Calicivirus Inactivation by Nonionizing (253.7-Nanometer-Wavelength [UV]) and Ionizing (Gamma) Radiation

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Noroviruses (previously Norwalk-like viruses) are the most common viral agents associated with food- and waterborne outbreaks of gastroenteritis. In the absence of culture methods for noroviruses, animal caliciviruses were used as model viruses to study inactivation by nonionizing (253.7-nm-wavelength [UV]) and ionizing (gamma) radiation. Here, we studied the respiratory feline calicivirus (FeCV) and the presumed enteric canine calicivirus (CaCV) and compared them with the well-studied bacteriophage MS2. When UV irradiation was used, a 3-log10 reduction was observed at a fluence of 120 J/m2 in the FeCV suspension and at a fluence of 200 J/m2 for CaCV; for the more resistant phage MS2 there was a 3-log10 reduction at a fluence of 650 J/m2. Few or no differences were observed between levels of UV inactivation in high- and low-protein-content virus stocks. In contrast, ionizing radiation could readily inactivate MS2 in water, and there was a 3-log10 reduction at a dose of 100 Gy, although this did not occur when the phage was diluted in high-protein-content stocks of CaCV or FeCV. The low-protein-content stocks showed 3-log10 reductions at a dose of 500 Gy for FeCV and at a dose of 300 for CaCV. The inactivation rates for both caliciviruses with ionizing and nonionizing radiation were comparable but different from the inactivation rates for MS2. Although most FeCV and CaCV characteristics, such as overall particle and genome size and structure, are similar, the capsid sequences differ significantly, making it difficult to predict human norovirus inactivation. Adequate management of UV and gamma radiation processes for virus inactivation should limit public health risks.

Human caliciviruses are the most important enteric agents of gastroenteritis in industrialized countries (13, 17). Many waterborne outbreaks associated with noroviruses have been reported (1, 3, 5, 10, 15). Usually, epidemiological data prove that water was the common source for the outbreak, and in some molecular tracing studies norovirus strains were successfully identified in the source water and identical viruses were found in stools from patients (4, 8, 11, 12, 16). Virus concentrations in source water may peak with sewage overflow due to heavy rainfall or leaking septic tanks or pipes (2, 20). Breakdown or malfunctioning of water treatment plants may lead to insufficient reductions in virus concentrations in drinking water (20). Moreover, European legislation is mainly based on bacteriological quality parameters that determine fecal contamination of food and water. Viruses, however, are more resistant to treatment (9), are generally harder to detect, and exhibit lower dose-response relationships than bacteria. Therefore, water may meet microbiological safety guidelines but still pose a considerable health hazard.

Irradiation treatment is being used more frequently for disinfection of food and water. Bacteriophages have been used as indicators for inactivation of viruses by ionizing and nonionizing radiation since bacteria are generally less resistant to UV than viruses. Since noroviruses cannot be cultured (6), it is difficult to assess their resistance to ionizing and nonionizing radiation. The rate of inactivation by radiation has to be studied by using molecular methods or model viruses. Detection by PCR underestimates the efficiency of radiation for inactivating viruses (23). In this study we determined the inactivation of two cultivable animal caliciviruses, feline calicivirus (FeCV) strain F9 and canine calicivirus (CaCV) strain no. 48, which have highly divergent capsid sequences, and compared the results with inactivation of bacteriophage MS2. In addition to inactivation by nonionizing (253.7-nm-wavelength [UV]) radiation, inactivation by ionizing (gamma) radiation was studied as a process for calicivirus reduction. Virus stocks with high and low protein contents were compared to establish the possible effect of stabilizing solute constituents on inactivation by radiation.

MATERIALS AND METHODS

Viruses and cells. Crandell Reese feline kidney (CRFK) cells and Madin-Darby canine kidney (MDCK) cells were cultured in Dulbecco’s minimal essential medium supplemented with 10% (vol/vol) heat-inactivated fetal calf serum, gentamicin (50 μg/ml), and 1% (vol/vol) nonessential amino acids (Invitrogen, Paisley, Scotland). The medium used for virus propagation was identical except that it contained a low serum concentration (0 to 2%, vol/vol). FeCV strain F9 (kindly provided by H. Egberink, Utrecht University, Utrecht, The Netherlands) was propagated in monolayers of CRFK cells. CaCV strain no. 48 (kindly provided by F. Roerink, Kyoritsu Shoji Corporation, Tokyo, Japan) was propagated in MDCK cells (19). Virus stocks used in the inactivation experiments were

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obtained by inoculation of young cells (within 36 h after seeding) at a multiplicity of infection of 0.1. 50% tissue culture infectious dose (TCID50) per cell. The suspensions were harvested after 24 h by one cycle of freezing and thawing and were clarified by centrifugation (10 min, 1,800 g). The clarified stocks were diluted 10-fold in tap water. Stocks FeCV1 and CaCV1 contained 1.3 × 10^6 and 1.6 × 10^6 TCID50 of FeCV and CaCV, respectively, and the protein contents were 3 and 4 μg/ml, respectively, as assessed by the Bio-Rad protein assay with bovine serum albumin as the standard. The 253.7-nm transmittance measured over 1 cm was 68 and 57% of the Viennese tap water transmittance for the FeCV1 and CaCV1 stocks, respectively.

Further purification was done by extraction with chloroform (10%, vol/vol) and subsequent pelleting of the virus by centrifugation (95,000 × g, 3 h), resuspension in Dulbecco’s minimal essential medium, and 1,000-fold dilution in tap water. This resulted in stocks FeCV2 and CaCV2. Clarified stocks FeCV2 and CaCV2 contained 2.5 × 10^7 and 1.6 × 10^7 TCID50 of FeCV and CaCV, respectively. The protein contents of stocks FeCV2 and CaCV2 were below the detection limit of the Bio-Rad protein assay (<0.2 μg/ml), with 100% 253.7-nm transmittance relative to tap water transmittance measured over 1 cm. The titers of infectious virus were determined by determining TCID50 in cell cultures (10× dilutions in the 96-well format).

Radiation experiments. Experiments were performed at room temperature. The main chemical characteristics of the tap water were pH 7.6, a conductivity of 285 μS/cm, 3.0 mg of KMnO4 demand per liter, 0.8 mg of total organic carbon per liter, 1.7 mg of chloride per liter, 4.6 mg of nitrate per liter, 8.0 mg of oxygen per liter, 0.01 mg of sulfate per liter, and 0.01 mg of ammonia in the water. Linear regression analyses were carried out for comparisons of virus inactivation rates.

(i) UV irradiation. A board on which 10 low-pressure mercury UV lamps (EK 36; length, 500 mm; wavelength, 253.7 nm; ozone-free; Katadyn, Wallisellen, Switzerland) were mounted was horizontally suspended over the irradiation vessel. An aperture (100 by 150 mm) was located directly below the UV lamps and provided a bundle of quasi-parallel radiation to the irradiation vessel. The fluence was about 1 J/m². The UV fluence at 253.7 nm was measured online with a research radiometer (IL 1700; SED 240; International Light), taking into consideration the absorption at 253.7 nm of the test suspension and the reflection at the surface. Experiments were performed at room temperature with continuous stirring.

(ii) Gamma irradiation. The gamma irradiation experiments were carried out by using an AECL gammacell 220 cobalt 60 source (AECL, Mississauga, Canada) with an average dose rate of 0.86 Gy/s during the experiment. Samples (15 ml) were irradiated in glass vials. Dose rate calibrations have been performed with different chemical dosimeters (7).

FIG. 1. Electron micrographs of negatively stained FeCV and CaCV. (Left panel) FeCV in culture suspension of CRFK cells infected with FeCV strain F9. (Inset) Virus from the same suspension displaying characteristic Caliciviridae morphology. (Right panel) CaCV in culture suspension of MDCK cells infected with CaCV strain no. 48.
200 Gy the amount of MS2 was reduced 5 to 7 log\textsubscript{10} units, whereas the amount of CaCV was reduced 2.4 log\textsubscript{10} units and the amount of FeCV was reduced 1.6 log\textsubscript{10} units. Moreover, the inactivation of the animal caliciviruses appeared to follow second-order kinetics, with faster inactivation occurring at doses between 0 and 400 Gy and slower inactivation occurring at doses from 400 to 800 Gy.

DISCUSSION

Since noroviruses cannot be cultured (6), their resistance to ionizing and nonionizing radiation was assessed by using animal caliciviruses as a model. Here, we studied not only the respiratory virus FeCV strain F9 but also the presumed enteric virus CaCV strain no. 48 and compared them with the well-studied bacteriophage MS2 in tap water.

Inactivation by UV irradiation. Similar reductions were observed for FeCV and CaCV, although inactivation of MS2 required a UV dose that was three times higher. Previously, Nuanualsuwan et al. compared the levels of UV resistance of different viruses in phosphate-buffered saline (22). A reduction of 90\% was obtained with UV fluences of 480, 370, 240, 230, and 160 J/m\textsuperscript{2} for FeCV, hepatitis A virus, poliovirus type 1, MS2, and φX174, respectively (22). Another recent study, however, showed that the resistance of MS2 was greater than that of FeCV in buffered demand-free water (90\% reduction at fluences of 230 and 60 J/m\textsuperscript{2}, respectively) and that the resistance of human adenovirus type 40 was even greater at a fluence of 500 J/m\textsuperscript{2} (25). The high level of resistance of bacteriophage MS2 to UV radiation has been reported by our groups (9, 24), as well as by other groups (18). The different results obtained in the studies on the efficiency of UV inactivation of FeCV may have resulted from differences in the FeCV strains used or from differences in the compositions of the suspensions. Little or no effect on UV inactivation was observed.

The virus characteristics, such as size, genome, and weight, are very similar for the animal and human caliciviruses (Table 1). FeCV strain F9 and CaCV strain no. 48 are even more closely related, clustering within the vesivirus genogroup (14). However, the capsid sequences are very different, with only
37% amino acid homology. In comparison, in the capsid region there is 85% amino acid homology between different FeCV strains and 16 and 17% homology between Norwalk virus and CaCV and FeCV, respectively. Recently, it was shown that the viral capsid is the primary target for UV inactivation by capsid epitope binding experiments (21). We found that the very high viral capsid is the primary target for UV inactivation by capsid CaCV and FeCV, respectively. Recently, it was shown that the resistance of CaCV and FeCV differed significantly from that observed for MS2 for inactivation by UV radiation (P = 0.0003 and P = 0.01, respectively) and gamma radiation (P = 0.01 and P = 0.03, respectively). Moreover, CaCV is significantly more resistant to UV inactivation than FeCV (P = 0.008), whereas FeCV shows significantly greater resistance to gamma radiation (P = 0.002). The threefold-higher reduction in the level of MS2 compared with the animal caliciviruses could make MS2 a very conservative indicator for UV inactivation of caliciviruses. Pending a culture method for human caliciviruses, we suggest that the most resistant calicivirus should be used as a surrogate to assess inactivation by radiation, which is different for different types of radiation. Human caliciviruses are more divergent from the feline and canine vesiviruses; however, even though there were significant differences between the inactivation profiles of CaCV and FeCV, we found that in contrast to MS2, the rates of inactivation of the animal caliciviruses after irradiation were the same order of magnitude. Extrapolation from animal to human caliciviruses may be justified. More importantly, the efficiency of gamma rays is hampered by scavengers in high-protein-content substances, as we show here. The gamma radiation doses of 2 to 4 kGy often used for bacterial control in food products may not be sufficient to reduce calicivirus infectivity, but this should be studied. We have shown that scavengers present no problems for the use of the ionizing radiation used to produce water fit for consumption. Adequate management of radiation processes in the food and water industry will be the only way to ensure protection of public health.

TABLE 1. Characteristics of caliciviruses

<table>
<thead>
<tr>
<th>Virus</th>
<th>Nucleic acid</th>
<th>Length of RNA (kb)</th>
<th>Capsid protein</th>
<th>Buoyant density in CsCl (g/cm³)</th>
<th>UV fluence (J/m²)</th>
<th>Gamma dose (Gy)</th>
<th>Size (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FeCV</td>
<td>Single-stranded RNA</td>
<td>7.7</td>
<td>668–671</td>
<td>1.33</td>
<td>120</td>
<td>500</td>
<td>30</td>
</tr>
<tr>
<td>CaCV</td>
<td>Single-stranded RNA</td>
<td>8.5</td>
<td>691</td>
<td>1.38</td>
<td>200</td>
<td>300</td>
<td>30</td>
</tr>
<tr>
<td>Norovirus</td>
<td>Single-stranded RNA</td>
<td>7.6–7.7</td>
<td>530–580</td>
<td>1.36–1.41</td>
<td>Unknown</td>
<td>Unknown</td>
<td>30–35</td>
</tr>
</tbody>
</table>

* Values for a 3-log₁₀ reduction in caliciviruses in low-protein-content stocks.

Virus reduction by radiation. Quantitative risk assessment may be a useful tool for protection of public health by exposure to pathogenic microorganisms in water and food. The virus concentration in drinking water is so low that it cannot be measured directly; it can be determined only by measuring the virus concentration in the source water and the subsequent reduction via treatment processes that are applied to reduce the source water virus concentration. Here, we show that two cultivable caliciviruses, which are very comparable in terms of their virus characteristics but differ in their capsid amino acid sequences, display comparable sensitivities to radiation. However, the resistance of CaCV and FeCV differed significantly from that observed for MS2 for inactivation by UV radiation (P = 0.0003 and P = 0.01, respectively) and gamma radiation (P = 0.01 and P = 0.03, respectively). Moreover, CaCV is significantly more resistant to UV inactivation than FeCV (P = 0.008), whereas FeCV shows significantly greater resistance to gamma radiation (P = 0.002). The threefold-higher reduction in the level of MS2 compared with the animal caliciviruses could make MS2 a very conservative indicator for UV inactivation of caliciviruses. Pending a culture method for human caliciviruses, we suggest that the most resistant calicivirus should be used as a surrogate to assess inactivation by radiation, which is different for different types of radiation. Human caliciviruses are more divergent from the feline and canine vesiviruses; however, even though there were significant differences between the inactivation profiles of CaCV and FeCV, we found that in contrast to MS2, the rates of inactivation of the animal caliciviruses after irradiation were the same order of magnitude. Extrapolation from animal to human caliciviruses may be justified. More importantly, the efficiency of gamma rays is hampered by scavengers in high-protein-content substances, as we show here. The gamma radiation doses of 2 to 4 kGy often used for bacterial control in food products may not be sufficient to reduce calicivirus infectivity, but this should be studied. We have shown that scavengers present no problems for the use of the ionizing radiation used to produce water fit for consumption. Adequate management of radiation processes in the food and water industry will be the only way to ensure protection of public health.

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REFERENCES


