RECOVERY OF POLIOVIRUS FROM CUT SURFACE OF STORED FRESH PAPAYA FRUIT

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Abstract. Poliovirus kept on the cut surfaces of fully ripe papaya cubes placed in an ice box showed a sharp and significant reduction in the recovery of infectious virus about 15 minutes after exposure. Thereafter, a very gradual decrease ensued and infectious residual virus was detected up to the end of the 6-hour exposure period. Papaya cubes washed or kept overnight before virus inoculation, and from less ripe fruits produced a similar survival pattern. A very small proportion of the inoculum was recovered from the mashed content of the inoculated papaya cubes thus suggesting that most of the non-recovered virus particles were inactivated. The results suggest that the importance of poliovirus-contaminated cut papayas as a transmission vehicle for the virus is greatly reduced by the rapid decline in the infectivity of a large proportion of the virus soon after contamination. Nevertheless, the potential to transmit remains as a small residual pool of infectious poliovirus is able to survive for a relatively long period.

INTRODUCTION

Tropical fruits that are large and with skins that cannot be removed by peeling are prepared for eating by cutting away the skin and then slicing the fruit into smaller pieces. These additional manipulations in preparing the fruits enhance the risk of contamination with enteric microbes. The potential sources of enteropathogens contaminating fruits prepared in this manner by street vendors who then display them on a cloth-wrapped ice block in a closed transparent box are the vendor's hands, the knife used in peeling and cutting the fruits, the cloth on which the fruits are displayed and the pail of water used to wash the fruits.

When an inert non-reactive surface is contaminated by a virus, only environmental factors, such as temperature, determine the survival rate of the virus. However, on a reactive surface, the nature of a surface is also an important influencing factor. In this regard, as cut surfaces of tropical fruits coated with their exudates are biologically reactive surfaces, the survival rate of an enteropathogen inadvertently planted on them is an important determinant of the potential of a particular cut fruit as a transport vehicle for the pathogen.

There are some reports on the survival of enteric viruses in fruit juices (Konowalchuk and Speirs, 1976 a, b; 1978). But there is a paucity of reports on their survival on fruits (Konowalchuk and Speirs, 1975; Niu *et al,* 1992) and none on cut tropical fruits kept under conditions practiced by street vendors. The last mentioned observation and the ubiquitous presence of street vendors selling fruits suggest that an investigation into the importance of cut fruits as a vehicle in the transmission of enteric viral pathogen is needed.

This study investigated the ability of poliovirus to survive on cut surfaces of papayas under a number of parameters: exposure period, fruit ripeness, washing or otherwise of cut fruits prior to virus inoculation, time interval between fruit preparation and contamination. The choice of papaya in this study was not something plucked from the air as this large tropical fruit with skin not eaten is a popular fruit sold by street vendors in the tropics. The findings from this study were discussed in the context of cut papayas eaten raw as a transmission vehicle for poliovirus.

MATERIALS AND METHODS

Cell culture

The BSC-I cell-line was kindly provided by Dr DA Anderson from Macfarlane Burnet Center for Medical Research, Australia. The cells were grown in Eagle minimum essential medium (GIBCO, FlowLab, Sydney, Australia) with 0.08% $NaHC0_3$, 20 mM HEPES (GIBCO), 0.2 mM NaOH, 40 µl/ml gentamicin sulfate (Miramycin; Atlantic Laboratories, Bangkok, Thailand), 5 µl/ml amphoteracin B (Fungizone; GIBCO) and 10% FCS (GIBCO). Cell cultures were maintained in the same medium with 1% FCS (maintenance medium).

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Virus

Poliovirus type I, Mahoney strain, was also provided by Dr DA Anderson. Seed poliovirus diluted 100 times in medium without FCS was inoculated on confluent BSC-1 monolayers and allowed to adsorb for 60 minutes at 37°C. The infected culture then was kept in maintenance medium until 90% or more cells showed cytopathic effect. The culture then was subjected to 3 freezing-thawing cycles, the virus suspension concentrated 10 times and clarified by centrifugation at 15,800*g* for 5 minutes. The supernatant was filtered through a 0.2 µm pore-size filter (Minisart, Sartorius AG, Goettingen, Germany) and the virus filtrate aliquoted and stored at -70°C. The stock virus has a titer of 4.7 x 10^7 plaque-forming units (pfu)/ml.

Papaya

Carica papaya Linn cultivar Eksotika grown locally were used throughout this study. Unripe fruits, purchased from the same source, were allowed to ripen at room temperature. Fruits that reached the required ripeness but not yet used were kept at 10°C. Fully ripe fruits have a skin color index of 6 (all skin yellow) while less ripe fruits were used at color index 4 (skin with more yellow than green).

Ice box

A plastic aquarium cover with louvered surface was wrapped in aluminum foil and placed with the inside facing up on a tray. A hole in the foil allowed water to drain from the cover into the tray. A block of ice (21 x 16 x 5 cm^3), wrapped with a wet towel, was placed on the inside of the cover. The clear plastic trapezium-shaped aquarium (top: 37.5 x 20.7 cm^2; bottom: 34.5 x 18.0 cm^2; height; 27.5 cm) was inverted over the ice block. The ice box was placed in the laboratory in a location away from direct sunlight. The room temperature was 25-27° C. The air temperature just above the ice stabilized to 16°C in about 30 minutes. The box was always set up 30 minutes before use.

Experiment protocol in the recovery of poliovirus from cut papaya surfaces

Papaya was rinsed with tap water and then dried with paper towel. A 1-cm wide cross section was cut from the widest girth of the fruit and the required number of cubes (2.0 x 2.5 x 1.0 cm^3) cut from it using a metal mold. Each cube was placed on a 35 mm polystyrene tissue culture dish (Costar, Cambridge, MA, USA). In the second part of this study, 2 treatments were done before the papaya cubes were used. Fully ripe papaya cubes were each placed in 30 ml sterile distilled water in a beaker and swirled for 10 seconds before being transferred to a filter paper placed on a 9 mm Petri dish. Excessive water on the surfaces of each cube was removed by folding a section of the paper over each surface and gently pressing on it. In the second treatment, a set of fully ripe papaya cubes were kept in a closed Petri dish at 8°C overnight.

A 20 µl of poliovirus suspension (~5.0x10^5 pfu) was dropped on the center of the top surface of each cube which then was placed in the ice box. Immediately and at 1, 3, and 6 hours a cube was removed and pierced on one side with the sharpened ends of 2 applicator sticks which acted as a handle for manipulation of the cube. The inoculated surface was rinsed with 1 ml of cell culture medium without FCS (washing medium) which was allowed to drain into the plastic container. The inoculated surface was scraped gently with a blade and the scrapping added to the rinsing. The content was vortexed, centrifuged (15,800*g* x 3 minutes), and the supernatant filtered. The volume recovered was noted and a series of dilutions made. The average time from rinsing to dilution preparation was about 15 minutes. Three different dilutions (4-6 replicate wells) were titrated by plaque assay to determine the total number of viable virus rinsed from the surface. The plastic surfaces of tissue culture dishes, used as containers for papaya cubes, served as the inert surface control. In each experiment the titer of the inoculum used was also determined.

The study on each variable was repeated 4 times, each using a different fruit. The total number of infectious virus recovered in replicate experiments was expressed as the mean total pfu ± standard deviation (SD). The survival rate was expressed as a percentage of the inoculum used.

Experiment protocol in the recovery of inoculated poliovirus from surface and mashed content of cut papayas

The top surfaces of 2 papaya cubes were each inoculated with 20 µl of virus. After 1 and 5 hours exposure, all inoculated surface of a cube were rinsed with 1.0 ml of washing medium and then gently wiped with a sterile blade. Any substance on the blade was added to the rinsing. The rinsed and wiped cube was placed in a sieve and mashed to a puree. During this process 4 ml of washing medium was added in stages. The total volume of the mash was noted before it was centrifuged (4,900*g* x 5 minutes). A series of dilutions was made from the filtered supernatant and titrated for virus. The actual titer of the inoculum used in each experiment was also determined. The study was done in triplicate, each using a different fruit. The total number of

viable virus recovered in replicate experiments was expressed as the mean total pfu ± standard deviation (SD).

Plaque assay

Each well of a 6-well tissue culture plate (Costar) was seeded with 1 x 10^4 cells in 3 ml growth medium. The confluent monolayers formed 4 days later were rinsed once with phosphate-buffered saline (PBS) and each inoculated with 0.1 ml virus inoculum or test samples. Virus was allowed to absorb for 60 minutes at 37°C with gentle shaking of the plates every 15 minutes. Each well then was overlaid with 3 ml maintenance medium with 1.5% agarose (SeaPlaque, FMC Bioproducts, Rockland, USA). After the agar overlays had solidified, the plates were stacked and wrapped in plastic cling wrap (Glad Cling Wrap, First Brands Guangzhou Ltd, China). Three days after incubation at 37°C, 3 ml of 4% formalin in PBS was added to each well for 60 minutes. After washing, the agar overlays were removed and the cells stained with 0.2% crystal violet in 20% ethanol for 15 minutes. The plates were rinsed with water and the nurnber of clear areas (plaques) counted.

Statistical analysis

Student's *t*-test from the computer program STATISTICA was used to assess significant difference between 2 means.

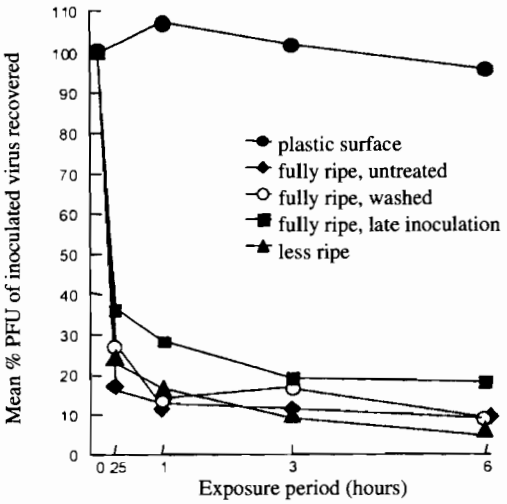
RESULTS

Recovery of poliovirus from cut papaya surface

Fig 1 shows that poliovirus was stable on plastic surface throughout the 6-hour period. However, when kept on the cut surfaces of fully ripe papayas, a sharp and rapid reduction in the number of infectious virus recovered was observed. Subsequently, there was no significant decline in the proportion of virus recovered and a residual population was detected up to 6 hours. The same pattern was observed for less ripe fruits, and also cut fruits rinsed in water or kept overnight before inoculation. At any period of exposure, the proportion of virus recovered from the surfaces of late inoculated papayas was higher than the other surfaces but the differences were not significant.

Recovery of poliovirus from surface rinsing and mash of the same cube

Table 1 shows that after 1 hour of exposure, a small proportion of inoculated virus was recovered from the papaya surfaces while about half this figure was detected from the same whole piece of papaya



Mean pfu of virus dose used (x10^5). Plastic surface 7.29; Fully ripe: untreated 7.55, washed 5.88, late inoculation 3.55; Less ripe: untreated 7.11

Fig 1–Recovery of poliovirus inoculated on the surface of cut papayas and plastic (control).

cubes after they were mashed. Inoculated cubes kept for 4 more hours showed nearly a 3-fold drop in virus recovered from the surfaces compared to 1 hour exposure. However, the proportion of virus recovered from the whole cubes was not very different between the 2 exposure periods.

DISCUSSION

Closed ice boxes are used by street vendors to maintain the freshness of cut fruits displayed for sale. However, they may also inadvertently enhanced the survival of poliovirus as the conditions of lower temperature and high humidity achieved are favorable to the virus survival (Abad *et al,* 1994). Notwithstanding the 2 conditions which support virus survival, the contact surface is also an important factor in survival. This was clearly shown by the stability of the virus throughout the entire exposure period of 6 hours when kept on a non-reactive plastic surface. In contrast, on the cut surface of papayas, the recovery of infectious poliovirus was greatly reduced after a short exposure. The loss in the recovery of infectious virus from contaminated papaya surface was attributed mainly to inactivation as the number of infectious virus recovered from the mashed papaya cubes after rinsing was far too low to account for the loss from the surface.

A number of factors may influence the survival rate of poliovirus on the cut surface of papayas. Ripeness is one as papayas can be eaten over

Table 1
Recovery of inoculated poliovirus from the surfaceand mashed content of cut papayas.

Exposure period to virus (h)	Total viable virus recovered from papaya	
	Surface washings	Mashed cubes
1	$6.51\pm3.94\times10^4$ (12.12%)[a]	$3.04\pm1.71\times10^4$ (5.66%)[c]
5	$2.24\pm2.16\times10^4$ (4.17%)[b]	$2.33\pm1.10\times10^4$ (4.43%)[d]

Figures given are the mean ± SD of 3 separate experiments, each using a different papaya.
Mean virus inoculum used was $5.37 \pm 1.19\times10^5$ ± pfu. Figure in parenthesis is the mean total number of infectious virus recovered expressed as % of the virus inoculum used.
[a-d]Significantly lower number of virus was recovered from papayas compared to the inoculum used: [a]($t = 6.528$, $df = 4$, $p = 0.0028$), [b]($t = 7.381$, $df = 4$, $p = 0.0018$, [c]($t = 7.300$, $df = 4$, $p = 0.0019$), [d]($t = 7.456$, $df = 4$, $p = 0.0017$).

a range of ripeness. During ripening, changes in composition (Lazan *et al,* 1989; Wills and Widjanarko, 1995) and pH (Lazan *et al,* 1989) that occur may effect the survival pattern of poliovirus. However, this study showed that over the range of ripeness in which raw papayas are pleasant to the human palate, any biochemical or physiochemical differences do not effect the survival of poliovirus.

Two events may meliorate the anti-poliovirus effect of cut papaya surface: the common practice of rinsing cut papayas with water after preparing them for eating, and late contamination of prepared fruits. Washing mechanically removes surface exudate and any biologically active molecules contained in it while late inoculation may allow the virus to be planted on a more hospitable surface if biological molecules with anti-poliovirus activities are active for only brief period. However, this study showed that both actions had no significant effect on virus inactivation and survival, and therefore the potential for papaya to transmit poliovirus.

From the perspective as a transmission vehicle for poliovirus, the rapid inactivation of most poliovirus particles soon after exposure to cut papaya surface reduces its efficiency in virus transmission-unless the fruits are eaten immediately after contamination. Although the potential as a transmission vehicle is thus greatly reduced, prolonged exposure which did not result in further significant decrease in virus infectivity left a small residual infectious virus population which may post a threat as a dose as small as 2 pfu of attenuated poliovirus was able to cause infection (Koprowski, 1955;1956).

In conclusion, preventing contamination is the best public health approach because once a papaya has been contaminated by poliovirus, a proportion of the virus is able to survive for an extended period unaffected by the ripeness of the fruits, time of contamination, rinsing the fruits before or after contamination.

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