Survival of Respiratory Viruses on Fresh Produce

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Abstract In addition to enteric viruses of fecal origin, emerging zoonotic viruses such as respiratory coronaviruses and influenza viruses may potentially be transmitted via contaminated foods. The goal of this study was to determine the recovery efficiencies and the survival of two respiratory viruses, namely, adenovirus 2 (Ad2) and coronavirus 229E (CoV229E), on fresh produce in comparison to the enteric poliovirus 1 (PV1). Adenovirus was recovered with efficiencies of 56.5, 31.8, and 34.8 % from lettuce, strawberries, and raspberries, respectively. Coronavirus was recovered from lettuce with an efficiency of 19.6 % yet could not be recovered from strawberries. Poliovirus was recovered with efficiencies of 76.7 % from lettuce, but only 0.06 % from strawberries. For comparison purposes, the survival of Ad2, CoV229E, and PV1 was determined for periods up to 10 days of storage. The enteric PV1 survived better than both respiratory viruses on lettuce and strawberries, with only \( \leq 1.03 \log_{10} \) reductions after 10 days of storage at 4 \(^\circ\)C compared to CoV229E not being recovered after 4 days on lettuce and reductions of 1.97 \( \log_{10} \) and 2.38 \( \log_{10} \) of Ad2 on lettuce and strawberries, respectively, after 10 days. Nevertheless, these respiratory viruses were able to survive for at least several days on produce. There is therefore the potential for transfer to the hands and subsequently to the mucosa via rubbing the eyes or nose. In addition, some respiratory coronaviruses (e.g., severe acute respiratory syndrome coronavirus) and adenoviruses are also capable of replication in the gut and there is thus some potential for acquisition through the consumption of contaminated produce.

Keywords Respiratory viruses · Coronavirus · Adenovirus · Survival · Produce · Fomites

Introduction

There are 1.5 million deaths from respiratory infections worldwide each year (World Health Organization 2006). In the United States, adults and children less than 5 years experience *1.5–3.0 and 3.5–5.5* respiratory illnesses per year, respectively (Monto and Sullivan 1993). Viruses account for up to 69 % of all respiratory infections. These include illnesses caused by influenza viruses (A and B), rhinovirus, respiratory syncytial virus, human parainfluenza viruses (1–4), human coronaviruses (severe acute respiratory syndrome—SARS, 229E, and OC43), adenoviruses (primarily serotypes 4 and 7), and Coxsackievirus A21 (Monto and Sullivan 1993; Couch 1995; Boone and Gerba 2007). Viruses are particularly significant as etiologic agents of respiratory infections in the young and the elderly (Klein 2004). In pediatric respiratory units, adenoviral infections can be devastating; the fatality rate in hospital-acquired cases is 91 %. The hands of hospital attendants are believed to spread the virus (Sattar et al. 2000).

Few respiratory viruses are transmitted by one route only (Gwaltney et al. 1978; Brankston et al. 2007). Respiratory viruses may be spread by direct contact (person-to-person), the airborne route via droplets, or by indirect contact (via hands and/or fomites) (Goldmann 2000;
Some respiratory viruses irritate the respiratory epithelium and induce coughing and sneezing, thereby facilitating their transmission by not only the airborne route, but also by environmental contamination with the generated aerosols/droplets (Hall et al. 1980; Barker et al. 2001; Boone and Gerba 2007). The primary route of transmission is at times difficult to determine and is often a point of contention among researchers. In addition, unusual circumstances or conditions may lead to an atypical route of transmission for respiratory viruses. For instance, environmental contamination by stools played a significant role in the transmission of SARS coronavirus in certain outbreak populations in China (Dowell et al. 2004; Chu et al. 2005; Thomas et al. 1996; Goldmann 2000; Barker et al. 2001; Dowell et al. 2004; Boone and Gerba 2005, 2007; Chu et al. 2005; Kramer et al. 2006; Winther et al. 2007). Fomites may become contaminated with respiratory viruses by contact with body secretions or fluids or by contact with soiled hands. Also, viruses may become aerosolized via talking, sneezing, coughing, or vomiting and can then be deposited on fomite surfaces (Boone and Gerba 2007). Viruses can easily be spread to the mouth when fomites and subsequently the hands become contaminated.

There are also emerging zoonotic respiratory viral agents such as coronavirus (SARS) and influenza virus which could potentially be transmitted by foods, depending on the produce structures (Klein 2004). During the SARS outbreak of 2002–2003 in southern China, there was concern that the SARS coronavirus could be transported from one country to another by food (Food-HACC.com 2003). The SARS coronavirus causes severe respiratory disease but may also have a gastrointestinal component. It is shed in the feces for more than 10 weeks (Leung et al. 2003).

Non-enveloped viruses are usually more resistant to harsh environmental conditions and to the action of antimicrobials than enveloped viruses (Watanabe et al. 1989; Barker et al. 2001). The purpose of this study was to determine the ability of an enveloped (human coronavirus 229E) and a non-enveloped (human adenovirus 2) respiratory virus to survive on fresh produce in comparison to a non-enveloped enteric virus (poliovirus 1). Coronavirus 229E is a respiratory virus that causes mild, self-limiting, upper respiratory tract infections (Myint 1994), and has been used as a surrogate for the SARS coronavirus (Bright et al. 2009). Human adenovirus 2 also causes respiratory illness (Brandt et al. 1969). No such previous research has been conducted on the survival of respiratory viruses on produce.

Materials and Methods

The purpose of this study was to determine the recovery efficiencies of two respiratory viruses, namely, adenovirus 2 and coronavirus 229E from lettuce, strawberries, and raspberries. In addition, the survival of these viruses on produce stored at 4 °C was assessed. Poliovirus 1, a human enteric virus known to be very stable in the environment, was included in all tests for comparison.

Virus Preparation

Human coronavirus strain 229E (CoV229E) (ATCC #VR740) was maintained on MRC-5 (fetal human lung fibroblast, ATCC #CCL-171) cell line monolayers with minimal essential medium (modified with Earle’s salts; HyClone Labs, Inc., Logan, UT, USA) containing 2 % fetal bovine serum (HyClone Labs, Inc.) and 1 mM sodium pyruvate at an incubation temperature of 35 °C with 5 % CO₂. Human adenovirus 2 (Ad2) (ATCC #VR846) was maintained in the same manner but incubated at 37 °C using primary liver carcinoma (ATCC #CRL-8024) cell line monolayers. These viruses and cell lines were obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA).

Poliovirus 1 (PV1) (strain LSc-2ab) was maintained on the buffalo green monkey kidney (BGMK) cell line at 37 °C. PV1 was obtained from the Department of Virology and Epidemiology at the Baylor College of Medicine, Houston, TX, USA. The BGMK cell line was obtained from D. Dahling at the United States Environmental Protection Agency, Cincinnati, OH, USA.

Cell cultures were frozen and thawed three times to release the viruses from the cells. The viruses were then purified by centrifugation (1,000×g) to remove cell debris followed by polyethylene glycol (9 % PEG, 0,5 mol/L NaCl) precipitation. PV1 and Ad2 were further purified by extraction with equal volumes of Vertrel XF (Dupont, Wilmington, DE, USA), emulsified, and centrifuged (7,500×g for 15 min). The upper aqueous layer containing viruses was then aliquoted into sterile 1-mL vials.

For Ad2 and CoV229E, viral titrations were performed using the Reed-Muench method (Payment and Trudel 1993) to determine the tissue culture infectious dose that affected 50 % of the inoculated wells (TCID₅₀). Cells were observed for cytopathogenic effects over 7 (CoV229E) to 12 (Ad2) days. PV1 was titered via the plaque assay.
method (Bidawid et al. 2003). All purified virus stocks were stored at $-80 \, ^\circ \text{C}$ until used in experiments.

Sample Preparation

Iceberg lettuce (*Lactuca sativa*), strawberries (*Fragaria ananassa*), and raspberries (*Rubus idaeus*) were purchased directly from local markets. The produce samples were placed in 250-mL autoclavable wide mouth bottles with screw caps (Nalgene, West Chester, PA, USA) to about one-third capacity in the following manner: The outer three or four leaves of lettuce were discarded. Whole interior leaves were cut into pieces ($\sim 2 \times 2 \text{ cm}$) with a sterile razor. Approximately 8–10 g of cut leaves were added to each lettuce sample bottle. Two whole strawberries weighing between 15 and 20 g (for a total of 30–40 g) and four whole raspberries weighing between 4 and 5 g each (for a total of 16–20 g) were added to each of the strawberry and raspberry sample bottles, respectively.

Recovery of Viruses From Produce

The efficiency of recovery of each virus from produce was first assessed. The produce was inoculated with either 10 mL of Ad2 ($\sim 2.5 \times 10^9 \text{ TCID}_{50}$ total), 5 mL of CoV229E ($\sim 5.0 \times 10^8 \text{ TCID}_{50}$ total), or 10 mL of PV1 [$\sim 1.8 \times 10^7$ plaque forming units (PFU) total] using a pipette to carefully and evenly add the viral solutions drop-by-drop onto the produce. The caps of the bottles were then replaced and the bottles were manually rotated to ensure total coverage of the produce by the virus suspension. The inoculated produce was then incubated for 15 min at room temperature before processing. All of the tests were performed in triplicate.

Viruses were eluted from the produce by the addition of 90 mL of phosphate-buffered saline (PBS; pH 9.0; Sigma-Aldrich, St. Louis, MO, USA) to the bottles for the PV1 and Ad2 experiments. Only 45 mL of PBS was added to the samples inoculated with CoV229E to minimize the amount the virus was being diluted since the initial stock concentration was fairly low. The bottles were then placed on an orbital shaker (New Brunswick Scientific, Edison, NJ, USA) at 200 rpm for 10 min.

The PBS was collected as a pipette and placed in sterile bottles. These eluates containing organic debris were clarified by centrifugation at 1,400×g for 15 min at 5 °C (JA-14 rotor; J2-21 centrifuge; Beckman Coulter, Inc., Fullerton, CA, USA) to remove large suspended materials (Butot et al. 2007). The pH was then adjusted to 7.0 to 7.4. Aliquots of 4–7 mL were filtered through cellulose nitrate membrane syringe filters with a pore size of 0.22 μm (Acrodisc; Pall Corporation, Ann Arbor, MI, USA) pre-wetted using 5 mL of 3 % beef extract (pH 9.0; Becton, Dickinson and Company, Franklin Lakes, NJ, USA) to prevent virus adsorption to the membrane (Croci et al. 2008). Since all of the samples could not be assayed at once, the filtered aliquots were frozen for subsequent virus assay at $-20 \, ^\circ \text{C}$ for Ad2 and PV1 and at $-80 \, ^\circ \text{C}$ for CoV229E since CoV229E does not survive very well at $-20 \, ^\circ \text{C}$. The TCID$_{50}$ method was used to assay the recovered Ad2 and CoV229E and the plaque assay method was used to assay the recovered PV1 as described previously.

Survival of Viruses on Produce

To assess the survival of these viruses on produce, the produce samples inoculated with virus (as described previously) were stored at 4 °C for up to 10 days for Ad2 and PV1, and 8 days for CoV229E. The following initial inocula titers were used: $\sim 2.0 \times 10^8 \text{ TCID}_{50}$ of Ad2, $\sim 1.2 \times 10^6 \text{ TCID}_{50}$ of CoV229E, and $\sim 1.4 \times 10^7$ PFU of PV1. Duplicate sample bottles were removed immediately (time = 0 h) and also after various time intervals (1, 2, 4, 8, or 10 days) and eluted for viruses as described previously. The filtered aliquots were also frozen for subsequent virus assay at $-20 \, ^\circ \text{C}$ for Ad2 and PV1 and at $-80 \, ^\circ \text{C}$ for CoV229E and assayed as described previously.

Statistical Analysis

A Student’s $t$ test (two-tailed, assuming unequal variances) was used to compare the survival of the viruses on different types of produce. A $P$ value of $\leq 0.05$ was considered to indicate a statistically significant difference between the levels of the recovered viruses.

Results

Recovery of Viruses From Produce

Table 1 shows the recovery efficiencies of Ad2, CoV229E, and PV1 from produce. Ad2 was recovered with an efficiency of 56.5 % from lettuce. Its recoveries from strawberries and raspberries were lower (32–35 %). The recovery efficiencies of CoV229E and PV1 were only determined from lettuce and strawberries; raspberries were eliminated from these experiments because of their short shelf-life before deterioration. PV1 had the greatest recovery efficiency from lettuce (76.7 %) of any of the viruses on any of the produce. Nevertheless, both PV1 and CoV229E were not recovered well from strawberries (0.06 %).
Survival of Viruses on Produce

Table 2 shows the survival of Ad2 on produce. After 8 days at 4 °C, the recovered Ad2 levels decreased by 1.75 log10 on lettuce, 1.65 log10 on strawberries, and 0.60 log10 on raspberries. After 10 days, these reductions increased to 1.97 log10-2.38 log10, and 1.14 log10, respectively.

Table 3 shows the survival of CoV229E on lettuce stored at 4 °C. Strawberries were not included in the survival experiment with CoV229E because of the inability to detect the virus during the initial recovery efficiency determination tests. CoV229E declined by 0.20 log10 after 2 days of storage and could no longer be detected by day 4 (>1.31 log10 reduction).

PV1 survival on lettuce stored at 4 °C was fairly stable (Table 4), with no observed reductions until day 8 (0.36 log10). By day 10, this reduction had increased to 1.03 log10. Even though the recovery efficiency of PV1 from strawberries (Table 1) was very low (0.06 %), the recovered virus levels could still be used to determine its survival. Reductions in PV1 were observed as early as day 1 on strawberries (0.84 log10); nevertheless, the measured reductions did not continue to increase over time during storage, fluctuating between a low of 0.39 log10 (day 10) and 1.24 log10 (day 8). This is likely due to the variability in the assay. In addition, it is possible that the number of viruses recovered from strawberries on day 0 was overestimated. Therefore, the reductions calculated using this number would be overestimated. This seems likely since the reductions observed were immediate, but then remained relatively stable for 9 days.

Discussion

In this study, we determined the efficiency of recovery of two human respiratory viruses, Ad2 and CoV229E, and one enteric virus, PV1, from lettuce, strawberries, and...
Table 4 Poliovirus 1 survival on produce stored at 4 °C

| Time (days) | Lettuce | | | | Strawberries | | | |
|---|---|---|---|---|---|---|---|---|
| | Virus recovered a (log_{10} ± SD) | log_{10} reduction b | | | Virus recovered a (log_{10} ± SD) | log_{10} reduction b | | |
| 0 | 6.87 ± 0.33 | 0.00 | | | 4.72 ± 0.37 | 0.00 | | |
| 1 | 6.91 ± 0.30 | 0.00* | | | 3.88 ± 0.70 | 0.84* | | |
| 2 | 6.96 ± 0.29 | 0.00 | | | 4.03 ± 1.08 | 0.69 | | |
| 4 | 6.90 ± 0.19 | 0.00* | | | 3.72 ± 0.60 | 1.01* | | |
| 8 | 6.50 ± 0.20 | 0.36* | | | 3.49 ± 0.66 | 1.24* | | |
| 10 | 5.83 ± 0.47 | 1.03 | | | 4.33 ± 0.39 | 0.39 | | |

SD = standard deviation

* Statistically significant difference between the survival on strawberries and lettuce (P ≤ 0.05)

a Average of six replicates from two experiments (assayed by the plaque forming method)

b Average Log_{10} reduction (−log_{10} N_t/N_0 where N_t is the titer of virus at the specified day and N_0 is the titer of virus at time = 0)

In addition, the survival of these three viruses on produce during storage at a refrigeration temperature of 4 °C was determined. Such survival data are important in determining the risks of exposure over time following the contamination of produce and its subsequent storage.

The recovery efficiency of 56.5 % for Ad2 from lettuce was comparable to the 55 % of enteric adenovirus type 6 recovered from lettuce by Ward et al. (1982) in a previous study; nevertheless, our recovery of Ad2 from strawberries and raspberries was lower than those reported for enteric viruses (~50 %) in other studies (Bidawid et al. 2000; Le Guyader et al. 2004; Butot et al. 2007). For example, Bidawid et al. (2000) reported a recovery of 81 % of hepatitis A virus from strawberries; however, they used immunomagnetic beads and positively charged Virosorb 1MDS filters to assist in virus capture and concentration.

PV1 was recovered with an efficiency of 76.7 % from lettuce in this study. This is greater than the 58 % recovery efficiency reported by Ward et al. (1982). In contrast, the recovery of PV1 from strawberries was only 0.06 %, demonstrating that virus recoveries from different types of produce can vary significantly. Similarly, the recovery efficiency of CoV229E from lettuce was 19.6 %, whereas the virus was not recovered from strawberries. Such differences between the recoveries of viruses from berries versus lettuce are likely due to the fact that fruits such as strawberries and raspberries have a porous surface texture which may trap viruses beyond the reach of chemical disinfectants or eluents (Richards 2001). Alternatively, the elution process itself may cause damage to the surfaces of soft fruits, resulting in greater virus retention. The low pH of fruits, which are typically below those of vegetables, may also affect the sorption of viruses to the produce surface and therefore their recovery. The pH of strawberries is 3.0–3.9, raspberries is 2.9–3.5, and iceberg lettuce is ~6.0 (Martin-Diana et al. 2005; Roberts et al. 2005a, b). Basic buffers (pH 7.4–9.5) are recommended as eluents to extract viruses from acidic fruits in order to prevent a drop in the pH of the eluent during the elution process which may reduce virus recovery (Croci et al. 2008). Le Guyader et al. (2004) reported that detecting viruses on berries, particularly raspberries, is difficult because of the presence of various inhibitors as well as the low pH. Butot et al. (2007) found recovery efficiencies of 1.99, 0.42, and 1.1 % of hepatitis A virus, norovirus, and rotavirus, respectively, from fresh strawberries, and similarly low efficiencies of 2.3, 3.1, and 1.3 % for these three viruses, respectively, from frozen raspberries.

During postharvest transportation, strawberry shelf-life can be increased to 5–10 days (postharvest) by using controlled atmospheres while maintaining the transport temperature between 0 and 5 °C (TransFresh Corporation 2011). Strawberry producers also recommend not washing berries until just before serving, and to avoid contact with moisture (e.g., misters) in the food market. Such moisture causes rapid breakdown of the berries. Raspberries are very perishable and should always be kept between 0 and 1 °C. Under ideal conditions, strawberries should have a shelf-life of 2–5 days and raspberries should have a shelf-life of 1–2 days in the consumer’s refrigerator. In the virus survival experiments, the produce samples were stored in covered bottles to avoid evaporation of the inoculum volume during storage; however, the fruits started to deteriorate before day 10 due to the excess moisture. Raspberry deterioration was much more pronounced than that observed with strawberries. For this reason, the survival of PV1 and CoV229E on raspberries was not determined.

Ad2 survived the longest on raspberries, followed by lettuce, and then by strawberries. Such observed differences in Ad2 decay rates between raspberries and strawberries may be due to an uneven virus distribution over the fruit surfaces or to differences in the berry surface characteristics. The Ad2 reductions between raspberries and lettuce were significantly different after 8 days of storage.
at 4 °C and between raspberries and strawberries after 1 day.

CoV229E was very sensitive to the elution process. The recovery efficiencies were low, with no virus recovered from strawberries and only 19.6 % recovered from lettuce. In addition, the CoV229E fell to below the detection limit of our assay within 4 days of storage at 4 °C. These results are comparable with those obtained by Gundy et al. (2009) who reported that coronaviruses die-off very rapidly in wastewater.

Enveloped viruses such as CoV229E are typically less stable in the environment than non-enveloped viruses such as Ad2 and PV1 (Watanabe et al. 1989; Barker et al. 2001). PV1 can withstand a wide range of pH and temperature variations (Jaykus 2000). The acidity of the strawberries may therefore inactivate CoV229E more readily than the non-enveloped Ad2 and PV1. In addition, some fruits, including strawberries, blueberries, and grapes, contain quercetin which has been shown to have antiviral properties in cell culture experiments (Davis et al. 2008).

In this study, the enteric PV1 appeared to survive longer than both respiratory viruses on lettuce and strawberries, with only ≤1.03 log10 reductions after 10 days of storage at 4 °C compared to no CoV229E being recovered after 4 days on lettuce and reductions of 1.97 log10 and 2.38 log10 of Ad2 on lettuce and strawberries, respectively, after 10 days. Survival studies of poliovirus on soft fruit and salad vegetables performed by Kurdziel et al. (2001) found a linear decline of poliovirus of 0.086 log10 per day for lettuce stored at 4 °C for 15 days. They reported a decline of 0.12 log10 of poliovirus on frozen strawberries stored at −20 °C for 15 days. Raspberry samples displayed severe deterioration by day 9, but no significant decline in poliovirus was observed.

The results of this study demonstrate that respiratory viruses may persist on fresh fruit and vegetables for several days at the refrigeration temperature commonly used to store fresh produce in the average consumer household. Contamination of fresh produce may therefore result in the transmission of not only the enteric viruses that are traditionally considered “foodborne” pathogens, but also possibly respiratory viruses such as adenoviruses, coronaviruses, and influenza viruses that can infect via contact with mucosal membranes. Considering the low infectious dose of many viral agents, there will always be a risk of infection from the exposure to or consumption of food contaminated with respiratory or enteric viruses if no regular preventive produce surface sanitization is performed. This is of particular concern for uncooked fruits and vegetables. In addition, food handlers that are ill with respiratory rather than non-gastrointestinal disease could still pose a potential health risk for food consumers, particularly while preparing “finger foods” that come into contact with the consumers’ hands which can then transfer the viruses to the mucosa via hand to mouth contact or by rubbing the eyes or nose (Hendley et al. 1973; Goldmann 2000). The World Health Organization has highlighted the importance of hygiene, particularly that of hands, in minimizing the spread of respiratory tract infections (Hendley et al. 1973; Rusin et al. 2002; Bell 2006). Numerous respiratory viruses can survive on hands for significant periods of time and may be transferred from hands to fomites (such as produce) and back again (Hall et al. 1980; Ansari et al. 1991; Morens and Rash 1995; Barker et al. 2001). In addition, some respiratory coronaviruses (e.g., SARS coronavirus) and adenoviruses are also capable of replication in the gut and there is thus the potential for acquisition through the consumption of contaminated produce.

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