Waterborne norovirus outbreaks

Leena Maunula
University of Helsinki,
PO Box 66 (Agnes Sjöberginkatu 2),
Department of Food & Environmental Hygiene,
Faculty of Veterinary Medicine, 00014 Finland
Tel.: +358 919 157 143;
+358 408 384 007;
Fax: +358 919 157 101;
leena.maunula@helsinki.fi

Noroviruses (NoVs) are the most common nonbacterial causative agents of waterborne outbreaks. Due to the mild and short-lived disease of gastroenteritis, even large epidemics may go unnoticed, since patients do not necessarily visit a doctor. NoVs have several means by which to survive both in the environment and in a population. The nonenveloped small virus retains its infectivity in the environment, and particularly in cold water, for a long time. Unlike most enteric viruses, it causes disease both in children and adults. A large number of genotypes combined with a small infective dose and short-term immunity guarantee efficient circulation of these viruses. The world of NoVs has been revealed to us predominantly by molecular methods. Having learned to detect these viruses first in patients, the emphasis is now in searching for methods sensitive enough to find them in environmental samples. In this review, the latest methods and their use in monitoring of these viruses are discussed.

Noroviruses (NoVs) have likely caused waterborne outbreaks far beyond the beginning of 1970 when they among several new viruses were discovered. Then, electron microscope was applied to direct analysis of stool specimens for viral pathogens. The Norwalk agent was first visualized in 1972 [1].

In the 1980s, researchers described several waterborne outbreaks, and demonstrated the epidemiological evidence between water consumption or bathing in contaminated water and gastrointestinal disease. The causative agent was shown to be the Norwalk agent due to a fourfold rise in antibodies against the virus in sera of the patients. Methods for finding viruses in water were still missing. In some cases, an increased amount of coliforms were found, but sometimes no indicators for fecal contamination were present in water. The Norwalk agent turned out to be a very challenging research subject, since it did not grow in available cell cultures. Many features of its routes of infection and stability were discovered in early tests with volunteers [1].

Electron microscopy (EM) studies revealed other new viruses, such as the rota- and astroviruses, in human patient and animal samples. Particles of approximately 30 nm were at the limit of EM resolution, and the difficulty to interpret or correctly classify visualised particles was obvious, taking into account phases of the same size present in stool samples. Immuno-EM, which used the antibodies in convalescent patient sera, increased the specificity, but these NoV antibodies were unavailable on a broad scale. In those days, nobody would have suspected that these viruses would demonstrate such enormous variety, or that all but genogroup I type 1 viruses that the Norwalk agent presented escaped reaction with those antibodies. The structural classification of small round-structured viruses (SRSVs) by Appleton was taken as a general guide for electron microscopists [2]. As more findings coincided with diarrheal outbreaks and Norwalk agent findings in patient stools, the significance of these viruses as causative agents in outbreaks was established. However, some confusion was caused by the low detection limit of EM, and viruses were usually detected only in one or two in ten samples from ill patients.

In the 1990s, a new page was turned in NoV research. Molecular methods were applied to these viruses and the genome of Norwalk virus was sequenced. It was classified in the family Caliciviridae, together with feline and other animal caliciviruses [3]. The gene amplification method invented by K Mullis enabled sensitive diagnostics for the noncultivatable NoVs [4]. Norwalk-like viruses (NLVs), such as Mexico, Hawaii and Snow-Mountain agents, which were morphologically similar but serologically distinct from Norwalk, could be characterised in detail. For the first time, this method could be used to detect viruses in environmental samples containing virus amounts below the detection limit of EM. Antigen and antibody tests for NLVs were also developed and improved. It was becoming very clear that the reporting and surveillance of outbreaks caused by NoVs had been severely underestimated.
Detailed reviews have been written about NoVs and viruses and/or pathogens causing waterborne outbreaks [5–8]. The emphasis of this review is to describe waterborne outbreaks caused by NoVs, and to review the information acquired during the past decade. In that time period, the number of publications and reports has multiplied. The application of real-time and quantitative gene amplification techniques and surveys in which viruses have been detected in water will be discussed.

**Noroviruses**

**Classification of noroviruses**

NoVs form one of the four genera in the Caliciviridae family in addition to three other genera: Sapovirus, Vesivirus and Lagovirus (Figure 1) [3]. Viruses in the Vesivirus and Lagovirus genera are animal pathogens, whereas Norovirus and Sapovirus genera include human viruses. Caliciviruses are unenveloped, icosahedral viruses that are 30–38 nm in diameter with a linear, positive-sense single-stranded (ss)RNA genome approximately 7.4–7.7-kb long. It has a polyadenylated tail at the 3′-end and a viral protein-linked genome (VPg) at the 5′-end.

NoVs have three open reading frame (ORF) regions: ORF1 codes for nonstructural proteins; ORF2 codes for capsid (VP1); and ORF3 codes for structural basic proteins (VP2) [9]. ORF1 contains a polymerase-coding region that has been widely used for gene amplification in noroviral diagnostics. In many aspects the genome structure resembles that of picornaviruses, with helicase, VPg, polymerase and proteinase coding regions.

The capsid protein has a molecular weight of approximately 58 kDa. The capsid protein consists of a protruding (P) domain and a shell (S) domain, which are connected by a flexible hinge [10]. The S domain is conserved and forms an icosahedral scaffold. The strain specificity and cell-binding determinants are located at the exterior of the capsid in the P2 subdomain of P, and have a large sequence variation. The P1 subdomain provides fine-tuning to position the P2 subdomain [11].

NoVs attach to specific ABH histo-blood group antigens [12]. Individuals with an O phenotype were more likely to be infected with Nov, whereas persons with a B histo-blood group antigen had a decreased risk of infection and symptomatic disease [13]. Studies have demonstrated that secretor status is the most predictive of risk for NoV infection [14]. In particular, H-type antigens served as ligands on the gastroduodenal epithelial cells of secretor individuals by recombinant NoV virus-like particles (VLPs) [15].

For reasons mentioned previously, NoV classification has been based more on similarity in nucleotide sequence identities among viruses, and to a lesser extent on the investigation of antigenic and antibody reactions. Viruses in Nov genera are grouped into five genogroups, which are further divided into genotypes [16]. Viruses of genogroups I and especially II are the most common human pathogens among NoVs, although some GII NoVs have also been detected in swine. Genogroup IV NoVs also cause disease in humans, but they are detected much more rarely than GI and II NoVs. Other animal NoVs found to date diverge from human NoVs; bovine viruses form genogroup III, and murine viruses form group V. Nucleotide sequence identities between genogroups usually comprise 70% or less.

Genotypes are deduced from the nucleotide sequence of the capsid gene, although genotyping by polymerase gene is possible. The nucleotide sequence identity is usually 90% or more, depending on the length of the gene area compared. Each genotype has a reference strain, such as GI.1 Norwalk, GII.1 Hawaii and so on [3]. Genogroups I and II can be divided into 14 and 17 genotypes [17]. In 2005, Zheng and coworkers proposed 8, 17, 2, 1 and 1 genotypes for genogroups I–V, respectively [16]. The number of genotypes, especially those of animal NoVs, is likely to increase [18].
Virus characteristics & inactivation
Most enteric viruses survive surprisingly well in the environment. The most important source of these viruses in the environment is sewage of human origin. The survival of infectious NoVs in cold water has been difficult to study, but it has been reported that many enteroviruses stay infective for a long time in cold water [19]. They can survive under ice cover and in frozen conditions.

Feline calicivirus (FCV) has been used as a surrogate for the study of inactivation of NoVs. FCV has been reported to survive for 28 days in tap water at 4°C [20]. In groundwater, viruses (hepatitis A virus, poliovirus coxsackievirus and echovirus) showed greater inactivation at greater temperatures, but largely at temperatures greater than 20°C [21]. The geometric mean inactivation rate for hepatitis A virus was 0.02–0.04 log10/day. The survival time of viruses also depends on the microbiological flora of natural water [19,21]. Solar ultraviolet (UV) light, which has been reported to inactivate viruses in alpine lakes [22], may decrease the number of NoVs present in the environment in Summer.

In a study with volunteers, as much as 10 mg/l of chlorine (3.75 mg was insufficient) in drinking water was needed to inactivate the Norwalk agent within a treatment time of 30 min [23]. Chlorine dioxide inactivated FCV at a concentration of 20–30 mg/l at pH 6 [24]. In wastewater, a chlorine concentration of 30 mg/l easily inactivated FCV by greater than 4 log10 within 5 min [25].

Ozone is efficient against NoVs: very low ozone residuals of less than 0.01 mg/l inactivated FCV under the studied conditions [26]. In drinking water, the Norwalk virus was inactivated (reductions >3 log10 within 10 s) by 0.37 mg ozone/l at pH 7.5 in a time period of 5 min [27].

In secondary wastewater, FCV was more resistant to UV irradiation than Escherichia coli. For 4-log10 reduction of FCV, a dose of 19 mw cm-2 was required; however, this dose was less than that needed to inactivate poliovirus or MS2-phage [25]. FCVs and NoVs have some differences in their inactivation, and thus NoVs appear to be more resistant than FCV [28].

Disease & transmission
NoVs infect people of all age groups, and is transmitted by the fecal–oral route [3]. They spread easily from person to person by touch or via air droplets, and so on, but can also be transmitted via food, the environment and water (a topic of this review). The disease caused by NoVs can be regarded as mild, and medical treatment is rarely needed. The disease is self-limiting, and the main symptoms include nausea, vomiting and diarrhea, often accompanied by abdominal cramps, headache, fever, chills, myalgias and sore throat. The incubation period is short (10–51 h), the disease usually lasts 24–48 h or sometimes several days, after which excretion of viruses continues for 1–2 weeks. Evidence from presymptomatic fecal excretion and post-recovery transmission causing foodborne NoV outbreak has been reported. It appears that only short-term immunity is raised after NoV infection. In addition, heterologous immunity may be developed only to some extent and not at all between genogroup I and II viruses.

The most common transmission route for NoV infection is person to person. Together with Campylobacter, NoVs are the most common causative agents in waterborne outbreaks in many countries. In New Zealand, NoV has been mentioned as a major cause for waterborne nonbacterial infections [29]. In waterborne outbreaks, it is always the raw material (water) that is contaminated, whereas in foodborne outbreaks the food may also be contaminated during preparation or while serving. In all NoV outbreaks, secondary infections are common. Virus transmission can also occur via different kinds of surfaces. Although waterborne outbreaks are not very common, in these events, many people, even hundreds, may become infected.

Disease caused by NoVs was previously called ‘Winter vomiting disease’ [3]. The name describes the seasonal nature of the outbreaks, since the epidemic peak occurs in Winter–Spring months in the northern hemisphere countries. Some epidemic seasons are much stronger than others depending on the circulating viruses and perhaps to some extent on the immune status of the population. In some years, an emerging or new variant may start the season earlier than usual, as the new GII.4-2002 variant did in many European countries [30]. A similar seasonal pattern occurs even more clearly with rotaviruses, which mainly infect children. It appears that all NoV genotypes can be met globally, but differences in their prevalence can be found in different geographical areas.

Evolution mechanisms: mutations & recombination
We have been able to follow and analyze the evolution of NoVs only for a short period of time, with the oldest samples originating from the 1970s. As with many other RNA viruses, NoVs have a lot of genetic variation, which is partly due to the characteristics of the RNA polymerase
that lacks proofreading ability. Recombination also occurs, as evidenced by several discrepancies of genotype determined by polymerase or capsid regions [31,32]. Recombination in the junction of Pol and Cap occur both in human and animal NoVs [33,34].

The sequence diversity within a genotype is considerable. Some genotypes clearly have several subtypes. The GII.4 genotype has many subtypes and appears to evolve all the time. It is by far the most common NoV and appears to be an endemic strain globally [35,36].

In recent years, the most common recombinant has perhaps been the GIIb strain. The polymerase type GIIb emerged in 2000 in Europe, and it recombines quite frequently with several capsid types, most commonly with GII.3 [37,38].

Methods used to detect noroviruses in water

Virus concentration

The virus content of contaminated water in drinking or natural waters is so low that the concentration of viruses by volume reduction is necessary in most cases. Detection methods have changed from previous cell cultures of enteroviruses to molecular methods of enteric viruses, which has affected concentration. For gene amplification, filtration of large water volumes may be unsuitable due to increased levels of inhibitors for DNA polymerase or RT enzyme.

A thorough review has been written about different concentration methods for viruses [5]. Here are described some recently published methods or improvements that have successfully been applied to find NoVs in water in outbreak situations. Traditionally, viruses have been concentrated by water filtration (adsorption elution), flocculation methods, polyethylene glycol (PEG) precipitation, ultra-centrifugation, freeze-drying, chromatography and ultrafiltration.

It has been known for a long time that viruses attach well to charged surfaces. Viral capsid proteins have a slightly negative net charge, although the charge in the outer surface area of virus particles changes regionally, which may explain why positively and negatively charged membranes can be used for concentration. Viral elution from the membrane is often relatively inefficient, and results in poor recovery of the virus.

Table 1 lists several methods designed for detecting NoVs in water. NoVs have been found in outbreak situations with these methods. Schwab and colleagues applied the adsorption-elution method together with RT-PCR [39]. They seeded 1 l of beef extract glycine eluate with enteric RNA viruses (Norwalk, poliovirus and hepatitis A virus) and purified it with several methods, such as PEG precipitation and ultrafiltration, in order to obtain positive results with RT-PCR. In comparison to the cell culture method, as little as three plaque-forming units (PFUs) of poliovirus 1 in 1 l of eluate could be detected with RT-PCR. Gilgen and colleagues used filter adsorption and ultrafiltration in 1-l water samples [40]. Virus capture by immunomagnetic beads has also been reported [41].

<table>
<thead>
<tr>
<th>Method</th>
<th>Concentration 1</th>
<th>Concentration 2</th>
<th>Further concentration</th>
<th>Examples of this method</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sobsey et al. (1979)</td>
<td>Electropositive filter</td>
<td>0.5 M lysine plus PEG precipitation</td>
<td>[71,74]</td>
<td>[117]</td>
<td></td>
</tr>
<tr>
<td>Gilgen et al. (1997)</td>
<td>Electropositive filter</td>
<td>Glycine plus beef extract, pH 9.5</td>
<td>Microconcentration</td>
<td>[81,87,90,93]</td>
<td>[40]</td>
</tr>
<tr>
<td>Fout et al. (2001)</td>
<td>Electropositive filter</td>
<td>1.5% beef extract</td>
<td>Celite precipitation</td>
<td>[69,85,96]</td>
<td></td>
</tr>
<tr>
<td>Dahling et al. (1986)</td>
<td>Electropositive cartridge (1 MDS)</td>
<td>0.05 M Tris, pH 9.3, 3% beef extract</td>
<td>Filtration, ultra-centrifugation, microconcentration</td>
<td>[118]</td>
<td></td>
</tr>
<tr>
<td>Lodder et al. (1999)</td>
<td>Fiber glass-epoxy cartridge</td>
<td>0.05 M Tris, pH 9.3, 3% beef extract</td>
<td>Organic flocculation</td>
<td>[80]</td>
<td>[120]</td>
</tr>
<tr>
<td>Katayama et al. (2002)</td>
<td>Electronegative filter</td>
<td>1mM NaOH, pH 10.5</td>
<td>Microconcentration</td>
<td></td>
<td>[42]</td>
</tr>
<tr>
<td>Wyn-Jones et al. (2000)</td>
<td>Electronegative cellulose nitrate</td>
<td>0.1 M Tris-glycine, 3% beef extract, pH 9.5</td>
<td>Organic flocculation</td>
<td>[54]</td>
<td>[121]</td>
</tr>
</tbody>
</table>

MDS: Myelodysplastic syndromes; PEG: Polyethylene glycol.
Katayama and colleagues have used a negatively charged filter to obtain recoveries of 38–89% for seawater, with cations eluted with 0.5 mM H$_2$SO$_4$ prior to viral elution with 1 mM NaOH [42]. Cation-coated filter method has been applied to NoV analysis in large volumes of freshwater [43], and for enteric virus analyses in surface water with a recovery of 56 ± 32% for the latter [44].

**Molecular detection methods**

Molecular methods such as RT-PCR were first developed for research and diagnostic purposes with NoVs in patient samples, but the detection stage could also be directly applied to environmental samples. The first RT-PCR detection methods were described a couple of years after Xi and colleagues had cloned the Norwalk virus genome and performed preliminary sequence analysis [45]. Gene amplification was 100-fold more sensitive than dot-blot hybridization [46].

For diagnostics, primers from a region in an RNA-dependent RNA polymerase region were frequently used, often in nucleotide positions between 4500 and 5000 [47,48]. They were designed to obtain genogroup I or II viruses. In spite of this, it was often necessary to use degenerative oligonucleotides as primers [49]. To confirm the specificity of the amplicons in hybridization, a panel of probes had to be used for recognition of all genotype strains, which made it laborious [50,51]. New NoV sequences were published regularly, and experienced specialists were needed to keep the tests updated. Conventional gene amplification is still a valuable tool in genotyping and sequence analysis of NoVs.

The development of real-time gene amplification made the detection of NoVs much faster and easier to perform. At that time, it had become evident that the polymerase gene area of NoVs was not very conserved, not even the primer sequences. In the junction of Pol and Cap, a long, more conserved nucleotide sequence region was available [52]. Using this area, it was possible to develop a RT-PCR with a specific probe. Some challenges still exist concerning the quantitativity of the tests.

RT gene amplification can be performed using different chemistries. Several applications of SYBR® Green I real-time RT-PCR, and melting curve analyses for NoVs were published in 2004 [53–57]. However, in recent years, the most popular technique of choice has been the probe NoV test using TaqMan® chemistry [52]. The following papers have been improvements for that test [44,58–61]. An optimised RT test is highly sensitive.

Environmental samples may contain inhibitory factors for PCR reactions. Purification of the nucleic acid from these inhibitors is of crucial importance. The method of Boom and colleagues and its commercial modifications are widely used [62]. Still, unless an internal control is added, gene amplification tests may give false-negative reactions.

Concerning environmental samples, enteroviruses were among the first viruses for which new molecular techniques were applied, since it was possible to compare results obtained by a cell culture method to those of a gene amplification method [39]. For NoVs, the only available detection method was found to be useful for environmental samples as well. Due to the low number of viruses in these samples, the sensitivity of the test is highly important, and much effort has been focused on the design of sensitive primers and on the optimization of the gene amplification reaction. To date, the exact quantitation of the amount has been considered less important, and when ct-values often range from 35 to 40, the error in quantity is likely to be high.

**Other detection methods**

When the causative agent is not obvious in outbreak situations, robust, economic and high-throughput test systems would be desirable for rapid screening. Assays based on antigen–antibody reactions, such as enzyme immunoassay (EIA), were difficult to develop for NoVs due to their immunological variability. The first broadly-reacting antigen EIA assays could be developed only after a panel of NoV VLPs of different genotypes had been prepared, [63–65]. However, for environmental samples, the sensitivity of EIA tests is generally too low.

**DNA microarray**

DNA microarray offers a nanoscale molecular method. The advantages of this method are high throughput and small format, which enables rapid testing. It suits genotyping well, since it is easy to include tens or hundreds of sequences in spots on one glass slide. Also, the detection of a panel of different pathogens is convenient.

In the future, DNA microarray-based techniques may provide an opportunity for the faster, larger scale analysis of viruses, especially if no preceding PCR is required or if it could be
performed on the slide. Several DNA microarray applications for NoVs have already been described [66,67].

Waterborne noroviral outbreaks
NoVs have a worldwide distribution, and thus waterborne outbreaks occur everywhere as well. They are less reported in developing countries, since the disease is mild and outbreaks caused by pathogens with more serious symptoms are surveyed.

The likelihood of waterborne outbreaks to occur depends on the water source and purification system. The survey and detection of NoVs in environmental samples is not presently systematic in any country, and, for this reason, data on viral contamination are lacking. In the literature, over 30 descriptions in English of waterborne NoV outbreaks can be found from 1977 to 2006 (Table 2) [68–99], in addition to larger surveillance reports [100,101]. The 32 case reports that describe outbreaks from 1978 to 2005 mainly from North America (ten outbreaks) or Europe (eight outbreaks), including several from the Scandinavian countries (seven outbreaks), reveal some features of NoV waterborne outbreaks. The annual and monthly distribution of the outbreaks in this material was approximately equal.

Waterborne NoV outbreaks were reported as community epidemics in seven cases, often in a small town with a few thousand inhabitants. Publications also described epidemics linked to tourist resorts (tourist saloon, ski resort, resort hotel and snowmobile lodge) [69,74,77,96]. Waterborne NoV outbreaks were linked to many kinds of recreational events (recreational park, caravan park, pool, canoeing and camp) [70,79,92,93] or travelling (hotels, bus trip and cruise ship) [71,102]. People can become infected by NoV when drinking or swimming in contaminated water; the latter transmission route was often associated with children. Some more widespread, even multistate epidemics, have been traced in which factories have unwittingly contaminated their products, such as custards or consumed ice [76]. Thus, contaminated water may be found behind many foodborne NoV outbreaks.

As Table 2 illustrates, the leakage of sewage containing NoVs is often the factor that triggers the start of a waterborne outbreak. The maintenance of septic tanks and sewage lines are often forgotten until an accident has occurred. NoVs may spread across long distances in suitable geological conditions. An example is an outbreak in the USA where ‘effluent from the resort’s sewage treatment facility seeped through fractures in the subsurface rock (with little filtration) directly into the resort’s deep well’ [89]. Surface water contamination may be connected to heavy rainfalls with the transient occurrence of flooding, and surface water contaminates the drinking water system. One common reason appears to be a failure of chlorination, which may be specifically due to a blocked chlorine feed tube or inadequate chlorination due to taste problems. Groundwater is particularly vulnerable during incidents of contamination, since usually no chlorination is used at all. All types of deficiencies in the water system may cause the outbreak, including pump failures or breaks in pipes.

The likelihood of waterborne noroviral outbreaks increases towards the end of the NoV epidemic season. Out-of-season outbreaks have occurred due to the long survival of the viruses in the environment and particularly in groundwater. Weather conditions may sometimes render the purification in water or sewage treatment plants less than optimal. Heavy rains can cause overflows in sewage treatment plants, which may enable contaminated water to flow into rivers [103,104]. In countries with harsh Winter conditions, the water distribution network undergoes stress due to the freezing and thawing of the ground.

<p>| Table 2. Most common factors that have caused norovirus waterborne outbreaks. |</p>
<table>
<thead>
<tr>
<th>Factor</th>
<th>No. of publications (n = 32)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sewage leak</td>
<td>9/32</td>
</tr>
<tr>
<td>Surface water contamination</td>
<td>4/32</td>
</tr>
<tr>
<td>Chlorination failure</td>
<td>4/32</td>
</tr>
<tr>
<td>Deficiencies/breakdown in the water system</td>
<td>4/32</td>
</tr>
<tr>
<td>Other (e.g., transient contamination, break in pipe or nonchlorination)</td>
<td>3/32</td>
</tr>
<tr>
<td>Unknown</td>
<td>8/32</td>
</tr>
</tbody>
</table>

Based on [68–99].

Molecular epidemiology, genotypes
The first reported detection of NoVs in water samples linked to a gastroenteritis outbreak was published in 1997 (Table 3) [71], and the method was sensitive enough to be applied to water analysis. Today, many countries have at least one laboratory that is capable of performing the viral analysis of water samples. Drinking water is one of the easiest environmental matrices for viral analysis.

Due to high genetic variation of NoVs, it is possible to indicate the likelihood of waterborne transmission of viruses in most cases by
comparing the nucleotide sequences of the viruses found in water and in samples of patients. Tables 3 & 4 describe reports in which NoVs and genotypes could be traced both in patient stool and water samples. In large outbreaks or in cases of strong sewage contamination, several NoVs were often found in water [85,87,90,96].

NoV genotype viruses found in water could be assumed to reflect the NoV types that have circulated in community during the NoV epidemic season. However, there appears to be a difference in the ratio of genogroup I and II viruses found in community versus waterborne outbreaks [101,105]. As Table 4 illustrates, NoV genogroup I viruses were relatively commonly detected in waterborne outbreaks (nine of 20 outbreaks), although they cause a minor portion of all NoV epidemics in community. In Finland, GII NoVs caused 86.9% of NoV outbreaks in 1998–2002 [105]. It can be speculated whether certain NoV genotype viruses might be more stable or resistant to chlorine in the environment than other types. Globally, the most common GII.4 virus was found in seven out of 20 waterborne outbreaks of Table 4 [85,87,90,101]. The factors that make GII.4 virus superior in person-to-person transmission might not favor its survival in water.

The genotype GI.3 virus, which has been one of the most common GI virus, was commonly detected in waterborne outbreaks [29,105,106]. Accidental events may play a big role in the occurrence of outbreaks, since even one ill person may excrete a high number of viruses.

### Conclusions

The significance of viruses in water- and food-borne infections has not been understood due to the lack of methods to detect the mostly very low amounts of viruses in the environment. A further reason might be that the presence of viruses only became evident when the role of the pathogenic enteric bacteria, such as cholera in water, decreased. An enteric virus that affected citizens in the modern environment and higher hygienic conditions was poliovirus, the eradication of which has been a success to date.

Like poliovirus and other enteric viruses, human NoVs circulate between humans and sewage of human origin via different transmission routes; one of which is water. The vast genetic variety of NoVs indicates a long co-existence in mankind [107]. The genetic variation is a result of both accumulating mutations, especially at antigenically important sites, and recombination. Sewage usually contains several NoV strains of both human and animal origin. There is an increased likelihood that different strains may infect one cell and lead to recombination. The zoonotic aspect must also be taken into consideration. To date, the detected animal NoVs diverge genetically from human ones. More research is needed concerning this aspect.

In conclusion, the breakthrough of molecular and genetic methods has directed NoV research. Despite increased research activity, our knowledge of NoVs is still defective, partly due to lack of interest and resources compared with the other RNA viruses frequently making headlines.

### Table 3. Recent outbreaks in which noroviruses have been detected in water and patients.

<table>
<thead>
<tr>
<th>Study</th>
<th>Country</th>
<th>Month/year</th>
<th>Place</th>
<th>Link</th>
<th>Relative risk*</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beller et al. (1997)</td>
<td>Canada/USA</td>
<td>June 1995</td>
<td>Restaurant</td>
<td>Drinking well water</td>
<td>5.3 (2.3–12.6)</td>
<td>[71]</td>
</tr>
<tr>
<td>Schwoerer et al. (1999)</td>
<td>France</td>
<td>January 1999</td>
<td>Re-education ward</td>
<td>Tap water</td>
<td></td>
<td>[98]</td>
</tr>
<tr>
<td>Kukkula et al. (1999)</td>
<td>Finland</td>
<td>March 1998</td>
<td>Community</td>
<td>Municipal water</td>
<td>3.5 (3.11–3.96)</td>
<td>[87]</td>
</tr>
<tr>
<td>Brown et al. (2001)</td>
<td>Bermuda</td>
<td>February 1998</td>
<td>Hotel</td>
<td>Eating or drinking at the hotel</td>
<td></td>
<td>[74]</td>
</tr>
<tr>
<td>Parshionikar et al. (2003)</td>
<td>USA</td>
<td>October 2001</td>
<td>Tourist saloon</td>
<td>Drinking water and/or consumption of ice</td>
<td></td>
<td>[96]</td>
</tr>
<tr>
<td>Anderson et al. (2003)</td>
<td>USA</td>
<td>February 2001</td>
<td>Snowmobile lodge</td>
<td>Water consumption</td>
<td>3.3 (1.4–7.7)</td>
<td>[69]</td>
</tr>
<tr>
<td>Hoebe et al. (2004)</td>
<td>The Netherlands</td>
<td>June 2002</td>
<td>Recreational water fountain</td>
<td>Playing in the fountain</td>
<td>10.4 (1.5–70.8)</td>
<td>[80]</td>
</tr>
<tr>
<td>Maunula et al. (2004)</td>
<td>Finland</td>
<td>July 2001</td>
<td>Wading pool</td>
<td>Swimming in the pool</td>
<td></td>
<td>[90]</td>
</tr>
<tr>
<td>Kim et al. (2005)</td>
<td>Korea</td>
<td>May 2004</td>
<td>Hotel</td>
<td>Water consumption</td>
<td></td>
<td>[85]</td>
</tr>
</tbody>
</table>

*Confidence interval: 95%.
**Future perspective**

The surveillance of waterborne pathogens is a complex and difficult task. In most countries, the microbiological quality of (drinking) water is controlled by sampling the water for coliforms or *E. coli*. Viruses are still considered too difficult to handle and, therefore, remain excluded from actual legal texts. For the time being, viruses are only indirectly acknowledged in legislation, which states that drinking water should cause no excess disease among consumers. In the UK, mandatory monitoring for *Cryptosporidium* was introduced in 2003, since it was considered the main contamination risk. The effect of this monitoring on predicting of viral contamination risks remains to be seen. An optimal indicator system of water should cover bacteria, viruses and parasites.

Interest in monitoring viruses in environmental samples has increased. Some of the interest originates from outbreaks of quite high impact on societies. In particular, viruses such as avian influenza, which can potentially be mediated by contaminated water, have alerted the authorities. Recent terrorist actions and the ease with which drinking water could be used as vehicle to create serious outbreaks have also drawn the attention of the authorities.

Notwithstanding the aforementioned risks, in industrialized countries, the most obvious threat associated with viral contamination concerns common enteric viruses, preferentially NoVs. As an anecdote, the aim at ultimately sterile water may have a drawback, since the increase in allergies and asthma in developed countries has been attributed to the lack of microbial challenges in the early years of life. However, general agreement prevails on the prevention of large waterborne viral outbreaks.

During the coming years, NoVs will be characterised more thoroughly and the circulating strains will be identified. This will reveal the extent to which antigenic drift and/or antigenic shifts (recombinations) occur. For several years, the total number of NoV genotypes has increased annually. In recent years, it appears that only a limited number of the strains, such as GII.4, GII.b and GII.7, cause the majority of outbreaks, and other genotypes are found more rarely. This might offer a chance for future immunization efforts.

Methods such as microarray technology may offer more standardized tools to control waterborne pathogens of concern (108,109). This will lower the cost of testing and render possible simultaneous bacterial, viral and parasitological monitoring. The significance of viruses in biofilms will be characterized in more detail. Improvements in disinfection of water, such as LED-UV lamps, will lower the costs of water purification, which may benefit especially the developing countries.

<p>| <strong>Table 4. Noroviruses detected in water and patients in waterborne outbreaks.</strong> |</p>
<table>
<thead>
<tr>
<th>Study</th>
<th>Viruses in patients</th>
<th>Viruses in water</th>
<th>Examples of primers used in RT-PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beller et al. (1997)</td>
<td>NV GII.4–1996</td>
<td>NV GII.4</td>
<td>[110] and MDSR33N, MDSR46N</td>
</tr>
<tr>
<td>Schvoerer et al. (1999)</td>
<td>NV GII, 85% SMA*</td>
<td>NV GII, 85% SMA</td>
<td>[98]</td>
</tr>
<tr>
<td>Kukkula et al. (1999)</td>
<td>NV GII.4, GII.6</td>
<td>NV GII.4</td>
<td>[49,111]</td>
</tr>
<tr>
<td>Häfliger et al. (2000)</td>
<td>NV GII.2, GII.2</td>
<td>NV GII.2</td>
<td>[112]</td>
</tr>
<tr>
<td>Brown et al. (2001)</td>
<td>NV GII.2</td>
<td>NV GII.2</td>
<td>[110]</td>
</tr>
<tr>
<td>Parshionikar et al. (2003)</td>
<td>NV GII.3, GII.6</td>
<td>NV GII.3</td>
<td>[106,113]</td>
</tr>
<tr>
<td>Nygård et al. (2003)</td>
<td>NV GIIb</td>
<td>NV GIIb</td>
<td>[114] and n12/n13</td>
</tr>
<tr>
<td>Anderson et al. (2003)</td>
<td>NV GII</td>
<td>GII</td>
<td>[106]</td>
</tr>
<tr>
<td>Hoebe et al. (2004)</td>
<td>NV GII</td>
<td>NV GII</td>
<td>[49,115] and JV12BH</td>
</tr>
<tr>
<td>Maunula et al. (2004)</td>
<td>NV GII</td>
<td>NV GII</td>
<td>[90]</td>
</tr>
<tr>
<td>Kim et al. (2005)</td>
<td>NV GII, 4, 1, GII.4, 5</td>
<td>NV GII, 4, 1, GII.4, 5</td>
<td>[116]</td>
</tr>
<tr>
<td>Maunula et al. (2005)</td>
<td>Three cases of NV GII</td>
<td>NV GII</td>
<td>[101]</td>
</tr>
<tr>
<td>(nine outbreaks)</td>
<td>Two cases of NV GII</td>
<td>NV GII</td>
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<td></td>
<td>Three cases of NV GII</td>
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<td></td>
<td>Two cases of NV GII</td>
<td>NV GII</td>
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</tbody>
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*Could be GII.1 (85% identity with SMA-strain).*  
SMA: Snow Mountain agent
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Executive summary
• Noroviruses (NoVs) are the most common cause of viral food- and waterborne outbreaks in the developed countries.
• NoVs transmit by fecal–oral route from person-to-person (or by fomites), but they also may circulate from sewage via water, food (and environment) back to humans.
• Sensitive molecular methods for detection of NoVs in water and environment have been developed.
• Monitoring of NoVs in water might prevent waterborne noroviral outbreaks.

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Waterborne norovirus outbreaks – REVIEW


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Affiliation
Leena Maunula
University of Helsinki, PO Box 66 (Agnes Sjöberginkatu 2), Department of Food & Environmental Hygiene, Faculty of Veterinary Medicine, 00014 Finland
Tel.: +358 919 157 143;
+358 408 384 007;
Fax: +358 919 157 101;
leena.maunula@helsinki.fi