Recovery of feline calicivirus infectious particles and genome from water: comparison of two concentration techniques

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Abstract The aim of this work was to determine the recovery rate of feline calicivirus (FCV-F9) infectious particles and genome from water after a concentration step using either adsorption elution on glass wool or filtration through an electropositive membrane. The results showed that the membrane filtration technique allowed a 75% recovery rate of FCV-F9 infectious particles while the yield was only 5.3% for FCV-F9 genome. Using the glass wool adsorption-elution technique, the recovery rate of FCV-F9 infectious particles was 0.5% while the yield was 102.5% with Poliovirus 1.

Keywords Caliciviridae; concentration; genome; water

Introduction

Enteric viruses are usually present at very low concentrations in environmental samples, requiring a concentration step for detection. The most common methodology for concentration of virus from water involves adsorption-elution techniques using different supports such as glass wool (Vilaginès et al., 1992), glass powder (Senouci et al., 1996) electropositive membranes (Ma et al., 1994) or electronegative membranes (Stetler et al., 1992). This primary concentration step can be followed by a secondary concentration step using PEG 6000 precipitation (Lewis and Metcalf, 1988), organic flocculation (Katzenelson et al., 1976) or ultrafiltration (Gilgen et al., 1997). Recovery rates of these methodologies have been well defined for infectious enteroviruses and especially for poliovirus type 1. The yield depends on different parameters including the kind of water, turbidity, virus serotype and adsorption support.

For other enteric viruses, such as human Caliciviridae and especially Norwalk-like virus (NLV), the recovery rate of infectious particles is unknown because they cannot multiply in cell culture. Nevertheless, animal Caliciviridae, such as feline calicivirus (FCV) or simian calicivirus (Pan 1), are able to multiply in specific cell strains (Slomka and Appleton, 1998; Myrmel et al., 1999; Huang et al., 2000) which allows these viruses to be used as models of human caliciviruses.

The aim of this work was to determine the recovery rate of feline calicivirus (FCV F9) infectious particles from water after a concentration step using comparative adsorption elution on glass wool and filtration through an electropositive membrane. Simultaneously, the recovery rate of infectious particles of poliovirus type 1 was studied using the same concentration techniques. Finally the recovery rate of FCV-F9 infectious particles and genome concentration on the membrane was determined.

Materials and methods

Viruses

Two kinds of viruses were used: feline calicivirus F9 (FCV F-9) and poliovirus 1 Sabin Lsc 2ab (PV1). Both viruses give a cytopathic effect when inoculated into cell cultures. Viruses
were quantified on cell culture using the MPN method (40 wells/dilution) with feline embryo-derived cells (FEA) and BGM cells for FCVF-9 and PV1 respectively.

**Water**

Autoclaved tap water, obtained after a classical treatment (pre-chlorination, sedimentation, sand filtration, ozonation, activated carbon and post chlorination), was used for all concentration experiments.

**Concentration techniques**

Two kinds of concentration techniques were used in this study. The first was adapted from Gilgen et al. (1997). Briefly, 1 L water was treated with 0.02 M MgCl₂ and filtered through a positively charged membrane (Zetapore NM047-01-045 SP). The adsorbed virus was eluted with 4 mL elution buffer (glycine 50 mM, beef extract 1% w/v, pH 9.5) by stirring at 250 rpm for 20 min. This constituted the concentrate. The second was a normalised method for virus concentration using glass wool (AFNOR XPT 90-451) described by Vilaginèes et al. (1992) where 10 L water were filtered through 50 g glass wool contained in a cartridge (Sartorius SM 16249). The adsorbed virus was eluted with 300 mL elution buffer (glycine 50 mM, beef extract 3% w/v, pH 9.5) and then precipitated with polyethylene glycol 6000 (10% w/v solution) for 12 h at 4°C. The precipitate was recovered in 10 mL phosphate buffer and constituted the concentrate.

**Viral RNA quantification**

RNA was extracted from 140 µL concentrate using the Qiamp viral RNA kit (QIAGEN ref 52904) according to the manufacturer’s instructions. The extracted RNA was recovered in 60 µL elution buffer. Synthesis of cDNA was performed as previously described (Gantzer et al., 1998) with 5 µL extracted RNA using AMV reverse transcriptase and 0.5 µM reverse primer in a final volume of 20 µL. DNA quantification was performed using 5 µL of reverse transcription product in a final volume of 50 µL containing 25 µL Master Mix (Perkin Elmer Ref 4304437), 0.5 µM reverse and forward primer and 0.25 µM Taq man probe (Table 1).

**Results**

**Adaptation of the filtration technique to FCV-F9**

The global recovery rate depended on three main parameters: the percentage of adsorbed virus, the percentage of eluted viruses and perhaps also the stability of the virus in the elution buffer (pH 9.5). To optimise the concentration method for recovering infectious FCV-F9 from water these parameters were tested under different conditions.

First, the percentage of adsorbed virus was evaluated with and without 0.02M MgCl₂ in the water to be concentrated. Water (1 L) was seeded with 5 × 10⁵ MPNCU and both water and filtrate were quantified on cell culture to evaluate the percentage of adsorbed virus. Four experiments were performed with or without MgCl₂ in the water before filtration. The results showed that viral absorption was 99.1% with MgCl₂, significantly higher than the 86.3% obtained without MgCl₂ (P = 0.0131, t-test).

Secondly, the stirring time for the elution was evaluated. Water samples (1 L) were seeded with 5 × 10⁵ MPNCU FCV-F9, filtered through a membrane and eluted by 20 min

<table>
<thead>
<tr>
<th>Table 1 Primers and probe for quantitative PCR of FCV-F9</th>
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<tr>
<td>Reverse primer</td>
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<td>Forward primer</td>
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<td>Probe</td>
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contact time, by stirring for 20 min at 250 rpm, by stirring for 20 min at 420 rpm or stirring for 40 min at 420 rpm. The recoveries for these four experiments were 1.3%, 52.4%, 69.3% and 37.8% respectively. There was a significant difference between the single membrane contact protocol and the other methods using stirring (P < 0.05). The same experiments (n = 10) were performed with poliovirus (5 × 10^5 MPNCU) with a mean recovery rate of 77% when using elution by simple 20 min contact.

Finally, the stability of FCV-F9 was studied in the elution solution. 10^6 MPNCU were seeded in 1 L elution solution at pH 9.5 and at pH 7. The viral concentration was followed for 1 h whilst being stirred. There was no significant difference between viral levels at pH 7 and 9 during the first 30 min (P > 0.41) but after 60 min contact there was a decrease at pH 9 (P = 0.049). However, even after 60 min there was less than a 2-fold difference between virus quantities at pH 7 and pH 9. These results were used to determine the concentration protocol described previously as defined for the membrane, which was used for all the following experiments.

**Recovery of FCV-F9 infectious particles after concentration using glass wool and electropositive membrane**

Poliovirus and FCV-F9 were seeded into tap water at quantities ranging from 5 × 10^3 to 5 × 10^5 in 1L for the membrane concentration technique and 10 L for the glass wool concentration technique. The quantity of infectious virus was determined in both water and concentrate using cell culture. The recovery percentages for each virus and each technique are given in Table 2.

For FCV-F9 virus, the recovery rate was very different depending on the concentration technique used. With the glass wool technique (n = 3) the yield was very low (0.5%) while with the membrane technique (n = 4) yield was high (75%). In contrast, recovery of poliovirus was satisfactory for both concentration techniques but was higher with the glass wool method (102.5%; n = 3) vs 69% with the membrane technique (n = 5).

**Recovery of FCV-F9 virus genome**

Taking into account the results obtained with infectious virus, the recovery of FCV-F9 genome was studied using only the membrane concentration technique. The yield of the membrane filtration technique was determined by quantifying viral RNA in water and concentrate. For this, a quantitative RT-PCR (QRT-PCR) was designed for FCV-F9. First, primer and probe optimal concentrations were defined (0.5 µM primers and 0.25 µM probe). The linear relation between the cycle threshold and the quantity of RNA was then verified in water and concentrate using standard curves. These curves enabled determination of the relative quantity of RNA in water and concentrates. Finally, QRT-PCR variability was estimated. When tested with and without the RNA extraction step for 10–10^5 MPNCU FCV-F9 (n = 5 for each level) the coefficient of variability was <5%. The yield for FCV-F9 using membrane filtration was about 5.3% (n = 4) significantly lower than the 75% obtained for infectious virus.

**Table 2 Recovery of infectious virus and viral genome after concentration using glass wool or membrane filtration**

<table>
<thead>
<tr>
<th>Virus</th>
<th>Glass wool Infectivity</th>
<th>Mean recovery (%)</th>
<th>Membrane filtration Infectivity</th>
<th>Genome</th>
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<tbody>
<tr>
<td>Feline calicivirus (FCV F9)</td>
<td>0.5% (0.3–0.8%)</td>
<td>75% (19–130%)</td>
<td>69% (59–76%)</td>
<td>5.3% (4.2–5.9%)</td>
</tr>
<tr>
<td>Poliovirus type 1</td>
<td>102.5% (96–107%)</td>
<td>75% (19–130%)</td>
<td>Not done</td>
<td></td>
</tr>
</tbody>
</table>

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Discussion

The aim of this work was to evaluate the recovery rate of infectious particles and genome of Caliciviridae after a concentration step using the glass wool and the membrane filtration technique with feline calicivirus (FCV-F9) as a model. With the membrane filtration technique it was shown that the addition of MgCl₂ at 0.02 M significantly increased adsorption of FCV-F9 on electropositive membranes (86.3–99.1%). The beneficial effect of antichaotropic agents for adsorption on the support is well known (Farrah, 1982; Lukasik et al., 2000) and was confirmed in this study. Overall, it has been shown that stirring during elution is a critical point with FCV-F9 (1.3% recovery without stirring and 69.3% with). Low yield for the membrane filtration technique has been previously reported for FCV-F9 (5–10%) with an elution by contact (Myrmel et al., 1999) whereas 73% yield has been reported for simian calicivirus (Pan-1) with elution after stirring (Huang et al., 2000). Using poliovirus it appeared that stirring was not essential to obtain a good elution yield since recovery rate was 77% without stirring.

Finally it was verified that FCV-F9 was stable at pH 9.5 during the elution time. Huang et al. (2000) observed a small decrease at pH 9.5 in 30 min (39%) for simian calicivirus (Pan-1) whereas we observed this decrease only after 60 min with FCV-F9. Consequently, inactivation during the elution time was negligible.

Considering these results, the membrane filtration technique was associated with a yield of about 75% of FCV-F9 infectious particles when filtering 1 L tap water with 0.02 M MgCl₂ and 20 min elution at pH 9.5 with stirring (250 rpm). Under such conditions approximately the same yield was obtained for poliovirus 1 (69%) which was similar to other studies reporting yields >50% (Sobsey and Glass, 1980; Dizer et al., 1982; Ma et al., 1994).

When using glass wool, for which a normalised technique is available, for concentrating enterovirus from tap water, our results confirmed the high yield obtained especially with poliovirus (102.5%). This method is well adapted for high-volume filtration (<1,000 L). Nevertheless, this technique would not be useful for concentration of FCV-F9 infectious particles as the yield is low, about 0.5%. Poliovirus 1 (enterovirus) and FCV-F9 (calicivirus) exhibit different behaviour during the concentration step. Perhaps, as the elution from glass wool was achieved only by contact (stirring of the cartridge being impossible), poliovirus 1 and not FCV-F9 would be eluted as observed for the membrane technique.

If molecular biology techniques were used, and especially QRT-PCR to detect virus genome, the yield obtained was much lower (about 5%). The difference of the nature of viral particles detected could explain this result. Cell cultures can be used to detect and quantify only infectious virus whereas the genome can also include non-infectious particles, such as naked RNA or RNA included in viral capsid, no longer capable of interacting with cell receptors. Moreover, it is usually accepted that non-infectious particles are about 100–1,000× more abundant than infectious particles (Kopecka et al., 1993), especially when virus is obtained from a cell culture supernatant as in this study. However, it seems that the yield of non-infectious particles concentration was much lower than that of infectious particles.

Conclusion

It was shown that poliovirus type 1, an enterovirus, and FCV-F9, a calicivirus, behaved differently during the concentration step. Specifically, adsorption-elution using glass wool is not at all adapted for concentration of FCV-F9 whereas with electropositive membranes a higher recovery of both infectious viruses was achieved. This may be the reason why Caliciviridae human pathogens, such as NLV, have been detected by RT-PCR mainly with techniques using such concentration methods (Gilgen et al., 1997). The recovery of FCV-F9 genome with the membrane filtration technique was only about 5%.
References


