Viruses Introduced by Foods

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This review describes the viruses (hepatitis A and E, Norwalk virus, rotavirus, astrovirus) and their detection in foods.

INTRODUCTION

Human enteric viruses introduced by foods replicate in the intestines of infected hosts and are excreted in the faeces. A distinctive property of enteric foodborne viruses is that they are generally adapted exclusively to human hosts.

Viruses are increasingly recognised as important causes of foodborne illnesses all over the world. Almost all enteric viruses contain RNA coated only with proteins. The particles cannot multiply in foods. The contamination may occur through contact with faecally contaminated water or poor personal hygiene practices of infected food handlers.

Human enteric viruses are environmentally stable and have low infectious doses. After enteric viruses are ingested some have their principal site of action in the lining of the small intestine. Others infect the liver and still others affect other parts of the host's body. Diseases results from killing of infected cells by the viral replicative process or from destruction of infected cells by the host immune response.

Viral gastroenteritis is reported as the most common foodborne illness in many countries. Recent data indicate that 10% of the cases of foodborne illness with unconfirmed etiology, reported from 1973 to 1987 in the U.S., were due to viral gastroenteritis. The apparent failure to confirm a viral aetiology in such outbreaks has been due largely to the lack of available tests, resulting in a drastic underestimation of the importance of foodborne viral infection.

The most common types of foodborne viral disease are infectious hepatitis A and acute viral gastroenteritis associated with Norwalk agent and other related small, round structured gastrointestinal viruses (SRSV). Rotaviruses, some adenoviruses and hepatitis E virus are important causes of outbreaks of waterborne disease, particularly in developing countries. Foodborne outbreaks due to SRSV, parvoviruses and astroviruses are occasionally reported. Foodborne outbreaks associated with human enteric viruses are almost always due to the consumption of faecally contaminated raw or undercooked shellfish and ready-to-eat products contaminated by infected food handlers.

HEPATITIS VIRUSES

Hepatitis A and E, which are different serologically, are transmitted enterically. Both of them infect the liver producing clinically indistinguishable illness (1) (2). The symptoms are jaundice, anorexia, vomiting and malaise. Hepatitis E is much more serious in pregnant women (mortality rate 17–33%) than hepatitis A (3). Hepatitis A is more likely to cause asymptomatic or mild infections in young children than in adults, whereas hepatitis E infects less often young children.

The virus is shed in faeces for 10 to 14 days before and 1 to 2 weeks after the onset of the illness during which time handling food items can lead to contamination.

Food-associated outbreaks of hepatitis have been recorded in cases where food was eaten uncooked or was contaminated just before it was eaten. There is no epidemiological evidence for the transmission of hepatitis E.

NORWALK (NV) AND RELATED VIRUSES

NV and related SRSV are a major cause of foodborne disease (4). Currently the Norwalk virus constitutes the fifth cause of foodborne illnesses among outbreaks reported in the U.S. NV has been proposed to belong to the Caliciviridae family and this family includes Snow Mountain agent, Hawaii agent and Tauton agent. Diagnosis is based on detection of the viral particles in patients' stool samples by electron microscopy, immune electron mi-

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croscopy, enzyme immunoassay or on demonstration of antibody against the virus in patients' serum samples by enzyme immunoassay. The antibody prevalence rates have been reported to be 50–70% of adults by the fifth decade of life in the United States, while the corresponding number for England is over 90% These high rates of anibody prevalence indicate that the population experience recurrent NV associated gastroenteritis since antibodies seem not to protect from illness (5).

The symptoms of the NV associated gastroenteritis are the common ones (nausea, diarrhoea, vomiting etc.). The incubation period ranges from 24–48 h. The faecal shedding may continue a week after the gastroenteritis. The viruses infect and kill cells of the small intestinal mucosa. The viruses are stable in food for approximately 3h at pH 8.7 at room temperature and for 60 min at neutral pH at 60°C. The causative agents of gastroenteritis have been found in shellfish eaten raw or slightly cooked. There is no vaccine for Norwalk-like viruses.

ROTAVIRUSES

Gastroenteritis caused by Rotaviruses serogroup A is common in infants throughout the world and is a significant cause of death in developing countries (6). Although most rotaviral gastroenteritis involves infants they can also affect older individuals. The incubation period is 1 to 3 days and the virus is shed during illness and no longer than 8 days. Infection begins in the enterocytes of the proximal small intestine but eventually involves the jejunum and ileum. Rotavirus survives for many days at 4 or 20°C in vegetables and is unstable outside the pH range of 3 to 10.

Group B rotavirus differs from the group A antigenically Group B rotavirus infections occur more frequently in adults than in children.

ASTROVIRUSES (7)

The astroviruses are among the small, round structured viruses seen with the electron microscope. Their incubation period is 3 to 4 days, diarrhoea is more typical than vomiting and the duration of the illness is 2 to 3 days. Shedding of the virus in feces lasts at least during the period of diarrhoea. The virus infects the small intestine and is resistant to acidic pH. In the United Kingdom 70% of children have antibody against astrovirus by 3 to 4 years of age.

Recent outbreaks of food-borne viral outbreaks are summarised in Table I.

DETECTION OF HUMAN ENTERIC VIRUSES IN FOODS

Traditional methods to directly detect viruses in foods after contamination have been based on the ability of enteric viruses to infect live mammalian cells in culture (6). Quantitative and enumerative methods using a variety of mammalian cell culture lines, generally from primate kidneys, have been reported. Such approaches have been limited because levels of contaminating virus generally are low (1–200 infectious units per 100 grams of shellfish) (8); residual food components interfere with assays and the epidemiologically important viruses do not replicate (SRSV viruses) or replicate poorly (hepatitis A virus) in mammalian cell culture. Alternative methods such as enzyme-linked immunosorbent assay (ELISA) and DNA/RNA probes have been reported but are limited by high detection limits ($>10^3$ infectious units), unavailability of reagents and poor sample quality (9). These difficulties are illustrated by the confirmation of viral contamination in a food in only two reported instances (10).

The in vitro enzymatic amplification method of polymerase chain reaction (PCR) offers an opportunity to enrich a single specific nucleic acid sequence up to a millionfold and hence provides a sensitive and specific method with a theoretical detection limit of one virus unit (11). This method is readily adaptable to the detection of RNA viruses by preceding the PCR with a brief reverse transcription (RT) step, hence the designation RT-PCR. The recent cloning of the Norwalk agent and related SRSV has provided an opportunity to develop effective molecular detection methods for these previously nondetectable agents.

The application of PCR methods to the detection of human enteric viruses in foods is an area of active re-

Table I

Recent outbreaks of foodborne viral disease (12)

Agent	Location	Date	No Cases	Food
HAV	China	Jan 1988	300 000	Raw clams
HAV	U.S.	July–Aug 1988	61	Raw oysters
SRSV	U.S.	Nov 1988	40	Raw oysters
SRSV	U.S.	Nov 1993	180	Raw/steamed oysters
SRSV	U.S.	Dec 1994	34	Steamed/roasted oysters
SRSV	U.S.	Jan 1995	3	Oysters
Norwalk	U.S.	Sept 1987	191	Commercial ice
Norwalk	U.S.	July 1988	1140	Celery/chicken salad

search. However, the development of such methods is complicated by low levels of contamination, high sample volumes and the presence of food components, which may interfere with enzymatic amplification reactions. There are three alternative approaches that have been used to simultaneously reduce sample volumes and the level of interfering compounds. The most frequently applied approach involves isolating and purifying RNA from the food sample before RT-PCR. A second approach combines capture of the virus with specific antibody followed by nucleic acid amplification by using RT-PCR. In the third approach, the intact virus particle is concentrated and purified from the complex food matrix resulting in sample volume reduction and removal of inhibitors, followed by subsequent heat release of viral nucleic acid from the virion capsid and RT-PCR. All three methods have been applied to various shellfish species and in some cases to other food items and naturally contaminated field shellfish specimens. Despite enormous strides in the ability to detect human enteric viruses with PCR the technique is still limited by the absence of effective concentration methods, the presence of enzymatic inhibitors and the inability to distinguish between infectious and noninfectious virions.

Avoiding faecal contamination of food is the surest way of preventing food-associated transmission of viruses. It is known that faecal and viral contamination occurs either from an infected food handler or from wastewaters.

Now that a vaccine against hepatitis A is available, it is advisable to immunise professional food handlers who are not already immune.

Enteric viruses are likely to persist for weeks in refrigerated foods and indefinitely in frozen foods. Proper hand washing and exclusion of ill persons from handling food are important preventive measures. Also the contamination of food with polluted water or wastewater should be avoided. The proper use of UV light and chlorine is able to inactivate viruses in water and on surfaces but the inactivation of viruses within a food can be successfully accomplished by cooking to temperatures that will kill vegetative bacterial pathogens.

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