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## Research Note

# Efficacy of Commonly Used Disinfectants for the Inactivation of Calicivirus on Strawberry, Lettuce, and a Food-Contact Surface

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## ABSTRACT

Norwalk and Norwalk-like viruses (NLVs) are important causes of foodborne gastroenteritis in restaurant-related outbreaks. Efficacy of common disinfection methods against these viruses on food-contact surfaces and fresh produce is not known partially because of their nonculturability. Seven commercial disinfectants for food-contact surfaces and three sanitizers for fruits and vegetables were tested against cultivable feline calicivirus (FCV). Disks of stainless steel, strawberry, and lettuce were contaminated with known amounts of FCV. The disinfectants were applied at one, two, and four times the manufacturer's recommended concentrations for contact times of 1 and 10 min. The action of disinfectant was stopped by dilution, and the number of surviving FCVs was determined by titration in cell cultures. An agent was considered effective if it reduced the virus titer by at least 3 $\log_{10}$ from an initial level of $10^7$ 50% tissue culture infective dose. None of the disinfectants was effective when used at the manufacturer's recommended concentration for 10 min. Phenolic compounds, when used at two to four times the recommended concentration, completely inactivated FCV on contact surfaces. A combination of quaternary ammonium compound and sodium carbonate was effective on contact surfaces at twice the recommended concentration. Rinsing of produce with water alone reduced virus titer by 2 $\log_{10}$. On artificially contaminated strawberry and lettuce, peroxyacetic acid and hydrogen peroxide was the only effective formulation when used at four times the manufacturers' recommended concentration for 10 min. These findings suggest that FCV and perhaps NLVs are very resistant to commercial disinfectants. However, phenolic compounds at two to four times their recommended concentrations appear to be effective at decontaminating environmental surfaces and may help control foodborne outbreaks of calicivirus in restaurants.

Norwalk and Norwalk-like viruses (NLVs) are members of the family *Caliciviridae* and are associated with as much as 40% of all foodborne outbreaks and 96% of the reported outbreaks of viral gastroenteritis in the United States *(4, 8)*. Consumption of contaminated food is an important mode of transmission of these viruses in hostels, hospitals, schools, recreational camps, and restaurants *(4, 8, 15)*. Fresh or frozen produce is contaminated by ill or asymptomatic-infected food handlers, who may contaminate food while preparing salads, cold food items, and frosted confectionery items *(4, 15, 21)*. Food-contact surfaces may also contaminate fresh produce during harvest, processing, or handling in restaurants.

Chemical disinfection of food-contact surfaces and rinsing food items with sanitizers are generally relied on to prevent and control foodborne outbreaks, but the efficacy of disinfectants for the inactivation of NLVs is not known. This is partially due to the lack of methods for propagation of these viruses in vitro. Feline calicivirus (FCV), on the other hand, grows rapidly in cell cultures and produces characteristic cytopathic effects. Both FCV and NLV belong to the same virus family, and their physicochemical properties and genome organization are also similar *(3, 9, 10)*. FCV was, therefore, used as a surrogate model to determine the efficacy of disinfectants against NLVs on fresh produce and food-contact surfaces. Commonly used disinfectants and food sanitizers were evaluated for their anti-FCV efficacy on artificially contaminated stainless steel surfaces and fresh produce (strawberry and lettuce), respectively.

## MATERIALS AND METHODS

**Viruses and cells.** FCV strain F9 was propagated in Crandell Reese feline kidney (CRFK) cells. Briefly, CRFK cells were grown in Eagle's minimal essential medium (Celox, St. Paul, Minn.) supplemented with 8% fetal bovine serum, penicillin (100 U/ml), streptomycin (100 μg/ml), fungizone (1 μg/ml), 15 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid), and 5 mg/ml of lactalbumin hydrolysate. Monolayers at 90% confluency were inoculated with FCV and after adsorption for 1 h at 37°C, and the cells were incubated in the maintenance medium (same medium without serum) under 5% $CO_2$. The cytopathic effects developed within 12 to 24 h. The stock virus was harvested by two freeze-thaw cycles, aliquoted, and stored at −70°C until use.

**Disinfectants and sanitizers.** Products tested for disinfection of food-contact surface were as follows: 5.25% sodium hypochlorite (Fox-chlor, Jane Fox, Minn.); 1.75% iodine and 6.5% phos-

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phoric acid (Mikroklene, Ecolab, St. Paul, Minn.); three quaternary ammonium compounds (QACs) (Microquat and Oasis 144, Ecolab, and UMQ, Chemical Specialties Lab, Fairmont, Minn.); 15% peroxyacetic acid and 11% hydrogen peroxide (Victory, Ecolab); and two phenolic products (Lysol IC, Reckitt & Colman, Montvale, N.J., and Microbac II, Ecolab). For fresh produce, the sanitizers tested were as follows: 5.25% sodium hypochlorite (Fox-chlor), QAC (Oasis 144), and 15% peroxyacetic acid and 11% hydrogen peroxide (Victory). All products were tested at one, two, and four times the manufacturers' recommended concentration. Disinfectants and sanitizers were diluted in sterile tap water immediately before use.

**Disinfection of food-contact surface.** The food-contact surface used in this study was stainless steel disk, approximately 1 cm in diameter, punched from no. 4 finish polished sheets. Before use, the disks were cleaned by sonication for 10 min in a detergent solution, washed in deionized water, and sterilized by soaking in 95% ethanol for 10 min as described *(12)*. The test procedure was modified from that used previously *(20)*. Briefly, the disks were placed in 24-well tissue culture plate, and 10 μl of FCV stock culture was deposited on the surface of each disk. The inoculum was allowed to dry for 30 min in a laminar flow hood. A total of 20 μl of each dilution of disinfectant under test was then placed on the dried inoculum on the disk in duplicate and tested for 1 and 10 min of contact time at room temperature. At the end of each contact time, 980 μl of tryptose phosphate broth was added to each reaction well to dilute the disinfectant and to elute the virus from the disk. Control disks were treated in an identical manner except that instead of disinfectant 20 μl of sterile water was used. Eluted FCV was titrated in CRFK cells. Corresponding dilution of each disinfectant in tryptose phosphate broth served as negative control. Each experiment was repeated two more times under the same conditions.

**Contamination of produce and recovery of FCV.** Strawberry and lettuce were artificially contaminated with FCV by adsorption-elution-precipitation method. Strawberries (100 g) were immersed in 100 ml of 0.05 M glycine buffer (pH 3.5) containing 7.5 $\log_{10}$ FCV. Virus-containing buffer without strawberries served as control. After 15 min, virus in the buffer that did not absorb on strawberries was titrated in cell culture. The experiment was repeated thrice, and under these conditions, 60 to 100% of virus was adsorbed on strawberries. For contamination of lettuce, 7.0 $\log_{10}$ of FCV was applied directly on the lettuce leaf (approximately 10 g) and allowed to dry for 15 to 20 min in a laminar flow hood.

For recovery of virus, the contaminated produce (100 g of strawberry or 10 g of lettuce) was transferred to 100 ml of 0.05 M glycine buffer (pH 9.5) containing 3% beef extract and stirred for 15 min to elute virus from the produce. The produce was discarded and virus in the eluate was precipitated by lowering the pH to 3.5. The virus was pelleted by centrifugation at 4,000 × *g* for 15 min, and the pellet was suspended in 1 ml of 0.1 M phosphate-buffered saline (pH 7.2). Using this method, we could recover 90 to 100% of the virus from both types of produce.

**Sanitization of fruits and vegetables.** The produce (100 g of strawberry or 10 g of lettuce) contaminated with approximately 7.0 $\log_{10}$ of FCV by the above method was immersed in appropriate dilution of sanitizer in 100-ml volume (in duplicate) for a contact time of 1 and 10 min. As a control, contaminated produce was treated in an identical manner except that it was immersed in 100 ml of sterile water instead of the disinfectant solution. The virus was recovered from the treated produce as described above and titrated. The experiment was repeated two more times under identical conditions.

**Virus titration.** To determine the efficacy of disinfectants, FCV in treated and untreated controls was titrated by inoculating 100 μl/well of serial 10-fold dilutions of all samples in CRFK cells grown in 96-well plates, using four wells per dilution. After 72 h of incubation at 37°C in a $CO_2$ incubator (5% $CO_2$ and 95% relative humidity), the cells were observed for cytopathic effects and 50% tissue culture infective dose calculated by the Reed and Muench method *(17)*.

## RESULTS

**Disinfection of FCV on food-contact surfaces.** Table 1 lists different disinfectant formulations tested for decontamination of FCV-contaminated surfaces and the results obtained with each. A disinfectant was considered effective if it caused 3 $\log_{10}$ (99.9%) or greater reduction in the number of infectious virus units compared with untreated control. The results are expressed as mean reduction (±SD) in $\log_{10}$ FCV titer of three repeat experiments after 10 min of contact compared with untreated control. Contact time of 1 min was not effective in any of the tests, and hence the data are not shown. None of the disinfectants was cytotoxic at the tested concentrations.

When tested at the manufacturers' recommended concentration, none of the disinfectants was effective on FCV-contaminated surfaces. We tested three QAC-based formulations for their efficacy on stainless steel surfaces. QACs alone were not effective against FCV at the concentrations tested. When QACs were used in combination with sodium carbonate, they were able to cause a more than 3-$\log_{10}$ reduction in FCV titer on stainless steel surfaces at twice the recommended concentration of disinfectant.

The peroxyacetic acid and hydrogen peroxide–containing formulation was effective only at four times (1:500) the recommended concentration and that too for a contact time of 10 min. The sodium hypochlorite solution was the least effective at concentrations of up to 800 ppm of free chlorine, although the maximum allowed for food-contact surfaces by the Food and Drug Administration (FDA) is 200 ppm of free chlorine. However, at 5,000 ppm of available chlorine, there was a 3.4-$\log_{10}$ reduction in virus titer (data not shown). Iodine-based compound (iodophor) was not effective even at 300 ppm of titratable iodine, although a maximum of 75 ppm of titratable iodine is recommended by manufacturers for environmental surface disinfection and 25 ppm for sanitizing food-contact surfaces. Treatment with two phenolic preparations reduced virus titer by more than 5 $\log_{10}$ when used at four times the manufacturers' recommended concentration.

**Disinfection of FCV on strawberry and lettuce.** Washing with water alone for 10 min reduced the recovered population of FCV from strawberries and lettuce by 2.0 $\log_{10}$. None of the three sanitizers tested was effective against FCV-contaminated strawberries and lettuce at manufacturers' recommended concentration for food and food-contact surfaces for up to 10 min of contact time (Table 2). When used at a fourfold higher concentration, only per-

TABLE 1. *Efficacy of chemical disinfectants against FCV on food-contact surfaces*

| Disinfectant tested | Dilution tested | $Log_{10}$ FCV reduction ± SD[a] |
|---|---|---|
| 9% QAC[b] | 1:200 (450 ppm)[c] | 0.3 ± 0.05 |
| | 1:100 (900 ppm) | 0.0 ± 0.0 |
| | 1:50 (1,800 ppm) | 2.3 ± 0.05 |
| 10% QAC | 1:256 (400 ppm)[c] | 0.7 ± 0.1 |
| | 1:128 (800 ppm) | 1.0 ± 0.1 |
| | 1:64 (1,600 ppm) | 2.0 ± 0.05 |
| 5% QAC and 2% sodium bicarbonate | 1:64 (780 ppm of QAC)[c] | 0.4 ± 0.05 |
| | 1:32 (1,560 ppm of QAC) | 3.3 ± 0.1 |
| | 1:16 (3,120 ppm of QAC) | 3.4 ± 0.05 |
| 5.25% sodium hypochlorite | 200 ppm of free chlorine[c] | 0.3 ± 0.05 |
| | 400 ppm of free chlorine | 0.3 ± 0.0 |
| | 800 ppm of free chlorine | 1.1 ± 0.05 |
| 15% peroxyacetic acid and 11% hydrogen peroxide | 1:2,000[c] | 0.4 ± 0.1 |
| | 1:1,000 | 0.6 ± 0.05 |
| | 1:500 | 3.0 ± 0.0 |
| 1.75% iodine and 6.5% phosphoric acid | 75 ppm of titratable iodine[c] | 0.0 ± 0.0 |
| | 150 ppm of titratable iodine | 0.0 ± 0.0 |
| | 300 ppm of titratable iodine | 2.0 ± 0.1 |
| 4.75% *o*-benzyl *p*-chlorophenol and 4.75% *o*-phenylphenol | 1:256[c] | 1.5 ± 0.05 |
| | 1:128 | 6.2 ± 0.2 |
| | 1:64 | 7.0 ± 0.2 |
| 5% *o*-benzyl *p*-chlorophenol and 10.5% *o*-phenylphenol | 1:200[c] | 0.4 ± 0.1 |
| | 1:100 | 0.4 ± 0.1 |
| | 1:50 | 5.6 ± 0.2 |

[a] The results are expressed as reduction in FCV titer (mean ± SD of three repeat experiments) following treatment with disinfectant compared with untreated control.
[b] QAC, *n*-quaternary ammonium compound of alkyl (50% $C_{14}$, 40% $C_{12}$, 10% $C_{16}$) dimethyl benzyl ammonium chloride.
[c] The concentration recommended by manufacturers for disinfection of environmental surfaces.

oxyacetic acid and hydrogen peroxide–containing formulations were effective in causing further 3-$log_{10}$ reduction in FCV titer on both strawberry and lettuce.

## DISCUSSION

The development of strategies to prevent gastroenteritis outbreaks due to the consumption of NLV-contaminated fruits and vegetables is hampered partially because of the nonculturability of NLVs. The use of surrogate cultivable viruses has been recognized by the Environmental Protection Agency for the testing of antiviral disinfectants *(16)*. Closely related FCV has been used previously as a model for inactivation studies of NLVs *(5, 22)*. Considering the physicochemical and structural similarities of both NLV

TABLE 2. *Efficacy of sanitizers against FCV on contaminated strawberry and lettuce*

| Disinfectant tested | Dilution tested | $Log_{10}$ reduction in FCV titer ± SD[a] | |
|---|---|---|---|
| | | Strawberry | Lettuce |
| 15% peroxyacetic acid and 11% hydrogen peroxide | 1:2,000[b] | 0 ± 0.0 | 0 ± 0.0 |
| | 1:1,000 | 1.0 ± 0.1 | 2.0 ± 0.1 |
| | 1:500 | 3.0 ± 0.06 | 3.0 ± 0.06 |
| 5.25% sodium hypochlorite | 200 ppm of free chlorine[b] | 0 ± 0.0 | 0 ± 0.0 |
| | 400 ppm of free chlorine | 0 ± 0.0 | 0 ± 0.0 |
| | 800 ppm of free chlorine | 1.0 ± 0.06 | 1.5 ± 0.05 |
| 10% *n*-alkyl (50% $C_{14}$, 40% $C_{12}$, 10% $C_{16}$) dimethyl benzyl ammonium chloride | 1:512 (200 ppm)[b] | 0 ± 0.0 | 0 ± 0.0 |
| | 1:256 (400 ppm) | 0 ± 0.0 | 0 ± 0.0 |
| | 1:128 (800 ppm) | 1.5 ± 0.1 | 2.0 ± 0.1 |

[a] The results are of three repeat experiments, expressed as mean reduction (±SD) in FCV titer on contaminated produce following sanitizer treatment compared with those treated with water alone.
[b] The concentration recommended by the manufacturers for food and food-contact surfaces.

and FCV, it is reasonable to believe that an agent shown to kill FCV will also kill NLVs when used under identical conditions. In a related study in our laboratory, the adsorption-elution method developed for recovery of FCV from contaminated surfaces worked equally well for recovery of NLV from artificially contaminated stainless steel surfaces as detected by reverse transcription and polymerase chain reaction (unpublished data).

There is no defined criterion for measuring the antiviral activity of disinfectants, although a 100,000-fold reduction in viable count (a 5-$\log_{10}$ kill) is considered efficacious for antibacterial activity *(7)*. However, it is very difficult to demonstrate a 5-$\log_{10}$ reduction for viruses on contaminated surfaces because of low virus titers. Generally, for antiviral efficacy testing, a 3- to 4-$\log_{10}$ reduction in virus titer compared with control is used *(12, 18, 24)*. The contact time between the virus and a disinfectant can range from 30 s to a few hours *(19, 24)*. For most disinfectants, the recommended contact time is between 1 and 10 min. We found that an increase in contact time beyond 10 min made little difference in antiviral activity of disinfectants (data not shown). Therefore, we used a maximum exposure time of 10 min for surface disinfection at room temperature in all experiments.

None of the QACs tested was effective against FCV on contaminated surfaces. This confirms the results of previous studies *(5, 11, 13, 20, 23)* in which QAC-based products were generally found to be unreliable virucidal agents and were specifically ineffective against hydrophilic, nonenveloped viruses, e.g., FCV, canine parvoviruses, and polioviruses. This is particularly important because QACs constitute more than 38% of disinfectants sold in the United States for hard surface disinfection *(20)*. However, QACs in combination with sodium carbonate were effective, albeit at higher concentrations, suggesting that anti-FCV activity might be due to sodium carbonate. Unfortunately, we did not pursue this line of investigation.

The maximum FDA-allowed concentration of sodium hypochlorite is 200 ppm of available chlorine when it is used as a no-rinse food contact surface sanitizer *(6)*. However, it was not effective even at four times the recommended concentration (800 ppm). When tested at 5,000 ppm of available chlorine (a concentration recommended for disinfection of virus-contaminated material in laboratories), sodium hypochlorite was able to cause more than a 3-log reduction in FCV titer on surfaces. The results obtained herein are in agreement with previous reports *(5, 11, 13, 18, 20)* and support the use of higher concentrations of sodium hypochlorite for surface disinfection when calicivirus outbreak is suspected.

Although phenolic compounds are effective bactericidal and fungicidal agents, their virucidal activity is not well established *(14)*. In the present study, they were found to be effective against FCV only at twofold to fourfold higher concentrations than recommended by the manufacturers. Although phenolic compounds are not much accepted as surface disinfectants because of their toxicity, they could be used as alternative agents to control the spread of foodborne viruses from food contact surfaces during calicivirus outbreaks.

In recent years, the frequency of outbreaks epidemiologically associated with raw fruits and vegetables has increased in the United States as a result of change in dietary habits and increased importation of food *(1, 15)*. To minimize the risk of infections associated with raw fruits and vegetables, decontamination of produce with different disinfectants has been tried *(2)*. Efficacy of these products in inactivating viruses is not known. To determine the efficacy of disinfectants to kill NLV on raw fruits and vegetables, strawberry and lettuce were artificially contaminated with FCV and treated with the commonly used antimicrobials for fruits and vegetable rinsing. FCV was resistant to all antimicrobial formulations commonly used for rinsing fresh fruits and vegetables at the FDA-permitted concentrations. This probably explains the reason for outbreaks of NLV due to consumption of fresh fruits and vegetables contaminated during harvest, processing, or handling in resaturants despite the use of commercial surface disinfectants and food sanitizers. Peroxyacetic acid and hydrogen peroxide–containing product could effectively decontaminate strawberry and lettuce but at a fourfold higher concentration than recommended by the manufacturers.

Results of these studies demonstrate that available sanitizing and disinfecting agents used in restaurants are not capable of removing calicivirus contamination from work surfaces or from produce at recommended use levels. Thus, preventing outbreaks of foodborne calicivirus depends on maintaining hand washing practices and excluding ill food workers from the establishment. However, in the event of an outbreak, decontamination of environmental surfaces may be accomplished using phenolic compounds at two to four times manufacturers' recommended concentrations with appropriate safety precautions. This practice may help control foodborne outbreaks of calicivirus in restaurants.

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