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Detection of small round structured viruses in artificially contaminated water using filter adsorption and reverse transcription polymerase chain reaction

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Abstract

The small round structured viruses (SRSV) are common causes of gastroenteritis worldwide. Fecally contaminated water is an important vehicle for transmission, but detection of SRSV in environmental samples has been hampered by the lack of sensitive detection methods. The present work describes the detection of SRSV in artificially contaminated deionized water and raw drinking water. SRSV-containing fecal extracts were added to water and virus was recovered by filter adsorption–elution, followed by flocculation. RNA was extracted and SRSV were detected by the use of reverse transcription polymerase chain reaction. The sensitivity of the method corresponded to a positive SRSV detection in 500 ml deionized water with an estimated concentration of 0.5–5 virus particles per ml.

Keywords: Norwalk-like viruses; SRSV; Gastroenteritis; Drinking water; Filtration

1. Introduction

The small round structured viruses (SRSV) are common etiologic agents of waterborne gastroenteritis. Outbreaks of SRSV-caused gastroenteritis have been associated with consumption of drinking water from private wells and community water systems, as well as the use of recreational waters (Kaplan et al., 1982a; Kappus et al., 1982; Lawson et al., 1991). Humans are the only natural hosts known for these viruses, thus water sources causing SRSV infections must have been contaminated with human feces. The SRSV belong to the Caliciviridae family and are denoted as human caliciviruses. The electron microscopy (EM) appearance and the symptoms of disease caused by different SRSV are similar. Four different serotypes have been described. Norwalk virus, Snow Mountain virus, Hawaii agent and

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Taunton agent are the prototypes of each serotype (Lewis, 1991; Lambden et al., 1993). The SRSV have been divided into two genogroups based on nucleotide sequence comparisons of the RNA polymerase gene. The prototype virus for SRSV, Norwalk virus, belongs to genogroup 1 (Ando et al., 1994, 1995).

Investigations of outbreaks of gastroenteritis with a suspected viral etiology are based on epidemiologic evaluation and virus detection in stool samples from patients. Drinking water is not tested for the presence of viruses on a routine basis, due to lack of sensitive methods. Virus detection in water could be of value in outbreak situations with unsatisfactory epidemiologic data. Sensitive detection methods for SRSV in environmental samples could also be important in surveys. Information about the level of SRSV contamination of the environment, combined with epidemiologic studies, would make it easier to asses the health significance of SRSV and to perform risk analyses.

It has not been possible to propagate SRSV in vitro. The mode of virus detection has been EM. However, the complete genomes of three different SRSV have been sequenced, enabling the use of reverse transcription polymerase chain reaction (RT-PCR) for detection of SRSV (Jiang et al., 1990, 1992; Lambden et al., 1993; Dingle et al., 1995). Other members of the Caliciviridae, for example feline calicivirus (FCV), are easily grown in cell cultures.

Several RT-PCR procedures are described for the detection of SRSV in feces and the use of RT-PCR in the detection of SRSV in environmental samples has recently been reported (Beller et al., 1997; Gilgen et al., 1997). In order to detect virus in drinking water, concentration of the virus is necessary. A rapid and sensitive filter based adsorption and elution method for the concentration of poliovirus and hepatitis E virus from environmental samples has been described (Jothikumar et al., 1992, 1993). In the present work a similar method for the detection of SRSV in artificially contaminated water, is evaluated. Concentration of virus was performed by filter adsorption–elution and flocculation. RT-PCR was used for virus detection. The culturable FCV was used as a model virus to estimate the virus recovery in the concentration step.

2. Materials and methods

2.1. Water samples

Deionized water was used for spiking with SRSV positive feces or FCV prior to filtration. To evaluate any interference from organic materials with detection of SRSV, samples from four drinking water reservoirs in Oslo and surrounding communities were also included. One additional sample originated from a bog pond that was heavily polluted with humic acids and not suitable as a drinking water source.

2.2. Model virus

FCV strain F9 was kindly provided by Dr. Jarret, Glasgow, UK. The virus was propagated in feline embryo lung cells with Earle's minimum salt solution complemented with 3% $NaHCO_3$, 1% L-glutamine, 0.5% penicillin/streptomycin, 10% fetal calf serum and 1% nonessential amino acids. After 2–3 days of incubation the FCV infected cells were freeze–thawed twice and the FCV containing medium was stored at $-70°C$.

2.3. Fecal extracts

The fecal samples originated from patients with gastroenteritis. Ten percent (v/v) fecal extracts were prepared in Hank's salt solution with 0.5% bovine serum albumin and antibiotics. The extracts were provided by the National Institute of Public Health, Oslo, Norway. The suspension was shaken for 15 min and cleared by centrifugation for 45 min at 1000 *g* (Kjeldsberg, 1986). The virus particles in the samples were classified as SRSV, classical human calicivirus, rotavirus or astrovirus according to EM morphology. The extracts were stored at $-70°C$. EM positive SRSV samples were further genogrouped in agreement with RT-PCR results (see below). Two EM and PCR positive SRSV samples (no. 2081 and no. 278, representing genogroup 1 and 2 respectively) were used in the filtration study. To estimate the amount of virus in the sample one EM negative, PCR positive SRSV extract (no. 2155, genogroup 1) was also included.

2.4. Filtration–elution and flocculation

The concentration of virus was performed as described by Jothikumar et al. (1993). The procedure is outlined in Fig. 1. $MgCl_2 \cdot 6\ H_2O$ was added to a final concentration of 1200 mg/l and the pH was adjusted. The influence of different pH values on virus recovery was evaluated on FCV and feces extract no. 2081. The pH of the water samples was adjusted to either 3.0, 4.0, 5.0 or 5.5 with 1 N HCl. The pH which gave the highest recovery was used in the subsequent study. The samples were filtered through 47 mm filters with a pore size of 0.45 μm (Millipore, Bedford, USA, AP20 047 00). Urea arginine phosphate buffer (UAPB) (pH 9) was used for virus elution. After 10 min of soaking, the eluates (10 ml) were run through the filter. Flocculation was achieved by adding 200 μl 1 M $MgCl_2 \cdot 6\ H_2O$. The samples were centrifuged for 30 min at 3000 *g* and the pellets were solubilized in 800 μl TRIzol (Gibco, NY, USA) (SRSV) or 800 μl McIlvaines buffer (pH 5) (model virus) (McIlvaine, 1959).

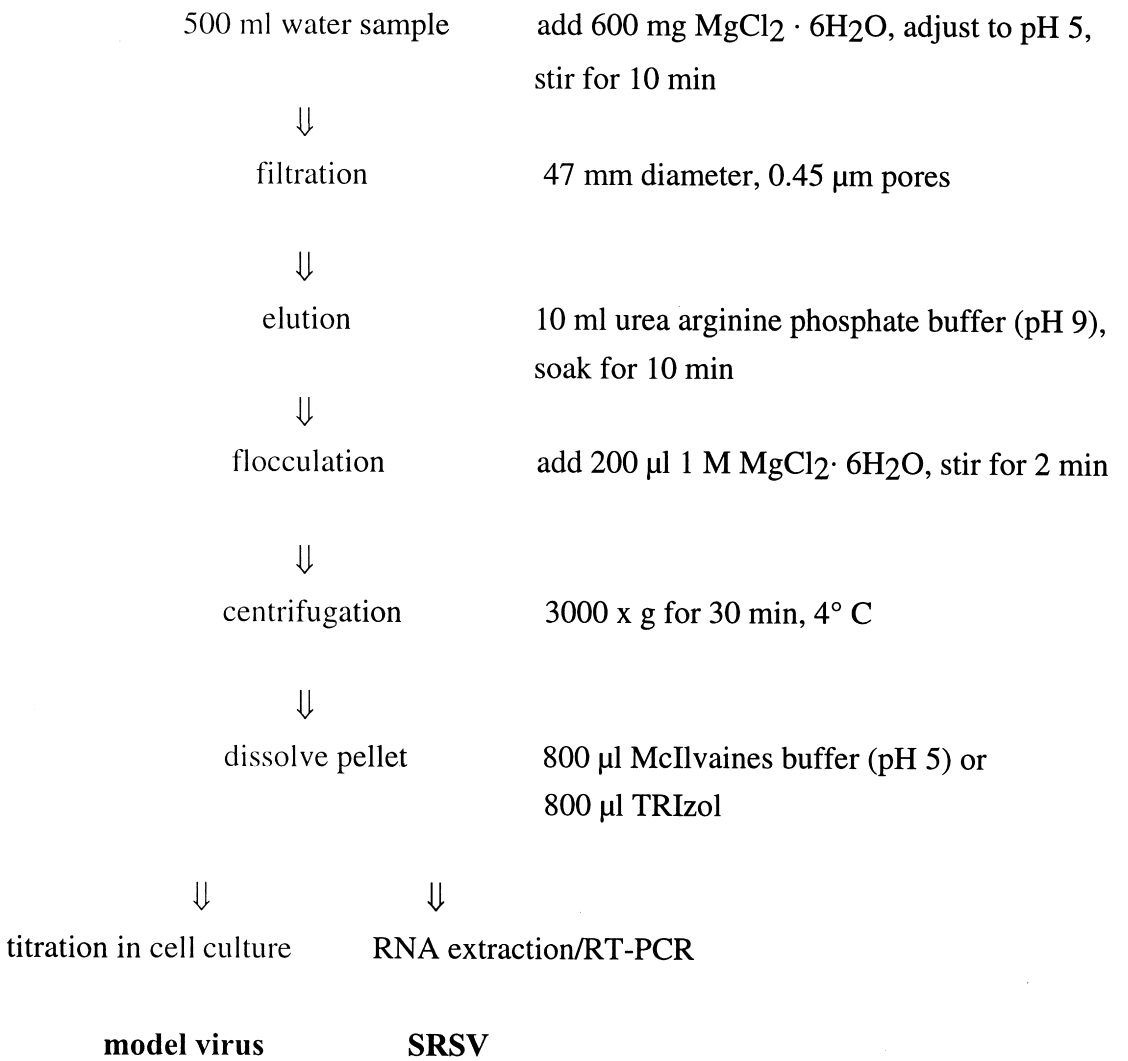

Fig. 1. Concentration of FCV and SRSV from water samples. Tenfold dilutions of FCV and fecal extracts (250 μl) were added to 500 ml of water. The pH of the water sample was adjusted and a divalent salt ($MgCl_2$) was added prior to virus concentration.

2.5. Filtration–elution and quantification of the model virus

To estimate the virus recovery in the concentration step, tenfold dilutions of the model virus were added to 500 ml deionized water prior to filtration. Four trials were performed. The virus was quantified in microtiterplates by endpoint titration of eight parallels. Earle's minimum salt solution, complemented with 3% $NaHCO_3$, 1% L-glutamine, 0.5% penicillin/streptomycin, 2% kanamycin, 0.5% fungizone, 2% fetal calf serum and 1% nonessential amino acids, was used as medium.

2.6. Specificity of the SRSV PCR primers

To test the specificity of the SRSV primers, cDNA from the FCV and from EM virus positive fecal samples were used as targets in the SRSV PCRs. Sixteen SRSV samples, one rotavirus sample, two astrovirus samples and two classical human calicivirus samples were included.

2.7. SRSV RT-PCR on fecal extracts

RT-PCR was run on feces extracts for SRSV genogrouping. Tenfold dilutions of extracts were included to estimate the cut off for virus detection in three samples (no. 2081, no. 278 and no. 2155). RNA was extracted from 250 μl sample by adding 800 μl TRIzol. The RNA pellet was washed with 75% ethanol, dried and dissolved in a 15 μl solution containing 30 mM dithiothreitol, 3 mM ribonucleoside vanadyl complexes (Sigma, St. Louis, USA) and diethylpyrocarbonate treated water (DEPC water). Prior to the RT-reaction, the RNA solution was heated to 55–60°C for 10 min to dissolve secondary structures. The RT-reaction was run in a total volume of 25 μl containing 5 μl RNA, 50 mM Tris–HCl (pH 8.3), 75 mM KCl, 3 mM $MgCl_2$, 5 μM random hexamers and 1 mM each of dNTP and 250 U M-MLV reverse transcriptase (Gibco). The reaction was run for 60 min at 37°C, then heated to 95°C for 10 min before the cDNA was used as target in PCR.

The PCR primer sequences and their locations related to the Norwalk virus genome are shown in Table 1. The primer pairs SOU1–SOU2 and SOU3–SOU4 were designed on the basis of the nucleotide sequences from Norwalk and Southampton virus. They were used for detection of the SRSV genogroup 1. The nested primers SOU3–SOU4 gave an amplicon of 183 base pairs (bp). SRSV genogroup 2 primers, SR33 and SR46, gave an amplicon of 123 bp (Ando et al., 1995).

For detection of genogroup 1 SRSV the PCR mixture (50 μl) contained 20 μM Tris–HCl (pH 8.4), 50 μM KCl, 4 mM $MgCl_2$, 0.5 mM SOU1 and 0.5 mM SOU2, 0.2 mM each of dNTP, 1.5 U Taq DNA polymerase (Gibco) and 5 μl cDNA. The reaction mixture was heated to 94°C for 3 min before cycling 40 times of 1 min at 94°C, 1 min at 50°C and 1 min at 72°C, before a final elongation for 7 min at 72°C. The PCR product (1 μl) was used as target DNA in the nested reaction. The $MgCl_2$ concentration was set to 2 mM and the primers SOU3 and SOU4 (0.5 μM each) were used. The annealing temperature was raised to 57°C for 0.5 min and 25 cycles were run.

For detection of genogroup 2, the $MgCl_2$ concentration was 2 mM and 0.25 μM SR33 and SR46 were used. Heating to 94°C for 3 min was followed

Table 1
Sequences of primers used for the detection of SRSV genogroup 1 and 2; the locations are related to the Norwalk virus genome (Genbank acc. no. M87661)

Identification	Genogroup	Sequence (5′–3′)	Location
SOU1	1	CCA GGA TGG CAA GCC ATG TTC C	5270–5291
SOU2	1	ATC ACC GGG GGT ATT ATT AGG G	5591–5570
SOU3	1	GTA AAT GAT GAT GGC GTC TAA GG	5354–5376
SOU4	1	ATT ATT AAC AAT CCA GGG ATC AAT	5534–5511
SR33[a]	2	TGT CAC GAT CTC ATC ATC ACC	4888–4868
SR46[a]	2	TGG AAT TCC ATC GCC CAC TGG	4766–4786

[a] Ando et al. (1995).

by 40 cycles of 1 min at 94°C, 1.5 min at 50°C and 1 min at 72°C before a final elongation for 7 min at 72°C.

A negative control consisting of sterile water was included in each PCR setup. The PCR products were separated in a 4% Nusieve 3:1 agarose gel (FMC BioProducts, Rockland, USA) and visualized by UV illumination after ethidium bromide staining.

2.8. Filtration–elution and detection of SRSV in deionized water

To assess the concentration method for SRSV, 250 μl of tenfold dilutions of SRSV-containing fecal extracts (no. 2081, no. 278 and no. 2155) were added to 500 ml water in triplicates. Dilutions of feces extract no. 2081 were also added to 5 l of water to test the influence of the filtration volume on the virus recovery. The pellets obtained by flocculation of the virus were dissolved in 800 μl TRIzol. Extraction of RNA and virus detection were performed as described for SRSV RT-PCR on fecal extracts.

2.9. Filtration–elution and detection of SRSV in heavily polluted water (bog pond) and drinking water reservoirs

To assess any interference from organic materials with the concentration procedure, water from a bog pond was included in the study. Feces extracts (250 μl of no. 2081) were added to the samples prior to filtration or to the filter eluate prior to flocculation to test if the virus was lost in any of these steps. Duplicate experiments were performed. To evaluate any inhibitory effects on the RT-PCR, SRSV positive RNA was added to RNA (1:3) extracted from concentrated bog pond samples devoid of feces extracts. Filtration, elution, flocculation and RT-PCR were performed as for virus detection in deionized water. The flocculation pellets were dissolved in 800 μl McIlvaine's buffer. One aliquot of 400 μl was used for RNA extraction directly. The other was put on a Chelex-100/Sephadex G-100 column and centrifuged at 400 *g* for 10 min to remove humic acids and metal ions prior to RNA extraction and RT-PCR (Abbaszadegan et al., 1993)

To evaluate the method for SRSV in raw drinking water, 250 μl of feces extract (no. 2081) were added to a 500 ml sample from four drinking water reservoirs. Concentration and RT-PCR were performed as for deionized water.

Each experiment included a parallel spiking and processing of deionized water as a positive control.

3. Results

3.1. Recovery of the model virus

The recovery of FCV by the concentration method is presented in Fig. 2. A strictly linear relationship was noted when more than 10^5 $TCID_{50}$ (tisue culture infective dose) was added, ($y = -1.86 + 1.09x$) and $R^2 = 0.93$, while a sudden drop in recovery was noted at lower $TCID_{50}$ levels. Different pH adjustments prior to filtration did not influence the recovery and pH 5 was used.

3.2. Specificity of the SRSV RT-PCR

SRSV in the feces extracts were grouped into genogroups 1 and 2 by PCR (Fig. 3). Virus samples giving positive PCR results using the primer pairs SOU1–SOU2 and SOU3–SOU4 and negative using SR33–SR46 were referred to as members of genogroup 1. Isolates that were positive with SR33 and SR46 only, were grouped as genogroup 2. Three of the sixteen EM positive SRSV samples were negative on RT-PCR. No amplification products were found when RT-PCR was performed on rota-, astro-, classical human calici-, FCV or the negative water control.

3.3. Sensitivity of the genogroup 1 nested RT-PCR on feces

Estimation of the sensitivity was performed on one EM negative feces extract (no. 2155) containing genogroup 1 SRSV. The highest dilution of this feces sample that resulted in a positive PCR product was 10^{-4}. As the estimated sensitivity of EM is 10^6–10^7 virus particles per ml of feces, this result indicates that the nested RT-PCR was capable of detecting less than 10^2–10^3 viral particles per ml of diluted feces.

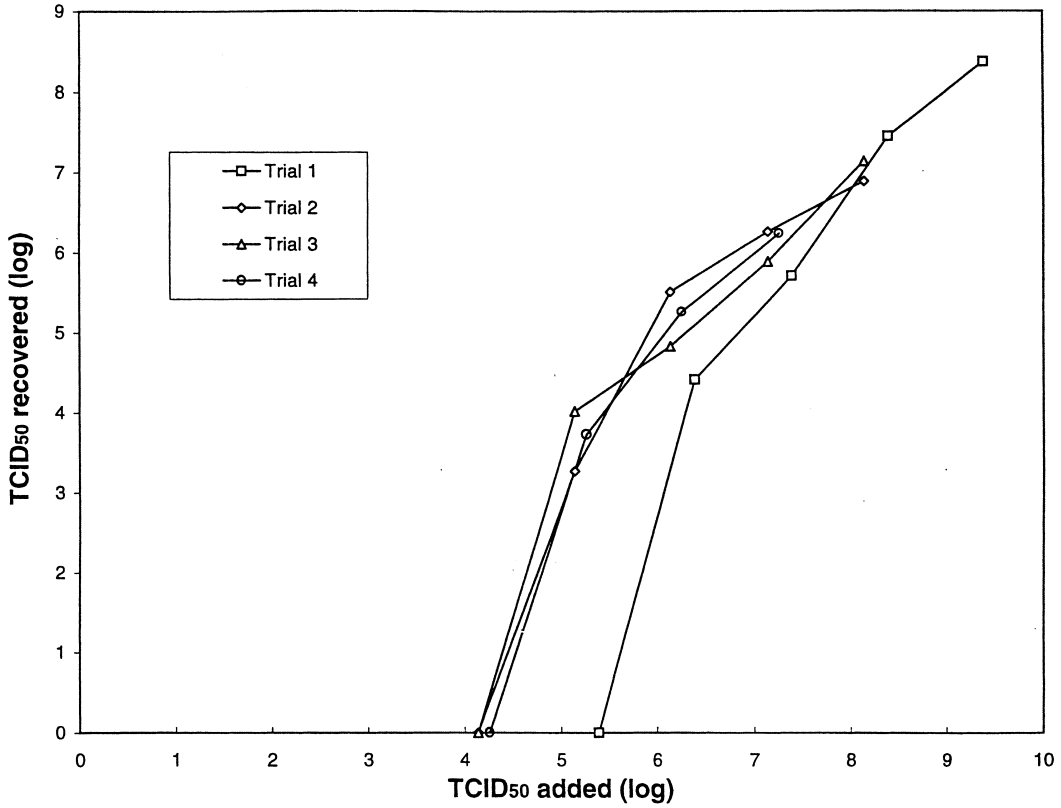

Fig. 2. Recovery of FCV (model virus) after filtration. Ten-fold dilutions of FCV were added to 500 ml water prior to filtration. Four trails were performed. Virus was recovered by isolation in feline embryo lung cells.

3.4. Sensitivity of concentration and detection of SRSV in deionized water

Adjustment of the water to pH 5 produced the strongest PCR amplification and this pH was subsequently used. The results of the filtration study are given in Table 2. The endpoint dilution for a positive PCR result on feces sample no. 2081, containing genogroup 1 SRSV, was 10^{-6}. A positive PCR result for this sample was obtained when a 10^{-5} dilution was added to 500 ml of water and when a 10^{-4} dilution was added to 5 l of water. Addition of a 10^{-3} dilution of sample no. 2155 (estimated as $2.5 \cdot 10^{2}$–$2.5 \cdot 10^{3}$ virus particles) gave a positive PCR result when added to 500 ml water. Generally a tenfold drop in sensitivity was found after dilution of the samples in 500 ml water and subsequent concentration, compared to detection directly from the samples.

3.5. Filtration and detection of SRSV in bog pond water and raw drinking water

The results with polluted water are shown in Fig. 4. Addition of feces extracts to the polluted water samples prior to filtration gave negative RT-PCR results [Fig. 4(A3 and A4)]. The opposite was obtained when feces were added to the eluates from polluted water (UAPB), indicating that the virus was lost during filtration [Fig. 4(B3 and B4)]. Addition of feces to the positive controls (deionized water) prior to filtration resulted in positive amplicons (183 bp) [Fig. 4(A1 and A2)], while inefficient flocculation and weaker amplicons were obtained when feces were added to the eluates from deionized water [Fig. 4(B1 and B2)]. Addition of PCR positive RNA to RNA extracts gave positive results for both deionized [Fig. 4(C1 and C2)] and polluted water [Fig. 4(C3 and C4)]. The Sephadex/Chelex spin column

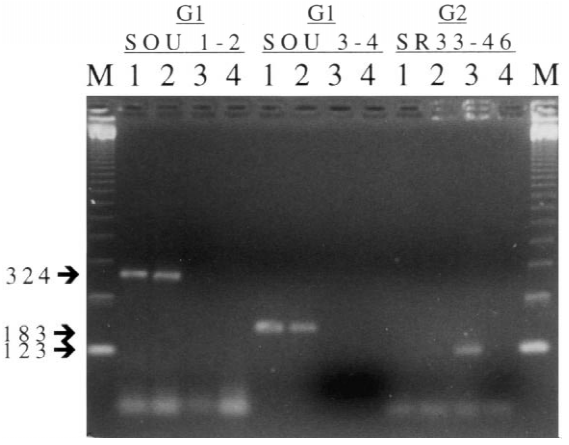

Fig. 3. Genogrouping of SRSV in feces extracts according to RT-PCR results. Genogroup 1 strains (G1) were detected by a nested PCR with primer pairs SOU1–2 and SOU3–4 (183 bp). The primers SR33 and SR46 were used to detect members of genogroup 2 (G2) (123 bp). M, DNA 123-bp ladder (Gibco, BRL); 1, Feces extract no. 2081; 2, No. 2155; No. 278; 4, Negative PCR control.

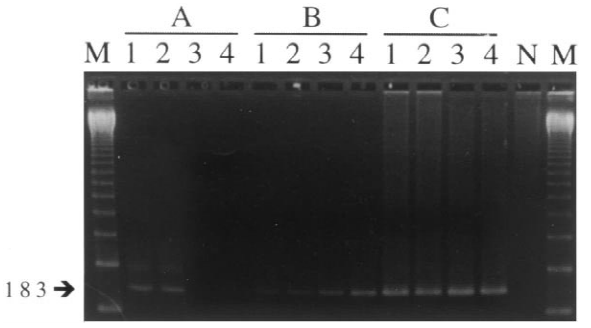

Fig. 4. Interference of organic materials with the detection of SRSV in water. To identify which step in the concentration procedure that was most sensitive to humic acids contamination, feces extracts (no. 2081) were added to heavily polluted raw water prior to filtration (A) and prior to flocculation (B). SRSV positive RNA was added to RNA extracts from the water samples to evaluate any interference from humic acids with the RT-PCR (C). One part of the concentrated water samples was used directly in RNA extraction. The other was put on a Sephadex/Chelex column to remove inhibitory substances prior to RNA-extraction and RT-PCR. A parallel spiking and processing of deionized water was used as a positive control. M, DNA 123-bp ladder; 1, Deionized water, direct RNA extraction; 2, Deionized water, Sephadex/Chelex column; 3, Raw water, direct RNA extraction; 4, Raw water, Sephadex/Chelex column; N, Negative PCR control.

enhanced the sensitivity when feces were added to the filter eluate originating from the polluted water [Fig. 4(B4)].

SRSV were detected when the fecal samples were diluted in water from the four drinking water magazines.

4. Discussion

The estimated recovery of the FCV model virus was in the magnitude 5–10% when the amount of added virus was above 10^5 $TCID_{50}$. This is rather low compared to the 82.6% recovery reported by Jothikumar et al. on poliovirus (Jothikumar et al., 1992). An explanation for this unexpectedly low recovery may be a rapid inactivation of FCV, not only a loss of virus particles. Conditioning of the water sample, the increase to pH 9 to facilitate elution from the filter and the subsequent decrease to pH 5 when dissolving the virus pellet may result in

Table 2
RT-PCR results on tenfold dilutions of SRSV feces samples and concentrated spiked water samples[a]

	Feces dilutions						
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}
Sample no. 2081 (G1, EM+)	+	+	+	+	+	+	−
added to 500 ml water	+	+	+	+	+	−	
added to 5 l water	+	+	+	+	−		
Sample no. 2155 (G1, EM−)	+	+	+	+	−		
added to 500 ml water	+	+	+	−			
Sample no. 278 (G2, EM+)	+	+	−				
added to 500 ml water	+	−					

[a] The diluted feces samples were added to deionized water and processed as outlined in Fig. 1. Virus was detected by nested PCR (genogroup 1, G1) or by singel PCR (genogroup 2, G2). EM+/EM−: positive/negative by electron microscopy.

loss of infectivity of FCV and partly explain the low recovery (Sobsey, 1982). As FCV is a respiratory pathogen, which is generally more sensitive to environmental changes than enteric viruses, it is likely more prone to inactivation than poliovirus. However, the RT-PCR used for the detection of SRSV will not be influenced by a loss of infectivity during the recovery step, unless breakdown of the viral RNA is also included. Consequently, the estimated recovery of FCV probably represents the minimum recovery of the SRSV.

The mechanism of recovery is adsorption of virus to the filter by hydrophobic and electrostatic interactions between the viral capsid proteins and the filter surface. The size of the filter pores is more than tentimes the size of the virus particles. To maximize adsorption, cations are added to the water sample and the pH is adjusted to between the isoelectric point of the virus and the filter (Gerba, 1984). Redman et al. has studied the effect of water pH on filtration of recombinant Norwalk virus capsid proteins in quartz sand (Redman et al., 1997). The quartz sand was negatively charged in the pH range 5–7 and the isoelectric point of the Norwalk particles was found at pH 5. Filtration of radioactively-labeled capsid proteins in 0.01 M NaCl solutions adjusted to pH 5 and pH 7 resulted in 99.92% and 17% retention of the particles on the quartz grains, respectively. These results indicate the importance of water pH on the electrostatic interactions between Norwalk virus capsid proteins and the filter media. The optimal model virus therefore should have the same isoelectric point as the SRSV in the feces extracts. A protein's isoelectric point depends on the composition of amino acids and may vary between different serotypes of a virus. The isoelectric points of the SRSV isolates used in this study are not known. The FCV model virus was chosen due to its relatedness to the SRSV and its ability to grow in cell cultures. However, optimalization of the filtration step should be possible provided recombinant capsid proteins representing different serotypes or large volumes of SRSV containing feces are available.

The sudden drop in recovery when less than 10^5 $TCID_{50}$ of FCV were added cannot be fully explained. The size and amount of virus aggregates are dependent on the virus concentration and thus may influence the recovery and the inactivation rate of the virus. Compared to the recovery of SRSV, as measured by RT-PCR, the amount of FCV added must be very high to get a recovery of 5–10%. This indicates that adhesion to particulate materials, which viruses in feces are capable of, facilitates the recovery at low concentrations of virus particles. In our experiment FCV was in cell culture fluid, devoid of particulate material.

Epidemiologic studies indicate that the SRSV infections have a low infective dose and that even low numbers of viruses in environmental samples may represent a threat to public health (Kaplan et al., 1982b). The development and use of sensitive detection methods is therefore important. A prerequisite for the use of RT-PCR is that the PCR primers will anneal to different strains of SRSV. The nucleotide sequence variation and the lack of knowledge about the geographical distribution of the strains may necessitate the use of several primer sets to ensure a PCR with satisfying specificity and sensitivity (Ando et al., 1995). The negative PCR results on three EM positive SRSV feces samples indicate that the primers used in this study were not able to detect all SRSV strains in the two genogroups. In feces extracts no. 2081, no. 2155 and no. 278 positive RT-PCR results were obtained in the 10^{-6}, 10^{-4} and 10^{-2} dilutions, respectively. A lower virus content (no. 2155 is EM negative) and the use of a single PCR on no. 278 (genogroup 2) may account for these differences. Estimation of the sensitivity of the RT-PCR has to rely on EM sensitivity due to the inability of SRSV propagation in vitro. Our estimate of being able to detect 10^2–10^3 SRSV particles per ml diluted feces (no. 2155) demonstrates a high sensitivity of the nested RT-PCR compared to the EM. By concentrating water polluted with feces it was possible to detect virus at a level of approximately 0.5–5 viral particles per ml water. This result indicates that the filtration/flocculation method has concentrated the SRSV with a factor of 200.

In this study SRSV were detected by RT-PCR after the filtration of 500 ml of deionized water containing 250 μl of a 10^{-5} dilution of feces sample no. 2081 (corresponding to 1 ml feces diluted in $2 \cdot 10^5$ liters of water). The sensitivity of the detection method decreased as the volume examined was expanded to 5 l. This result may be explained by a higher washing effect when the same filter size is used on larger water volumes. The average concentration of thermotolerant coliform bacteria in feces is 10^6–10^7 per ml. At the lowest level of fecal contamination which gave a positive PCR result in

the filtration study, the recovery of less than five thermotolerant coliform bacteria per 100 ml water could be expected, while SRSV could be present at 50–500 viral particles per 100 ml. The presence of thermotolerant coliform bacteria is traditionally used as an indicator of fecal contamination of water. No thermotolerant coliform bacteria are accepted in the water leaving the water works, thus making treatment and disinfection of most raw water mandatory. However, water treatment may break down for periods and most enteric viral pathogens, including SRSV, seem to be more persistent in the aqueous environment than the indicator bacteria (Berg and Metcalf, 1978). The absence of thermotolerant coliform bacteria therefore does not prove that the water is free of viral pathogens. In the described situation, with less than five indicator bacteria per 100 ml of water, the number of bacteria could drop below the detection level while infective virus would still be present in the water. This work therefore indicates that the described method could detect SRSV in bacteriologically acceptable drinking water.

Conditioning of the water sample to increase virus adsorption to the filter may be impractical if larger volumes are processed. Adjustment to low pH facilitates clogging of the filters due to formation of metal complexes and precipitation of humic acid and thus prefiltering may be necessary (Farrah et al., 1976). Conditioning of the water may be avoided if electropositive filters are used. These filters adsorb viruses efficiently over a wide pH range and the virus recovery is at the same level as that of electronegative filters (Sobsey and Jones, 1979; Sobsey and Glass, 1980; Sobsey et al., 1981). In a study on hepatitis A virus in environmental samples, positively charged filters, UAPB and flocculation were used for virus concentration (Jothikumar et al., 1998). Subsequent immunomagnetic capture and RT-PCR achieved the detection of 0.04 plaque forming units (PFU) (an estimate of 3.16 viral particles) in an initial volume of 100 ml water. This result indicates that the recovery of hepatitis A virus with positively charged filters was higher than our recovery of SRSV with electronegative filters. However, comparative studies including several filter types have to be performed with environmental samples containing SRSV to evaluate any differences in virus recovery.

A negative effect of organic materials on the concentration process and the RT-PCR has been described (Sobsey and Hickey, 1985; Tsai et al., 1993). The inhibitory effect was confirmed in this study by the use of water samples from a bog pond. Organic material decreased the filtration efficiency, which may be caused by competition with virus particles for binding sites on the negatively charged filters or by interference with virus elution. However, the flocculation of virus particles was facilitated. Removal of humic acids and metal ions by spun column chromatography enhanced the sensitivity slightly when feces was added after filtration, but prior to flocculation. This indicates an inhibitory effect of organic material on RT-PCR, although no effect was observed when PCR positive RNA was added to the extracts prior to RT-PCR. A difference in the number of targets for the RT-PCR could explain these results. The conclusion is that the sensitivity of the method is decreased by large amounts of organic materials. However, a water reservoir containing large quantities of humic acids is not acceptable as a drinking water source. The positive RT-PCR results on the samples from the four drinking water magazines suggest that the method can be of practical use.

The method we describe is simple, rapid and could be scaled up with the use of large filters. The combination of filter adsorption, flocculation and RT-PCR seems to be a feasible tool for detection of SRSV in raw drinking water. The estimated sensitivity indicates that the method could be useful in the investigation of an outbreak situation.

Acknowledgements

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