ABSTRACT

Viruses contaminate foods and sometimes cause illnesses in consumers. Methods have been needed for detection of foodborne viruses both in routine field samples and in samples associated with outbreaks of disease. Viruses are detected by inoculating living hosts such as tissue cultures or laboratory animals. Food samples, made fluid if necessary, can be inoculated directly into the test host. This approach has resulted in several isolations of viruses from field samples of foods. If greater sensitivities are desired, larger samples must be tested. This usually requires that the sample be concentrated before inoculation into the test host, and concentration can be performed only if food solids in the suspension are at a minimum. A family of methods has been developed for extraction and detection of enteroviruses from food samples. More recently, a procedure for dislodging enteroviruses from food surfaces has been devised. These procedures do not possess all of the desired properties of an ideal test method. In particular, they cannot detect all of the viruses known to be transmitted in foods. They do offer the possibility that several samples could be tested per day, with good sensitivity, at a moderate cost per sample. The means for routine surveillance of foods for virus contamination are at hand. Growing points for further development of surveillance methods are discussed.

Viruses are sometimes transmitted by foods (7). Most food-borne disease is preventable with the means at hand, and the same is true of food-borne virus disease. Food-borne diseases occur, however, and surveillance methods are needed to assist in controlling them.

Surveillance of viruses presents some special problems because viruses are submicroscopic in size and are totally incapable of multiplying outside of appropriate living host cells. This means that viruses cannot multiply in foods and are seldom present in the quantities typical of many other foodborne disease agents. Another consequence of these properties is that, for most purposes, virus can only be said to have been detected when it produces a demonstrable infection in some living host.

Depending upon the virus sought, a suitable living host might be a human, a laboratory animal, or a tissue culture. Tissue cultures have been used exclusively in the authors' laboratory because of their convenience, uniformity, and relatively low cost (8). All living hosts are expensive, however, and none is ideal in other respects. Humans have varying immunological experience, and no laboratory host is susceptible to all of the viruses which infect man.

There are two situations in which foods might have to be tested. These have been designated "field sampling" and "epidemiologic investigations," depending principally upon whether or not people have been made ill by the foods. Properties to which to aspire in developing tests for each of these situations are shown in Table 1. Two of the entries need qualification. First, the 50% end-point of a test procedure is defined as the least quantity of virus which, if present in a sample, is at least 50% likely to be detected. Second, the "possible" virus types are those which might have caused the symptoms seen in an outbreak or which might occur in the food under scrutiny. Enteric viruses (those most common to the human intestinal tract) have usually been emphasized in developing detection methods for foodborne viruses, for several reasons.

DIRECT TESTING

Foods to be tested may be categorized as fluids, surface-contaminated solids, and permeable solids or semisolids likely to be contaminated in depth. In any event, the sample must be fluid before it can be inoculated into a test host. A laboratory diluent may be used as added fluid (3), or liquid from the food itself (e.g., shell liquor from oysters; 22), may be employed. Foods contaminated throughout may simply be homogenized with the diluent, or attempts may be made to extract the virus into the diluent (2, 27), leaving the food solids behind. As will be discussed below, surface-contaminated solid foods can probably best be sampled by dislodging the contaminant into the fluid phase with as little of the food substance as possible.

The fluid suspension thus obtained might be inoculated directly into the test host (13). If it is toxic or shows a high level of microbial contamination, further treatment may be necessary before inoculation. This might include centrifugation (20), filtration, addition of antibiotics (26), or treatment with an organic solvent (10). The last of these may not be applicable to certain situations in which lipid-containing viruses are sought, for organic solvents inactivate such viruses. However, the enteric viruses

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are not inactivated by most of the organic solvents. The approach which has been described may be called direct testing. The food sample receives a minimum of treatment and is then inoculated directly into a suitable living host. It works: several isolations of viruses and rickettsia from field samples of food have been achieved in this way. Viruses of the tick-borne encephalitis complex have been isolated from the milk of naturally infected goats in Russia (23) and Czechoslovakia (12) by mouse inoculations. The rickettsia of Q fever (Coxiella burnetii) has been demonstrated in samples of raw cow's milk and cream and, in one instance, a pasteurized mixture of the two by inoculation of guinea pigs (4, 11). The bovine strain (SF-4) of parainfluenza virus type 3 has been detected in the milk of at least 14 cows by tissue culture inoculation (17). Direct tissue culture inoculation has resulted in isolations of enteroviruses from market samples of mussels (3) and of ground beef (27), from vegetables from a sewage-irrigated field (1), and from foods and food-contact surfaces in an outbreak of illness at a children's day-care center (23).

Direct testing does place some practical limits upon the sensitivity which can be attained, but this may not be important. Unfortunately, there is no completely objective way of deciding how sensitive a test procedure for food-borne virus must be. One almost never knows how much of a virus is required to produce an infection by the oral route. Even if this information were available, it would seem unrealistic to say that a food was "unsafe" if it caused illness in everyone that ate it, but was "safe" if it infected only one of every 10 consumers. There are no enrichment techniques for viruses, so greater sensitivity can be achieved only by testing larger samples. This may not be possible if larger quantities of the food are not available; almost all of these are destructive tests. If larger samples of food can be obtained, one must then consider the quantities of tissue cultures or other laboratory hosts which would be required to test them by the direct method.

**Testing after Concentration**

It seemed unlikely that the level of sensitivity specified in Table 1 could be achieved by direct testing because of costs (15). The most attractive alternative approach was to concentrate the food suspension before inoculating it into tissue cultures. Such concentration simply reduces the volume of sample to be tested. Many concentration methods have been examined and ultracentrifugation (9) and dialysis against hydrophilic substances (5) appear to be the most reliable. Each has been used successfully by others to detect viruses in field samples of shellfish (18, 21). The two have now been combined. A 25-g food sample is concentrated first by dialysis against polyethylene glycol 20,000 and then by ultracentrifugation so that the final volume (0.5 ml) can be tested in a single tissue culture (15). Most of the food solids must be eliminated from a sample suspension if this great a concentration is to be performed.

**Foods contaminated in depth**

Several solid and semisolid foods were inoculated with laboratory strains of enteroviruses. The virus was assumed to be within the food, rather than on its surface. Some foods gave the best separations and virus recoveries if treated with trichlorotrifluoroethane (Freon TF, DuPont) and bentonite, while others (usually those low in protein) were better treated with Freon and agamma serum (Fig. 1). Using the method found to be best for each of the foods, the 50% end-point for detection of three 'model' enteroviruses was in the range of 2 to 4 infectious units per sample (16): It was also determined that neutralized enteroviruses, such as might be shed during the convalescent phase of an infection, were reactivated by the Freon treatment.

These methods are complex but are not difficult to learn. It is estimated that an experienced operator, with proper equipment, could test 12 to 24 samples per day at a direct cost of $4 to $8 per sample. These

**Table 1. Detection procedures for food-borne viruses:**

<table>
<thead>
<tr>
<th>Property</th>
<th>Routine field sampling</th>
<th>Epidemiologic investigations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size</td>
<td>10 - 100g</td>
<td>100 - 1,000g</td>
</tr>
<tr>
<td>Sample number</td>
<td>10-50/operator-day</td>
<td>4-10/operator-day</td>
</tr>
<tr>
<td>Direct cost/sample</td>
<td>$5</td>
<td>$25</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>50% end-point = 1 infectious unit/sample</td>
<td></td>
</tr>
<tr>
<td>Spectrum</td>
<td>100% of possible virus types</td>
<td></td>
</tr>
<tr>
<td>Elapsed time</td>
<td>≤7 days</td>
<td></td>
</tr>
</tbody>
</table>

**Figure 1. Detection methods for enteroviruses which contaminate foods in depth (schematic summary).**
procedures will have to be adapted further to permit their use in detecting agents other than the enteroviruses. Larger or more labile viruses tend to be inactivated or lost during the processing.

**Surface contaminated foods**

The situation is somewhat different with surface-contaminated foods. It seemed that it should be possible to dislodge the virus from the contaminated surface into a fluid suspension without including any of the substance of the food. This, in turn, should simplify preparation of the sample for concentration and might even permit use of simpler, more rapid methods of concentration than those applied to clarified food suspensions. Model foods used in the initial studies on surface sampling included tomatoes, peaches, and (in the winter) well-worn tennis balls. These were contaminated with standard quantities of feces from a child who was undergoing immunization with trivalent oral poliomyelitis vaccine. The fecal specimens had titers in excess of $10^6$ plaque-forming units (PFU) of poliovirus per gram as determined in tissue culture by methods described previously (8).

The exploratory phases of the study included tests of surface-active agents, an ultrasonic probe, and brushing as means of dislodging the virus from the food. It was found that essentially all of the virus could be dislodged by rubbing a glass stirring rod over the inoculated area, and that none of the more elegant techniques listed was quite as good. The disadvantage of the glass rod was that it was not well suited to sampling the entire surface of a food when the point of contamination was unknown. An apparatus devised for this purpose is shown in Fig. 2. The sample (a tomato) is placed in a 2-qt., wide-mouth fruit jar of square cross section. The lid of the fruit jar has been punctured off-center and fitted with a steel shaft which is driven through a flexible rubber connection by an electric motor and continuously variable transmission. The shaft on the jar lid is passed through an improvised bearing, and the butt end of the jar is supported by floating it in a pan or sink of cold water (not shown). Also inside the jar are approximately 20 common glass marbles and 100 ml of 0.03 M phosphate buffer (pH 7.2). The jar is turned at approximately 100 rev/min for 5 min, and the fluid washings are collected.

It was found that essentially all of the contaminating virus was recovered from the surfaces of the test foods by this method. Limited tests were also done with pears, strawberries, and lettuce. Periods of storage between contamination and testing were found to have little influence upon the tenacity with which the virus adhered to the food surface. Food solids were not present in the washings unless portions of the surface were badly decayed. Surface dirt, fecal solids from the model contaminant, and a variety of bacteria appeared in the washings, but these were of no concern at this stage of the work.

The next task was to concentrate the washings to permit detection of lower levels of contamination. Since there were no food solids in the suspension, it seemed possible that the virus could be collected by adsorption to membrane filters and eluted in a small volume of fluid (6). Unfortunately, dirt from the food surface made the suspension very difficult to filter; and even when enough of the dirt had been eliminated to permit filtration, the virus did not adsorb efficiently to the membrane filters. The aqueous polymer two phase system, which has been used to concentrate viruses from water and sewage (19, 24), was also tested. The dextran sulfate in the virus concentrate was found to inhibit strongly some of

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**Figure 2.** Apparatus for dislodging viruses from contaminated food surfaces.

**Figure 3.** Recovery of CB-3 virus from experimentally contaminated tomatoes.
the enteroviruses used in evaluation experiments (14). It was decided that for the time being the concentration procedure outlined in Fig. 1 would be used with surface washings. The first model agent in these studies was Coxsackie virus type B-3 (CB-3), strain Nancy.

Two problems were encountered at times with the concentrated samples. First, the quantity of inoculated feces used to contaminate the tomato surface was sometimes sufficient to interfere with concentration. Second, surface microflora were sometimes encountered which concentrated with the virus and could not be suppressed by any of a number of antibiotics. Serial membrane filtrations solved neither of these problems very efficiently. Centrifugation at 7,500 rev/min for 20 min was found to eliminate fecal solids when necessary. Microbial contaminants were suppressed by adding 1 ml of chloroform to the wash water at the time that dislodgement of virus from the sample surface was begun. This complicated the process somewhat, because the chloroform is incompatible with the polyallomer ultracentrifuge tubes used in concentration and is highly toxic to tissue cultures. Cellulose nitrate tubes were used during the polyethylene glycol concentration step with samples containing chloroform. Surplus chloroform was removed at the end of this step by centrifugation at 3,000 rev/min for 20 min, and the supernatant fluid was collected by aspiration. Air, sterilized by membrane filtration, was sparged through each sample for 10 min to entrain any residual, dissolved chloroform. The final ultracentrifuge concentration shown in Fig. 1 was then carried out as usual.

Over a period of time, a total of 32 tomatoes contaminated with low levels of CB-3 virus have been tested in this manner. Chloroform was used in the majority of these tests. The results are shown in Fig. 3. The 50% end-point for this series of determinations is somewhat less than 2 PFU per tomato, and the ratio of virus recovered to virus inoculated is of the order of 49%. This suggests that the method is somewhat more sensitive than that developed for internally contaminated solid foods, as judged by either parameter; however, recoveries in limited trials with another enterovirus, ECHO virus type 6 (EC-6), strain D’Amori, have not been as good. EC-6 had proved to be an unusually difficult enterovirus in past studies involving other properties (8, 14, 16). Tests are continuing.

**Discussion**

Methods are now available for the surveillance of many foods for enterovirus contamination. Neither the procedure for internally contaminated foods, reported previously, nor the method for surface contaminated foods, described here for the first time, entirely fulfills the hopes expressed in Table 1. Direct costs have been estimated at $4 to $8 per sample for the methods summarized in Fig. 1, and those for the surface sampling procedure should be comparable. Though the sensitivities of the two systems still leave something to be desired, they appear to be significantly better than any of those described by others. The size of an internally contaminated sample in a field testing situation was arbitrarily standardized at 25g, while tomatoes for the surface sampling procedure have been as large as 100 to 140g. These can be tested at the rate of 12 to 24 per operator-day. Larger samples, such as might be acquired when investigating an outbreak, would have to be divided. This would result in a proportionate loss in the number of samples which could be tested per day.

There are several growing points for future development of detection methods for food-borne viruses. One of the most significant is speed. Any serological, physical, or chemical method which would detect virus would be faster than the infectivity process, though probably with a sacrifice of 100-fold or more in sensitivity. These alternative methods might well require more expensive equipment, whereas the authors’ research program is dedicated to devising procedures which allow use of less expensive facilities, but the trade-off might be justified in some circumstances.

More immediate goals are to adapt the test methods to a broader range of foods and to permit detection of a broader spectrum of viruses. Many of the direct test methods will permit detection of a broader range of viruses at some loss of sensitivity to any one type. The manipulations involved in preparing samples and concentrating them would inactivate some viruses outside the enterovirus group. The laboratory hosts in which infectivity is demonstrated also impose a significant limitation upon spectrum: there is no one of them which is susceptible to all of the viruses which infect man.

It seems highly unlikely that the perfect test method, able to detect all viruses in all foods at a low cost per sample, will ever materialize. Still, the means at hand could be expected to provide some very worthwhile information if they were applied to foods in the field on a routine basis.

**Acknowledgements**

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