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Assessment of Cooked Prawns as a Vehicle for Transmission of Viral Disease

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ABSTRACT

A method has been developed for recovery of viruses from cooked peeled prawns. The method involves elution of viruses from the surface of prawns using a suitable buffer, clarification of the extract by centrifugation and concentration of viruses present by ultracentrifugation. In trials with laboratory-contaminated samples of prawns the method recovered at least 70% of added poliovirus 1. The survival of poliovirus 1 on the surface of cooked peeled prawns was followed during storage at 4-6°C and -20°C for up to 15 d and up to 300 d, respectively. A substantial proportion (22-75%) of added virus remained infective for the periods that this product is usually stored either during transport and distribution or in the home. Thirty retail samples of cooked peeled prawns were examined for presence of viruses infective for man. Viruses were not isolated from any sample

Several surveys have indicated that cooked prawns marketed in Australia are often produced and handled unhygienically, and suffer considerable bacterial contamination after cooking (*1,10*). This mishandling could also lead to contamination of cooked prawns with human enteric viruses which are transmissible by food, for example hepatitis A virus and poliovirus. If such viruses are able to remain infective on cooked prawns for the periods that this product is stored during transport and distribution and in the home, cooked prawns could act as a vehicle for viral disease. Studies with some other flesh foods suggest that enteroviruses might be quite stable on cooked prawns and other crustaceans. However, it is not possible to predict confidently the behavior of enteroviruses in a food until appropriate experiments have been performed, since relatively minor components of a food can markedly influence virus stability (*2*). During this study an attenuated strain of poliovirus type 1 was used as a model virus for development of a method for recovery of enteroviruses from cooked peeled prawns and for assessing the stability of enteroviruses on this product. Retail samples of cooked peeled prawns were examined for presence of enteroviruses.

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MATERIALS AND METHODS

Virus and cell cultures

The human diploid fibroblast cell line MRC-5 was obtained originally from Commonwealth Serum Laboratories (CSL), Melbourne, Australia. Primary monkey kidney epithelial cell cultures derived from *Macaca fascicularis* (Cynomolgus) monkeys were prepared from tissue suspensions supplied by CSL. The LS-c, 2ab strain of poliovirus 1 was used. Poliovirus suspensions were prepared in MRC-5 cell cultures and diluted to an appropriate titer in cell culture maintenance medium after low-speed centrifugation to remove cell debris. The virus and cell cultures were propagated and handled using routine virological procedures (*5,9*).

Qualitative detection of viruses

MRC-5 and primary monkey kidney cell cultures were prepared in 150 × 16 mm culture tubes. The growth medium was removed, 0.3 ml of sample extract was placed on the cell monolayers and the cultures were incubated at 37°C for 90 min with frequent rocking. The inoculum was removed, maintenance medium was added and the cultures were incubated at 37°C. The cultures were examined for cytopathic effects every 2 d for 2-3 weeks. Each culture was blind-passaged at least once. Inoculated cultures contaminated with bacteria or fungi or showing evidence of sample toxicity were frozen and thawed three times to disrupt intact cells and then passaged after membrane-filtration when necessary. During the survey, half of each extract was tested in each type of cell culture.

Poliovirus plaque assay technique

The sample to be assayed was serially diluted with cell culture maintenance medium. The growth medium was removed from confluent monolayers of MRC-5 cells grown in 50-mm diameter petri dishes and the monolayers were washed with maintenance medium. The dishes were placed on a levelled bench and 0.1 ml of sample dilution or maintenance medium (virus-free control) was delivered to the center of each dish. After 30 min at room temperature the inoculum was removed and each culture was overlaid with 6 ml of medium 199 (CSL) containing 1% (v/v) fetal calf serum and 0.75% (w/v) agarose at 42°C and allowed to set. The cultures were incubated for 3 d at 37°C in a humidified atmosphere containing 5% (v/v) CO_2. After incubation the monolayers were stained with the vital stain neutral red, plaques were counted and the number of plaque-forming units (PFU) of poliovirus in the original sample was calculated. Dilutions counted had an average of no more than 40 plaques per dish, and, where possible, at least 100 plaques were counted in each assay. Each of the above operations was performed in a highly standardized manner.

Recovery of viruses from prawns

Cooked peeled prawns were purchased chilled or frozen in retail packages containing at least 100 g, transported to the laboratory in insulated containers and stored at 4°C until tested within 24 h. Frozen samples were thawed overnight at 4°C. A 25-g sub-sample of prawns was placed in 80

TABLE 1. *Recovery of poliovirus from cooked peeled prawns.*

Batch of prawns	Storage time (h)[a]	Poliovirus added (PFU/sample)	Poliovirus recovered (PFU/sample)	Recovery of virus (%)
A	0.5	580	460	79
B	3	350	290	83
B	3	18	16	89
C	16	700	530	76
C	16	33	23	70

[a]Elapsed time between contamination of prawns and commencement of recovery procedure.

ml of glycine-NaOH buffer, pH 8.8 (0.09 *M* glycine, 0.01 *M* NaOH) and shaken vigorously by hand for 2 min then for 1 min in every 5 min until 30 min had elapsed. This suspension was clarified by centrifugation (12,000 × *g* for 30 min at 4°C) and viruses present in the supernatant fluid were concentrated by ultracentrifugation (105,000 × *g* for 5 h or 193,000 × *g* for 4 h at 4°C). The pellet was resuspended in 2 ml of cell culture maintenance medium and stored at -20°C until tested for the presence of viruses using the qualitative technique or the plaque assay above. When smaller samples were tested, the above volumes were adjusted accordingly. All operations were performed aseptically.

Assessment of the virus recovery method

Samples of prawns were treated as above with the exception that 0.1 ml of poliovirus 1 suspension or 0.1 ml of maintenance medium (uncontaminated control samples) was placed on the surface of the prawns in each 25-g sample. The samples were then stored at 4°C for various periods before addition of glycine-NaOH buffer (Table 1). An uncontaminated control sample was tested in parallel with each contaminated sample.

Storage trials

Freshly cooked prawns were purchased, peeled aseptically and 10-15 g of prawns (about 6 prawns) were placed in each of 20 sterile 100-ml jars. Four jars were set aside as uncontaminated control samples. The surface of the prawns in each of the remaining jars received 0.1 ml of poliovirus 1 suspension. The jars were sealed and stored in a domestic refrigerator at 4-6°C or in a freezer at -20°C. The survival of poliovirus was assessed after various periods (Table 2).

Examination of retail samples of prawns

Thirty samples of cooked peeled prawns were purchased over a 1-year period from 9 retailers and examined as described above.

RESULTS

Preliminary qualitative trials indicated that the method described would recover poliovirus 1 that had been added to prawns, yielding an extract which was usually non-toxic and free from bacterial or fungal contaminants which interfered with testing in cell cultures. Table 1 shows the virus recovery efficiency of the method in experiments with three different batches of prawns. Poliovirus was also detected qualitatively in each of these extracts using MRC-5 cell cultures. No cytopathogenic agents were recovered from control samples of prawns at any time during this study.

The results of the storage trials are in Table 2. Virus inactivation was more rapid at 4-6°C than at -20°C, but a substantial proportion of added virus remained active under both storage conditions. Prawn samples held at 4-6°C for 8 d showed signs of spoilage (unpleasant odor and appearance), and yielded extracts that were mildly toxic to cell cultures. Prawn samples held at 4-6°C for 15 d were very badly spoiled and their extracts were very toxic to cell cultures. Although quantitative testing of the latter extracts was not possible, infective poliovirus was detectable in 0.1 ml of the concentrated extract from these samples.

TABLE 2. *Survival of poliovirus 1 on cooked peeled prawns during storage.*

Storage temperature (°C)	Storage time (d)	Recovery of virus × 10^2PFU/sample[a]	Recovery of virus %
4-6	3	7.8	60
		8.8	68
	8	2.9	22
		3.1	24
	15	+[b]	
-20	8	9.7	75
		7.0	54
	15	7.4	57
		8.3	64
	30	5.9	45
		4.9	38
	180	6.4	49
		6.0	46
	300	3.6	28

[a]1.3×10^3 PFU of poliovirus were added to each sample.
[b]Infective virus present.

Viruses were not isolated from any of the retail samples of cooked peeled prawns. Four of the 30 samples purchased could not be tested adequately in cell cultures because of microbial contamination or severe toxicity of the extracts towards cell cultures.

DISCUSSION

The results indicate that the virus recovery method described here is capable of recovering realistic numbers of infective enterovirus particles from cooked peeled prawns. The method is based on techniques previously used successfully for elution of viruses from food surfaces (*6,7,11*) and concentration of viruses (*3,6*). The sensitivity of the method allowed use in the storage trials of more realistic levels of poliovirus contamination than have been used in many earlier studies of virus stability, where more than 10^6 infective units of enteroviruses have been added per gram of food. In practice, contamination resulting from unhygienic food handling is unlikely to lead to more than a few hundred PFU of virus being transferred to 100 g of food.

The storage trials illustrate the hardiness of human enteroviruses in environments outside their host. The reduction in poliovirus titer observed in prawns stored frozen or chilled was insufficient to substantially affect the public health risks associated with contamination of this nature. A proportion of added poliovirus remained infective at 4-6°C despite gross microbial spoilage of the prawns. The rate of inactivation of enteric bacteria, e.g. salmonellae, in frozen prawns is likely to be considerably faster than the rate of inactivation of poliovirus observed here (*4*).

The above conclusions are probably also applicable to human enteroviruses other than poliovirus 1. Although the various enteroviruses do differ in their behavior in foods, the differences are usually not large and the behavior of poliovirus 1 is usually representative of the behavior of a range of other enterovirus types.

Although several viral diseases are known to be transmitted by food, foods other than molluscan shellfish have rarely been surveyed for contamination with human enteric viruses. The evidence for involvement of cooked foods in transmission of viral disease is almost entirely epidemiological. The failure to detect viruses in cooked prawns during the present survey is in accord with the experience of Kostenbader and Cliver (*8*), who obtained negative results in the most thorough recorded attempt to isolate viruses from retail food samples. After examining 60 retail food samples and a variety of other related samples, they concluded that the incidence of viruses in foods marketed in the U.S.A. was probably so low that surveys of this kind are unlikely to yield positive results. Thus, although foods such as cooked prawns are quite capable of transmitting viral diseases, the available evidence suggests that such transmission is not a frequent occurrence.

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