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Efficacy of oxidizing disinfectants at inactivating murine norovirus on ready-to-eat foods

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ABSTRACT

Noroviruses are the leading cause of foodborne illness, and ready-to-eat foods are frequent vehicles of their transmission. Studies of the disinfection of fruits and vegetables are becoming numerous. It has been shown that strong oxidizing agents are more effective than other chemical disinfectants for inactivating enteric viruses. The aim of this study was to evaluate the efficacy of oxidizing disinfectants (sodium hypochlorite, chloride dioxide and peracetic acid) at inactivating noroviruses on fruits and vegetables, using a norovirus surrogate, namely murine norovirus 3, which replicates in cell culture. Based on plaque assay, solutions of peracetic acid (85 ppm) and chlorine dioxide (20 ppm) reduced the infectivity of the virus in suspension by at least 3 $\log_{10}$ units after 1 min, while sodium hypochlorite at 50 ppm produced a 2-log reduction. On the surface of blueberries, strawberries and lettuce, chlorine dioxide was less effective than peracetic acid and sodium hypochlorite, which reduced viral titers by approximately 4 logs. A surprising increase in the efficacy of sodium hypochlorite on surfaces fouled with artificial feces was noted.

1. Introduction

Noroviruses (NoV) are responsible for 18% of all cases of gastroenteritis and about 58% of all outbreaks of foodborne illness worldwide (Ahmed et al., 2014; Scallan et al., 2011). These enteric viruses are transmitted via the fecal-oral route directly by infected individuals, or indirectly via contaminated fomites, water and food. Ready-to-eat foods are now recognized as a major vehicle of NoV transmission, based on analysis of international outbreaks involving multi-ingredient foods (40.2%), produce (16.5%), seafood (13.0%) and bakery items (8.7%) among others (Greig and Ravel, 2009). Although present in low numbers in foods, NoV are highly infectious (Teunis et al., 2008). Ready-to-eat foods, especially fruits and vegetables, can be contaminated at any point of the production chain (irrigation with contaminated water, fertilizer use, harvesting by workers with poor hygiene) and these products are not necessarily washed before consumption to reduce microbial contamination. In recent years, the frequency of outbreaks associated epidemiologically with raw fruits and vegetables has increased in the United States as a result of changes in dietary habits and increased importation of foods (Altekruse et al., 1997; Ponka et al., 1999). To minimize the risk of infections associated with raw fruits and vegetables, decontamination of produce using different disinfectants has been applied (Beuchat, 1998). Numerous studies on oxidizing agents have been published, mainly on bacteria (Kreske et al., 2006; Rodgers et al., 2004). Studies of the use of such products against enteric viruses are becoming more numerous. The majority of these have been conducted using surrogates. In the case of noroviruses, there have been many attempts over the years to obtain a model system able to support the propagation of human noroviruses (Duizer et al., 2004; Straub et al., 2007), but without real success. Recent assays have shown promise by using different cell lines like human epithelial colon rectal adenocarcinoma cell line Caco-2 (Papafragkou et al., 2014), human epithelial cell line INT-407 (Takanashi et al., 2014) and B cells with the help of enteric bacteria (Jones et al., 2014) but the reproducibility of these models has not been confirmed. Since human NoV are difficult to propagate in cell culture, surrogates such as feline calicivirus (FCV), F-specific coliphage MS2, murine norovirus (MNV) and Tulane virus (TuV) are used widely to study the persistence and inactivation of human NoV (Allwood et al., 2004; Arthur and Gibson, 2015; Cannon et al., 2006; Cromeans et al., 2014). It has been demonstrated that oxidizing disinfectants are more effective than alcohols or quaternary-ammonium-based disinfectants against NoV attached to stainless steel surfaces (Girard et al., 2010). Their effectiveness on foods should be explored. The effects of oxidizing disinfectants have been tested on MS2 and FCV as viral models for the wash of fruits and vegetables (Allwood et al., 2004; Gulati et al., 2001). These studies conclude that the dosage required to obtain significant inactivation of the virus is either too high for subsequent human consumption depending on the concentrations allowed by the authorities or affects product appearance.

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Before drawing such conclusions about NoV, chemical inactivation should be investigated using a murine norovirus (MNV), which appears to be the best surrogate identified so far and is phylogenetically closer than FCV to human NoV (Cannon et al., 2006). It is known that the presence of organic matter (e.g. feces) can have a protective effect on viruses and reduce the efficacy of a disinfectant such as chlorine. Since surrogate viruses are rarely if ever studied in association with such organic matter, greater attention should be given to testing the efficacy of disinfectants under realistic conditions (Poschetto et al., 2007; Sattar et al., 2006). Cannon et al. (2006) used a reconstituted artificial fecal material (Feclone) to mimic realistic environmental conditions in a study conducted with MNV and FCV.

In this study, the reduction of the infectivity of MNV-3 treated with oxidizing disinfectants in solution and the efficacy of these agents in reducing the infectious viral load on fresh-cut romaine lettuce, strawberries and blueberries were investigated.

2. Material and methods

2.1. Viral stock

MNV-3 was propagated in RAW 264.7 cells as described previously (Wobus et al., 2004). Cell line and virus were kindly provided by Kirsten Mattison (Health Canada, Ottawa, ON, Canada). The titer of the MNV-3 stock suspension was estimated at 7×10^7 plaque-forming units (pfu) per ml based on plaque assay (Girard et al., 2010) and stored at −80 °C until use. Virus stock was mixed with reconstituted artificial feces (Feclone™ BFPS-4, SiliClone Laboratories, Valley Forge, PA) just before use in experiments. The Feclone was prepared according to the manufacturer's instructions and diluted 1/10 in sterile water to improve the consistency. Feclone BFPS-4 is composed of water, fibrous cellulosic plant derivatives and polyoxyethylene.

2.2. Chemical inactivation of NoV in solution

Industrial disinfectant products commonly used in the food sector were kindly provided by a local supplier. Two supermarket-purchased natural products for washing fruits and vegetables at home were also tested. The tested disinfectants and their respective concentrations are listed in Table 1. Each disinfectant was freshly prepared according to ASTM E1052-96 method and was assayed with appropriate kits according to the manufacturer's instructions to achieve the desired concentrations before each experiment.

Briefly, 30 µl of viral suspension was mixed with 60 µl of disinfectant for 0.5, 1 and 5 min at room temperature. Sodium thiosulfate (100 µl of 1% $Na_2S_2O_3$) was then added to neutralize the disinfectant and the volume was brought to 300 µl with Earle's Balanced Salts Solution, pH 7.2. The neutralizers used for each disinfectant are listed in Table 1. The solution was serially diluted in the same buffer and the infectious viral particles were enumerated by plaque assay.

2.3. Cytotoxicity of the tested compounds

The cytotoxicity of each disinfectant and neutralizer used in this study was tested (data not shown). The experimental protocol was the same as for viral inactivation in solution except that the viral suspension was replaced with Earle's Balanced Salts Solution and the disinfectant/neutralizer solution was applied to the cells exactly as in a plaque assay. Cells were observed under a microscope immediately after direct contact with the disinfectant/neutralizer and after 1 and 2 days to visualize cytopathic effects. The cytotoxicity and antiviral effect of the Feclone were also tested (no effect was observed, data not shown).

2.4. Chemical inactivation of MNV on ready-to-eat foods

Based on results obtained in solution, only disinfectants containing sodium hypochlorite, peracetic acid or chlorine dioxide were tested, at concentrations consistent with the consensus among regulatory bodies regarding possible applications on foods. Neutralizer was not used.

2.5. Food surfaces

All products were purchased in a local supermarket and prepared as follows: Lettuce (romaine type) — Green portions (3 × 3 cm) were washed three times with sterile de-ionized water and then dried for 30 min under a laminar flow hood (including 20 min under UV) before adding MNV-3; Strawberries — Red portions (2.5 × 2.5 × 0.5 cm) were washed as described for lettuce; Blueberries — Whole blueberries were washed as described above. Samples were inoculated with 50 µl of undiluted MNV-3 suspension (7×10^7 pfu/ml) or 50 µl of MNV mixed with Feclone (approximately 10^6 pfu/ml). For all the samples, MNV-3 was allowed to attach for 30 min under a laminar flow hood. Foods with virus were then gently immersed in 10–15 ml of disinfectant for 1 min without agitation. The food surface was rinsed with 1 ml of Earle's Balanced Salts Solution, pH 7.2. Serial dilutions were made immediately in culture medium containing FBS to stop possible reaction with residual disinfectant and the infectious viral particles were quantified by plaque assay. Log_{10} reductions in viral pfu were obtained by subtracting the log pfu recovered after the disinfection treatment from log pfu recovered from produce not subjected to disinfection.

2.6. Statistical analysis

Results from triplicate experiments were analyzed statistically using GraphPad Prism 6 software (GraphPad Software Inc., La Jolla, CA, USA). Significant differences ($p < 0.05$) among mean values were tested by analysis of variance (ANOVA) and Tukey's multiple comparisons test for each treatment condition.

3. Results

Results obtained using bromine and natural products are not shown in tables or figures since these agents were less effective in reducing

Table 1
The disinfectants tested in this study and their respective neutralizers.

Product	Active compound	Concentrations tested	Neutralizer
Industrial			
1	Peracetic acid (15%) and hydrogen peroxide (10%)	20, 85, 250 ppm of peracetic acid	Sodium thiosulfate 1%
2	Sodium hypochlorite	50, 200, 500 ppm of free chlorine	Sodium thiosulfate 1% + HEPES
3	Chlorine dioxide	2, 20, 50 ppm of free chlorine dioxide	Sodium thiosulfate 1%
4	Bromine	0.362–1 ppm	Sodium carbonate 1 M
Natural			
1	Mild emollients of palm and coconut oils	Dilution proposed by the manufacturer	Lecithin (4%) and Tween 80 (28%)
2	Sodium lauroyl sarcosinate and witch hazel	No dilution	Lecithin (4%) and Tween 80 (28%)