Development of a Method for Recovery of Rotavirus from the Surface of Vegetables

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ABSTRACT

Rotaviruses cause waterborne disease outbreaks of gastroenteritis from sewage contaminated water, but methods have not been available to assess the potential for transmission of rotavirus by uncooked foods. A method was developed for recovery of rotavirus from the surface of vegetables. The simian rotavirus SA-11, used as a model for the human rotavirus, was adsorbed onto lettuce and the effects of various eluents tested for its recovery. The maximum recovery of rotavirus occurred with a solution of 3% beef extract at pH 8.0 after 5 min of exposure. Longer exposure times did not enhance virus recovery. Recovery of rotavirus and poliovirus type 1 (LSC) averaged 80 and 65%, respectively. Recovery of rotavirus from non-leafy vegetables was lower, averaging 44% for celery, carrot and radish. This method should prove useful for assessing the occurrence and survival of rotavirus on uncooked foods.

Epidemiological evidence suggests that a wide variety of human pathogenic viruses may be transmitted by food (6). Foods provide good media for persistence of viruses, but it is unlikely that virus multiplication takes place in any food (2). Methods and economic limitations have restricted most studies to detection of some human enteroviruses (10). Hepatitis A virus and Norwalk agent have been the most commonly documented viral food contaminants which infect man (3). However, these viruses cannot be grown easily or consistently in cell culture (10).

Rotaviruses can cause life-threatening gastroenteritis in humans and animals (7). Although they primarily cause diarrhea in children, serious outbreaks have occurred in adults (15). Rotaviruses are excreted in large numbers in the feces from infected individuals (9), and have been isolated from water contaminated with sewage (13). Crops which are irrigated with raw or treated wastewater could potentially transmit rotaviruses to humans since rotaviruses can survive conventional sewage treatment (13). Of particular concern are vegetables which have a relatively short growth period (lettuce and radishes) and are eaten raw. There have been a few studies on survival and recovery of some enteric viruses on vegetables (1,8). However, occurrence and persistence of rotavirus has only been studied in water (13), and methods have not been available for rotavirus recovery from vegetables. This study was designed to develop a method for recovery of rotaviruses from vegetables.

MATERIALS AND METHODS

Cell culture

The MA104 cell line (MA Bioproducts, Bethesda, Md.), was used for rotavirus assays and Buffalo Green Monkey (BGM) cells for poliovirus assay. The cells were grown in minimal essential medium (MEM, Flow laboratories #10-101-22) supplemented with 8% fetal bovine serum, 0.075% sodium bicarbonate, 100 U of penicillin/ml, 100 µg of streptomycin/ml, 50 µg of gentamycin/ml and 25 U of mycostatin/ml.

Viruses

Because of the difficulties with growth and assay of human rotavirus, the method was developed using the simian rotavirus SA-11, which is morphologically identical to the human rotavirus and shares many antigenic, physical and chemical properties. It has been used previously as a model for development of methods for human rotavirus detection in water (13). Rotavirus SA-11 was kindly supplied by H. H. Malherbe (11) and was grown in MA104 cells. Virus samples were diluted before assay in Tris-buffered saline solution which contained 1.58 g Trizmabase/L, 4.09 g of NaCl/L, 0.18 g of KCl/L, 0.028 g of Na_2PO_4/L, 0.10 g of dextrose/L, 100 U of penicillin/ml, 100 µg of streptomycin/ml and 25 U of mycostatin/ml.

Virus samples were enumerated by plaque assay, using MA104 cells and an agar overlay supplemented with trypsin (ICN, PLAINVIEW, N.Y.) 15 µg/ml and DEAE-dextran 100 µg/ml (12).

Vegetables

Fresh lettuce, celery, carrot, and radish for the laboratory studies were obtained from local grocery stores in Tucson, Arizona. Lettuce, celery stalk, and carrot were cut into small pieces with a surface area no greater than 10 cm² before addition of virus suspension to their surfaces. Radish was used as a complete bulb root.

Eluents

Four virus eluents commonly used for enteric virus elution from surfaces were tested for their ability to recover virus from vegetables. These eluents were 3% beef extract (GIBCO, Grand Island, N.Y.), 3% tryptose phosphate broth (TPB) (DIFCO

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Shake and take samples + 50 ml of eluent virus sample

Elution of virus from vegetables
Twenty grams of vegetable were placed in a flask and 100 ml of SA-11 virus suspension (containing 10⁴ to 10⁵ PFU/ml in Tris buffer) were added. This was then mechanically shaken for 20 min to allow virus adsorption and the supernatant fluid was decanted. A sample was taken from the decanted portion to determine the amount of unadsorbed virus.

Elution of the virus adsorbed to the lettuce was evaluated immediately by mixing 50 ml of each eluent with the lettuce for various periods up to 60 min. Eluents were immediately neutralized, filtered through 0.2-µm pore size membrane filter, and assayed. The entire procedure is shown in Fig. 1.

Celery, carrot, and radish were also evaluated for rotavirus recovery with 3% beef extract at pH 8.0 and a contact time of 5 min.

Data analysis
The quantity of virus adsorbed by a given vegetable preparation was estimated by the difference between the quantity of virus present in the initial inoculum which was added to the vegetable and the quantity of virus in the decanted supernatant liquid. The amount of virus eluted from a contaminated vegetable preparation was expressed as percent of that which was estimated to be adsorbed.

The data were analyzed by multiway analysis of variance with trial and culture plate replication (14). Transformation of the data to arcsin √percent/100 was used to satisfy the requirements for appropriate analysis of variance (16). For convenience, some analyses were made with a DEC-10 computer (University of Arizona Computer Center) using the program FACTAN (14). Confidence limits (CL) for means were calculated by the equation: CL = mean ± t(df) × error mean square.

RESULTS AND DISCUSSION

Effect of eluents and pH on rotavirus recovery
Four eluents commonly used to recover enteric viruses from filters and other solid surfaces were evaluated for virus recovery from lettuce. The eluents evaluated were 3% beef extract, 3% TPB, 0.05 M glycine buffer, and distilled water. They were evaluated at pH 8, and 9 after 10-, 30-, and 60-min exposure times and at pH 10 after 5, 10, and 15 min. The amount of virus eluted from lettuce at pH 8 and 9 was dependent upon the type of eluent, as well as contact time.

The 3-way interaction of conditions is illustrated in Fig. 2 in which the arcsin transforms of percent recovery averaged over the four trials are shown for the different conditions of the experiment. The analysis of variance on data illustrated in Fig. 2 was performed according to a 3-factor factorial with trial and sample replication. Ten minutes of exposure to beef extract at pH 8 was superior to any other combination of treatments. Distilled water or glycine buffer were obviously inferior, irrespective of pH and time of exposure.

Treatment of contaminated lettuce with these eluents at pH 10 was analyzed separately since exposure periods were reduced to 5, 10, and 15 min. The percent recovery of adsorbed viruses was generally lower than those observed at pH 8 and 9. A two-way interaction between eluent and time was significant and is illustrated in Fig. 3. At pH 10, the effects of increasing exposures on virus recovery were less pronounced than revealed at pH 8 and 9. This probably reflects the initial sensitivity of rotavirus to inactivation at pH 10 (4). The analysis of variance on data illustrated in Fig. 3 and 4 was performed according to a 2-factor factorial with trial and sample replication.

Shorter periods of eluent exposure were evaluated using beef extract and TPB at pH 8. Significantly more virus was recovered using beef extract with a 5-min contact time than other treatment combinations. The significant interaction between eluent and contact time (Fig. 4) shows that after 20 min of exposure, recovery of virus at pH 8.0 was lower than at 5 and 10 min and was essentially the same for beef extract and TPB.

Optimal recovery of rotavirus occurred at pH 8.0 with a solution of beef extract after a 5-min exposure. The recovery of rotavirus from celery, carrot, and radish was significantly lower than from lettuce and averaged 44%
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I  pH 8.0
VZZ  pH 9.0

Figure 2. Effect of eluent, pH, and contact time on elution of rotavirus from contaminated lettuce.

Figure 3. Effect of contact time and eluent type at pH 10.0 on the elution of rotavirus from contaminated lettuce.

Figure 4. Influence of contact time and type of eluent on recovery of virus at pH 8.0 from contaminated lettuce.

(Table 1). Optimal recovery of poliovirus from lettuce also occurred after a 5-min exposure time with beef extract and averaged 65% (Table 2).

These results suggest that rotaviruses are sensitive to increasing alkalinity, and that elution and recovery of infective virus at pH 8.0 is strongly influenced by choice of eluent and time of contact.

Rotaviruses are a major cause of childhood diarrhea and can cause life threatening disease in adults (5). They have been implicated as a major cause of traveler’s diarrhea in several studies, and have been responsible for numerous outbreaks of waterborne gastroenteritis worldwide (5). We have investigated at least one probable foodborne outbreak caused by rotaviruses, but methods for their detection in food were not available. This study provides methods for detection of rotavirus on food, and these methods are currently being used to study the occurrence of rotavirus on vegetables irrigated with wastewater, survival times on vegetables, persistence on food surfaces, and in the investigation of foodborne outbreaks of gastroenteritis.

TABLE 1. Recovery of rotavirus from contaminated vegetables using beef extract at pH 8.0 for 5 min.  

<table>
<thead>
<tr>
<th>Vegetable</th>
<th>Initial virus adsorbed, PFU (x 10^4)</th>
<th>Average virus recovered, PFU (x 10^4)</th>
<th>% Recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>Celery</td>
<td>9.1</td>
<td>4.5</td>
<td>49</td>
</tr>
<tr>
<td>Carrot</td>
<td>10.1</td>
<td>3.4</td>
<td>34</td>
</tr>
<tr>
<td>Radish</td>
<td>8.4</td>
<td>4.2</td>
<td>50</td>
</tr>
</tbody>
</table>

*Average of three experiments

REFERENCES


TABLE 2. Effect of time on poliovirus elution from lettuce with beef extract and TPB.

<table>
<thead>
<tr>
<th>Eluents</th>
<th>5 min</th>
<th>10 min</th>
<th>20 min</th>
<th>30 min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PFU (x 10^4)</td>
<td>% Recovered</td>
<td>PFU (x 10^4)</td>
<td>% Recovered</td>
</tr>
<tr>
<td>Beef extract</td>
<td>1.30</td>
<td>65^b</td>
<td>0.82</td>
<td>41</td>
</tr>
<tr>
<td>Tryptose phosphate broth</td>
<td>0.95</td>
<td>47.5</td>
<td>0.80</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>PFU (x 10^4)</td>
<td>% Recovered</td>
<td>PFU (x 10^4)</td>
<td>% Recovered</td>
</tr>
<tr>
<td>Beef extract</td>
<td>0.75</td>
<td>37.5</td>
<td>0.60</td>
<td>30</td>
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<tr>
<td>Tryptose phosphate broth</td>
<td>0.63</td>
<td>31.5</td>
<td>0.58</td>
<td>129</td>
</tr>
</tbody>
</table>

^Average of two experiments.

Approximately 2 x 10^4 PFU of virus was adsorbed to each lettuce section.
Middlemiss, et al., con't. from p. 260