Survival of Poliovirus on Soft Fruit and Salad Vegetables

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ABSTRACT

A series of studies were performed, using poliovirus, to ascertain the potential for enteric pathogenic viruses to survive on various foodstuffs. The extraction protocols, which could be performed in just a few hours, were developed for use with quantities of food that would normally constitute a portion for consumption. The protocols were based on elution of viruses from food surfaces, followed by differential centrifugation to remove food debris and concentrate viruses. The studies were mostly performed using fresh produce stored at refrigeration temperature for 2 weeks or so, which was considered to represent the maximum time elapsing between purchase and consumption. Each food sample was inoculated with a viral suspension, and samples were analyzed immediately and at intervals throughout the experiment. Statistical analyses were performed on the results, and the decimal reduction times (D-values), or number of days after which the initial virus numbers had declined by 90%, were calculated. In summary, the resulting D-values were as follows: lettuce, 11.6 days; green onion, no decline; white cabbage, 14.2 days; fresh raspberries, no decline; and frozen strawberries, 8.4 days. The results showed that enteric viruses may persist on fresh fruit and vegetables for several days under conditions commonly used for storage in households. Therefore, if contamination has occurred before purchase, there will always be a risk of infection from consumption of the food.

There have been several studies on the survival of human enteric viruses in various environments. However, very few studies have been performed to ascertain their survival on foodstuffs, mainly due to the difficulty of extracting the viruses. Previous studies have (4, 8, 9) used either small pieces of foodstuff, which would not reflect quantities likely to be consumed, or large samples (5, 14), for which full experimental details were not provided (5) or the extraction techniques were laborious and time-consuming (14).

In the study reported herein, we monitored the numbers of infectious poliovirus inoculated on various foodstuffs held at refrigeration temperatures for periods reflecting their storage in the household. The size of the samples was determined by the quantity of the foodstuff that would normally constitute a portion size in the United Kingdom (12). The purpose of the study was to acquire information to help determine whether viruses can persist on foods long enough, i.e., from preparation through storage to consumption, to present a risk to susceptible individuals. We used a rapid extraction method based on centrifugation, which can be performed in a few hours.

MATERIALS AND METHODS

Virus strains. Poliovirus type 1a was kindly provided by Professor C. R. Madeley and Mr. F. Laidler of Newcastle-upon-Tyne Public Health Laboratory Service.

Preparation of virus suspensions. MA104 cells (European Collection of Cell Cultures) were used to propagate poliovirus. A total of 225 cm² of MA104 cells were grown in M199 medium with Earle’s modified salts, L-glutamine, 1.25 g liter⁻¹ of sodium bicarbonate (Life Technologies Ltd., Paisley, Scotland) supplemented with 10% heat inactivated fetal calf serum (Life Technologies), and the addition of 200 U ml⁻¹ of penicillin, 200 μg ml⁻¹ of streptomycin (Life Technologies), and 2.5 μg ml⁻¹ of Fungi-zone (Life Technologies). The flasks were inoculated with poliovirus and incubated at 37°C until a visible cytopathic effect was produced. The contents of the flasks were pooled, and the cell debris removed by centrifugation at 5,000 × g for 30 min. Poliovirus particles were then purified from the infected cultures as follows. Sodium chloride was added to 1 M, and the culture shaken at 37°C until the NaCl had dissolved. The culture was spun at 5,000 × g for 30 min to pellet cell debris. The supernatant was transferred to a sterile bottle, and 105 g liter⁻¹ of polyethylene glycol 6,000 was added. After the polyethylene glycol 6,000 had dissolved, the suspension was left at 4°C overnight to increase occlusion of virus particles. The suspension was spun at 5,000 × g for 30 min, and the supernatant was discarded. The pellet was resuspended in phosphate-buffered saline, and an equal volume of chloroform/isoamyl alcohol (24:1) was added. The suspension was shaken vigorously and then spun at 5,000 × g for 30 min. The chloroform extraction was repeated. The final virus suspensions were stored at 4°C until use.

Inoculation and sampling. Webb’s lettuce, green onions, white cabbage, strawberries, and raspberries were used. All food samples were obtained from local shops. Samples of 30 g of Webb’s lettuce leaves were placed in stomacher bags (Seward, London, UK) and inoculated with 1 ml of a purified poliovirus suspension. The bags were shaken to disperse the inoculate and then stored unsealed at 4°C. Triplicate samples were taken for analysis on days 0, 1, 3, 6, 9, 12, and 15.

The typical weight of each green onion was 9 to 10 g. One onion was used per replicate. Onions were placed individually in stomacher bags, and each was inoculated with 1 ml of poliovirus suspension by pipetting directly into the middle of the stalk.
FIGURE 1. Survival of poliovirus on fresh lettuce stored at 4°C for 15 days. The TCIU recovered from each sample were log_{10} transformed and are plotted on the y axis. Days are plotted on the x axis. Dashed lines represent 95% confidence intervals.

FIGURE 2. Survival of poliovirus on green onion stored at 4°C for 15 days. The TCIU recovered from each sample were log_{10} transformed and are plotted on the y axis. Days are plotted on the x axis.

bags were stored unsealed at 4°C. Triplicate samples were taken for analysis on days 0, 1, 4, 6, 9, 12, and 15.

Samples of 90 g of white cabbage leaves were placed in stomacher bags, and each was inoculated with 1 ml of poliovirus suspension. The bags were shaken to disperse the inoculate and then stored unsealed at 4°C. Triplicate samples were taken for analysis on days 0, 1, 5, 8, 11, and 14.

Strawberries were inoculated while fresh and then frozen for storage. Samples of 100 g of fruit were placed in stomacher bags, and 1 ml of poliovirus suspension was added. The bags were shaken to disperse the inoculate and then sealed for storage. The samples were stored at −20°C. Triplicate samples were taken for analysis on days 0, 1, 4, 6, 8, and 15.

Samples of 60 g of raspberries were placed in stomacher bags, and 1 ml of poliovirus suspension was added. The bags were shaken to disperse the inoculate and then stored at 4°C. Triplicate samples were taken for analysis on days 1, 4, 5, 8, 9, and 15.

**Extraction of viruses.** Ninety milliliters of 1 M sodium bicarbonate was added to each lettuce sample, which was then stomached for 20 s. The liquid was drained thoroughly and centrifuged at 28,000 × g for 30 min to remove food debris. The supernatant was spun at 240,000 × g for 1 h to pellet virus particles. The pellet was suspended in a total of 2 ml of M199 cell culture medium. Ninety... food types, such as green onions, that had fairly solid surfaces. The liquid was drained thoroughly and spun at 240,000 × g for 1 h. The pellet was suspended in 2 ml of cell culture medium. Ninety... food solids (10) and the homogenate spun at 28,000 × g for 3 min. One milliliter of Catacloc TL was added and the homogenate spun at 28,000 × g for 3 min. The supernatant was spun at 240,000 × g for 1.5 h. The resulting pellet was suspended in a sufficient volume of cell culture medium. The final volume was measured and factored into the determination of recovered infectious viruses.

**Enumeration of recovered infectious viruses.** This was performed by the method of Reed and Muench (13). The result is expressed logarithmically as TCID_{50} ml^{-1}, which signifies that dilution of a viral suspension that causes a cytopathic effect in 50% of the cell cultures (wells in this case) it challenges. This can then be defined as tissue culture infectious units (TCIU), approximating to infectious virus units, per sample by calculating the antilog and factoring the final extract suspension volume into the calculation.

**Statistical analysis.** Linear regression analysis was performed using the Minitab package. All counts were log_{10} transformed before analysis.

**RESULTS**

Figure 1 shows the survival profile of poliovirus on lettuce at 4°C throughout 15 days. There was a significant linear decline of infectious virus of 0.0865 log_{10} (SE, 0.0162; t = 5.34 with 19 df; P < 0.001) or 18% per day. This corresponds to a D-value of 11.6 days. There was no significant decline in poliovirus numbers was observed (Fig. 2). Figure 3 shows a decline in infectious poliovirus on inoculated white cabbage stored at 4°C for 15 days. A degree of nonlinearity is introduced by the low values at day 5, but overall there is a significant linear decline of poliovirus of 0.0691 log_{10} (SE, 0.0154; t = 4.47 with 16 df; P < 0.001) or 15% per day. This corresponds to a D-value of 14.2 days. The raspberry samples had displayed severe deterioration by day 9, but no significant decline in poliovirus numbers was observed (Fig. 4). Infectious poliovirus on strawberries frozen and...
stored at −20°C for 15 days showed a significant decline of 0.1186 log_{10} (SE, 0.0312; t = 3.80 with 7 df; P < 0.007) or 24% per day (Fig. 5). This corresponds to a D-value of 8.4 days.

**DISCUSSION**

The results of the above experiments showed that enteric viruses, as represented by poliovirus, have the potential to persist on fresh fruit and vegetables for several days under commonly used household storage conditions. Therefore, if contamination has occurred before purchase, there will always be a risk of infection from consumption of the food.

A previous study by Ward and Irving (14) examined poliovirus survival on large batches (0.5 to 3.0 kg) of celery and spinach harvested immediately after 2-h irrigation with inoculated wastewater and stored at 4°C in a humid atmosphere. Virus survival of at least 76 days on celery and 55 days on spinach was observed. There was an approximately 2-log decline in recovered infectious virus in each case, although erratic virus inactivation was observed on celery. It was considered that this may have represented uneven virus distribution in the bags of celery collected; the use of more replicates (apparently only single samples were taken at each time point) may have allowed a more even decline to be observed, but the lengthy extraction procedure involving several rounds of filtration and organic flocculation would not make increased sampling facile.

On green onion and fresh raspberries no decline of virus was observed, but on lettuce and white cabbage poliovirus showed a 90% decline by at least 12 days. Konowalchuk and Speirs (9) and Badawy et al. (4), evaluating survival of enteric viruses on various vegetable samples, reported that viruses survived longer on lettuce and attributed this to the moist surfaces of this foodstuff. However, their studies were mostly performed using small samples (2 to 15 cm²) under conditions that probably kept the surface moist throughout the experiment. In our studies, where the virus inoculum was dispersed throughout larger samples of foods, it may have been that, with lettuce and white cabbage, the flat surfaces allowed greater evaporation of the suspending fluid than the pitted or more complex surfaces provided by green onion or raspberries. In a study on the survival of viruses on soft fruit, Konowalchuk and Speirs (8) found that coxsackie B5 virus, echovirus, and reovirus could survive up to 6 days on strawberries, cherries, and peaches under humid conditions without any reduction in inoculated numbers; however, if the fruit samples were allowed to dry, only 1% of the inoculate could be recovered. It has been shown (1, 3, 11) that desiccation has a negative effect on enteric virus survival on surfaces, and it will undoubtedly be an influencing factor for virus persistence on foodstuffs. Poliovirus is less resistant to desiccation than hepatitis A virus or rotavirus (1), and these latter viruses might thus persist longer on food surfaces.

Infectious poliovirus declined in frozen strawberries, with a 90% reduction in inoculated numbers being observed by 8.4 days. It would have been interesting to be able to compare this with hepatitis A virus survival, since evidence indicates that it can survive prolonged freezing in frozen strawberries. The outbreak in Michigan in early 1997, where many people contracted hepatitis from strawberries, was attributable to fruit that had been frozen for several months before consumption (7).

The present studies used the prototypic enteric virus type of poliovirus. It would be very useful to perform the experiments with astrovirus, enteric adenovirus, hepatitis A...
virus, and rotavirus, and the method described in the present report should be highly applicable. The study of small round structured viruses, the most significant viral enteric pathogen in the United Kingdom (2), awaits the availability of suitable cell culture propagation techniques.

Although it did not concern enteric viruses, the study by Bardell (6) found that herpes simplex could persist on contaminated pieces of tomatoes and lettuce at refrigerator temperature (2°C) for at least 1 h with no significant reduction in titer. The author stated that unhygienic practices by food handlers may result in transmission of viral agents, such as herpes simplex, that infect the mouth and respiratory tract and recommended that contamination of food by such viruses should be studied. The methods used in our study should be highly suitable for this.

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REFERENCES