detection of viruses by culture methods from the ultrafiltered concentrates has the advantage that only infectious viruses are detected, and because a large volume can be tested, it is a sensitive method as well. Nevertheless, it should be considered that the BGM cells used in the culture method in our study were not susceptible to infection by all enteroviruses (9), which may result in an underestimation of the numbers of infectious enteroviruses present in the tested water samples.
To detect the enteroviruses which cannot (or not efficiently) infect the BGM cells, and therefore were not detected in our assay, other cell lines or even molecular methods might be used.

Inhibition of the molecular detection assay in a large number of samples, as indicated by our internal control RNA in the rotavirus and norovirus RT-PCRs, suggested that further purification steps are required to remove inhibitors or, alternatively, bovine serum albumin and dimethyl sulfoxide could be added (5). In our study, a number of samples which were negative in the norovirus RT-PCR may be false negative, because in these negative samples, 88% of the undiluted and 41% of the 10-fold-diluted RNA samples showed RT-PCR inhibition. For rotavirus, these numbers were 94% and 70%, respectively. Although the RNA samples used for both the norovirus and rotavirus RT-PCRs originate from the same RNA extract, it seems that the rotavirus RT-PCR is more influenced by inhibitory factors than the norovirus RT-PCR. Rutjes et al. (35) described a method comparison to determine an optimized method for the detection of viruses in eluate and ultrafiltered concentrate obtained from large water volumes and had shown that the extraction of viruses from the ultrafiltered concentrate is a more suitable method. The resulting RNA samples were more purified from inhibitory factors and therefore were less prone to inhibition in the RT-PCR. In spite of the smaller volume that can be processed, the method has been shown to be more sensitive. Reduction of inhibition is important to avoid false negatives, and appropriate use of controls and standardization are essential (7). Sampling, concentration, and detection methods may largely influence virus concentrations in source waters for drinking water production and in that way influence the anticipated public health risk.

Although some samples might be false negative for the presence of viruses (or viral RNA), several samples tested positive for the presence of 2 or even 3 pathogenic viruses. Twenty-two of 69 (32%) samples tested positive for the presence of both norovirus and rotavirus RNA, 25 of 69 (36%) samples contained both norovirus RNA and infectious enteroviruses, and 27 of 69 (39%) samples contained both rotavirus RNA and infectious enteroviruses. In 19 of 69 (28%) samples, all three viruses were detected, which might suggest that people who are exposed to drinking water which is processed from these source waters have a greater risk of obtaining a viral infection when the treatment processes are less efficient or failing.

The high virus concentrations determined by RT-PCR (mean concentration, 10 PDU/liter) may be explained in part by the detection of virus RNA instead of infectious particles. Indeed, reoviruses and enteroviruses, which can be cultured, were present at much lower levels (mean concentration, 10⁻¹ PFU/liter). It is difficult to determine the concentrations of infectious rotaviruses and noroviruses, because these concentrations are dependent on different factors, like the water temperature and UV exposure. As shown in other studies (12, 37), the ratio of defective viruses to infectious virus particles changes due to various environmental characteristics and can vary from a factor of 20 to 10⁴. In volunteer studies, it has been shown that the infectious dose for both noroviruses and rotaviruses may be as low as 10 viral particles (41), and therefore even low concentrations of these infectious viruses present in the environment may cause a public health risk. These viruses cause gastroenteric disease, which is in general a mild illness, although for small children and elderly people, an infection with these viruses might be life threatening.

In the present study, surface waters were sampled throughout the year, with the most extensive screening period in the colder period of the year, ranging from November to April. Although less virus data were generated in the period from May to October, lower virus concentrations were found in this period than in the colder period of the year, suggesting that there is a seasonal influence. For noroviruses, this can be explained by the seasonality of noroviruses, which are more prevalent in the population in winter (31). For viruses in general, it has been shown that they survive better at lower temperatures (12) and with little UV exposure (11).

In a previous study (48), it has been shown that noroviruses occur in short-term fluctuations, and no correlation was found with the presence of enteroviruses and F-specific phages. When these peak concentrations occur, and when these viruses are infectious, there can be a higher exposure to these viruses. If, at that moment, the treatment is insufficient, viruses may be present in the drinking water at levels that cause an unacceptable risk to public health. The previous study, as well as the current study, shows the importance of monitoring for waterborne pathogenic viruses. Detection of viral indicators only would be less accurate in estimating the possible public health risk. Rapid molecular detection methods for pathogenic viruses are increasingly being developed and used for analyzing environmental samples (18–20, 28, 34, 37, 39, 49). Nevertheless, because of their abundant presence in surface waters and because their characteristics—size, charge, and inactivation rate—correspond with human pathogenic viruses, phages are still very useful organisms for determining the removal efficiency of different treatment processes, which can be used in a risk assessment to evaluate whether the treatment is sufficient. Tenius et al. (40) described the applicability of three models to characterize different treatment processes which can be used in quantitative microbial risk assessment. Although risk assessment, with data on virus concentrations in the source water and the treatment efficiency, is an important tool to estimate the possible risk of infection, there is no actual surveillance system in the Netherlands to determine the actual cases of infected individuals through transmission of these viruses via water. To further assess the risk of transmission of viruses via water, it is necessary to obtain more information about the identity and the viability of the viruses present in different water samples.

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