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Human Enteric Viruses as Causes of Foodborne Disease

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ABSTRACT: Recent epidemiological evidence indicates that enteric viruses are the leading cause of foodborne disease in the U.S.A. and, indeed, worldwide. Certainly, advances in epidemiology and molecular biology have improved the ability to study this previously elusive group of foodborne pathogens. The purpose of this article is to review the agents, transmission routes, epidemiology, persistence, diagnosis, and detection of foodborne viruses and their diseases, with specific reference to the role that contemporary technologies have had in improving our understanding of this important group of emerging foodborne pathogens.

Emergence Of Viral Foodborne Disease Agents

Since the discovery of Norwalk virus (NV) in 1972 (Kapikian and others 1972) and rotavirus in 1973 (Bishop and others 1973), the viral etiology of acute nonbacterial gastroenteritis has been well established, and a considerable number of enteric viruses are now recognized as human pathogens (Chiba 1996; McCarthy and others 2000). Recently, estimates of total illnesses, hospitalizations, and deaths due to foodborne diseases in the U.S.A., based on the compilation of data from multiple surveillance systems, approximated 30.9 million (80%) of the 38.6 million total foodborne illnesses annually to be attributable to viruses, making them the leading cause of foodborne illness in the U.S.A. (Mead and others 1999). Over the y, however, the prevalence of viral gastroenteritis in the U.S.A. and worldwide has been drastically underestimated for a number of reasons. For instance, laboratory confirmation of viruses as the cause of foodborne illness has been based on the detection of viral particles or antigen in stool or by seroconversion, that is, a rise in specific antibody to the virus (Hedberg and Osterholm 1993). These laboratory methods for antigen or serological testing continue to be developed and refined and are not always available, and laboratory confirmation of etiology has not been common practice until recently (Hedberg and Osterholm 1993). In most cases, viral contamination in food is not detected, either due to the lack of an appropriate method or the unavailability of food specimens (Cliver 1995). There is also a general reluctance by public health officials to classify foodborne outbreaks as viral solely on the basis of epidemiological criteria (Bean and Griffin 1990), and a general failure to report and investigate outbreaks of mild gastrointestinal disease. Taken together, these factors have contributed to an underestimation of the true scope and significance of foodborne viral infection (Hedberg and Osterholm 1993).

Fortunately, recent advances in epidemiology and molecular biology have improved the ability to study this previously elusive group of foodborne pathogens. The purpose of this article is to review the agents, transmission routes, epidemiology, persistence, diagnosis, and detection of foodborne viruses and their diseases, with specific reference to the role that contemporary technologies have had in improving our understanding of this important group of emerging foodborne pathogens.

Viral Foodborne Disease Agents

Viruses that are known to be transmissible through foods, and are of concern to human health, are shed in the feces of infected humans and transmitted via the fecal oral route (Table 1). Human enteric viruses have properties that make them quite different from the common bacterial agents of foodborne disease. As obligate intracellular parasites, they require live mammalian cells in order to replicate. To protect the viral genome from inactivation outside of infected cells, virus particles have properties that make them environmentally stable to the extremes of pH and to enzymes that are present in the human gastrointestinal tract. This stability enables virus particles to survive a variety of food production, processing, and storage conditions, making virtually any type of food product a potential vehicle for transmission of viral pathogens (Jaykus 2000b). The inability of human enteric viruses to replicate in foods, and the fact that they are generally present in low numbers, does not ensure product safety, as the infectious doses (10^0 to 10^2 infectious units) are presumed to be low (Iversen and others 1987; Moe and others 1998; Jaykus 2000a).

Human enteroviruses

The human enteroviruses, classified within the *Picornaviridae* family, have been shown to be present in human feces and in domestic sewage (Cliver 1994b). Approximately 66 immunologically distinct serotypes of the human enteroviruses are known to cause infections in humans, including the polioviruses, group A and B coxsackieviruses, and the echoviruses, and the more recently designated enterovirus serotypes 68 to 71 (Andreoletti and

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Table 1–Viruses associated with food and waterborne disease outbreaks

Astroviruses
Human Enteroviruses (polioviruses, groups A and B coxsackieviruses, and echoviruses)
Hepatitis A virus
Hepatitis E virus
Norwalk-like viruses (NLVs, for example, Norwalk and Snow Mountain)*
Sapporo-like viruses (SLVs)
Parvoviruses
Rotaviruses
Small round viruses

*Norwalk virus is the prototype genogroup I member of the NLVs;
Snow Mountain agent is the prototype genogroup II member of the NLVs. Other representative members include Grimsby, Hawaii, Desert Shield, and many others.

others 1996; Muir and others 1998; Lees 2000). The virion is a smooth, round, nonenveloped particle of about 27 nm dia with a positive sense, single stranded, nonsegmented RNA genome. Poliovirus, the prototype of this family, was the 1st virus shown to be foodborne after transmission by contaminated water and unpasteurized milk (Svensson 2000). While vaccination has virtually eradicated poliovirus in the U.S.A., it remains a problem in developing countries (Cliver 1997). Many of the infections caused by human enteroviruses are asymptomatic, although, when symptomatic, a wide range of clinical syndromes, including fever, paralysis, meningitis, poliomyelitis, respiratory disease, and diarrhea, have been reported. Numerous studies have reported the detection of human enteroviruses in molluskan shellfish from estuarine areas, both open and closed to harvesting, and they are, by far, the most commonly isolated viral agent in shellfish (Denis 1973; Fugate and others 1975; Gerba and Goyal 1978). While other members of the enterovirus group have been reported in association with food and water, only a small number of foodborne disease outbreaks caused by coxsackie and echoviruses have been recorded (Cliver 1997). Unfortunately, there is no correlation between the presence of human enteric viruses and the level of fecal coliforms, the common index of sanitary quality in shellfish or their harvesting waters (Chung and others 1996; Griffin and others 1999). Because of their high prevalence in human sewage associated with routine vaccination, and their ease of culture, it has been suggested that poliovirus could be used as an alternative indicator of viral pollution in harvesting waters (Pina and others 1998). Additionally, poliovirus is typically used as a model during the development of new virus detection methods for food and environmental samples (Jaykus 1997). However, with recent efforts toward worldwide poliovirus eradication, it is unlikely that this virus will be used as a model in future y.

Hepatitis A virus

Hepatitis A virus (HAV), which was first classified in the genus *Enterovirus* and has since been reclassified in the genus *Hepatovirus*, is a member of the *Picornaviridae* family and is among those enteric viruses most often transmitted by contaminated foods. Unlike the other enteric viruses, HAV targets the liver, and the disease has an incubation period of about 4 wk (range 2 to 6 wk). Proper hygiene and safe food handling practices are essential, because asymptomatic infected food handlers, including those shedding virus during the last 10 to 14 d of their incubation periods, can serve as a significant source of contamination and subsequent disease propagation through the community. The severity of HAV infection is age-associated; children under 6 y of age usually have asymptomatic infections, whereas older children and adults typically have symptomatic infections that may be as high as 95% during outbreaks (Lednar and others 1985; Ciocca 2000). Acute illness may last from 1 to several wk, and typical symptoms include fever, nausea, malaise, anorexia, headache, and jaundice (Ciocca 2000). Hepatitis A virus is more resistant to heat and drying than are most other human enteric viruses, having been shown to survive in seawater from a few d to several wk (Cliver 1997; Croci and others 1999) and to retain infectivity for at least 2 wk in feces (Cromeans and others 1994). While HAV infection is generally regarded as one of the more severe of the foodborne diseases (Cliver 1997), in most cases, recovery is complete and infection confers lifelong protection against reinfection (Ciocca 2000). An effective, economical vaccine is available which will likely reduce the long-term public health significance of this virus (Jaykus 2000a).

Agents of acute viral gastroenteritis

In 1972, a 27 nm particle was visualized in an infectious stool filtrate by immune electron microscopy (IEM) from an outbreak of gastroenteritis that occurred 4 y earlier in Norwalk, Ohio, U.S.A. (Kapikian and others 1972). The so-called "Norwalk virus" (NV) became the 1st recognized human gastroenteritis virus of medical importance. Norwalk virus is the prototype member of a group of morphologically similar viruses that are commonly seen in the stools of infected individuals by electron microscopy (EM) and have been associated with outbreaks of gastroenteritis (Cukor and Blacklow 1984; Kapikian and Chanock 1990). Through the y, these viruses have been collectively referred to as the "small round structured viruses" (SRSVs), although recent taxonomic changes are outlined below.

Until the cloning and characterization of the NV (Jiang and others 1990, 1993) and other related SRSV (Lambden and others 1993; Lew and others 1994a, 1994b) genomes, limited information was available on the molecular characteristics of the SRSVs. Furthermore, the inability to propagate any of the SRSVs in cell culture, and the absence of effective animal surrogates with which to study these viruses, has further complicated characterization of these agents. Recent genetic sequencing work (Jiang and others 1990, 1993; Lambden and others 1993; Lew and others 1994a, 1994b) has established the SRSV group as members of the family *Caliciviridae*, even though some of them lack the characteristic cup-shaped morphology seen by electron microscopy. Human enteric caliciviruses have a positive-sense, single stranded, nonsegmented RNA genome. These nonenveloped, icosahedral virurses have a dia of 27 to 40 nm, a buoyant density of 1.33 to 1.41 g/ml, and a viral capsid composed of 180 copies of a single structural protein (encoded in open reading frame (ORF) 2) that ranges from 58000 to 62000 Da in molecular weight (Kapikian and others 1996; Hardy 1999). Based on sequence analysis of the RNA-dependent RNA polymerase region and the capsid protein region of representative SRSVs, the human caliciviruses (HuCV) have been divided into 3 genogroups: genogroup I (NV prototype); genogroup II (Snow Mountain agent prototype); and genogroup III (Sapporo virus prototype) (Jiang and others 1997; Nakata and others 1998). Molecular epidemiological studies of gastroenteritis outbreaks in the U.S.A. and the U.K. have identified the genogroup II strains as being the most prevalent in recent y (Fankhauser and others 1998; Maguire and others 1999). Unlike genogroup I and II HuCVs which appear as SRSVs under the EM, genogroup III viruses have the typical animal calicivirus morphology (Matson and others 1995). Very recent virus taxonomy now subdivides the *Caliciviridae* family into 4 genera: (1) the Vesiviruses, represented by vesicular exanthema of swine virus and feline calicivirus; (2) the Lagoviruses, represented by rabbit hemorrhagic disease virus and Europian brown hare syndrome virus; (3) the "Norwalk-like viruses" (NLVs), having the Norwalk virus as the prototype strain; and (4) "Sapporo-like viruses" (SLVs), represented by the Sapporo virus (van Regenmortel and others 2000). In

general, the Vesiviruses and Lagoviruses are considered animal caliciviruses, posing no known human disease risk, while the NLVs and SLVs are the genera most often responsible for epidemic gastroenteritis.

According to recent epidemiological estimates, the NLVs account for over 60% of cases, 33% of hospitalizations, and 7% of deaths among all of the illnesses that are attributable to foodborne pathogens (Mead and others 1999). Year-round outbreaks of Norwalk and the NLVs have affected schoolchildren and adults in a variety of settings, including schools, restaurants, hospitals, and nursing homes (Griffin and others 1982; Guest and others 1987; Gordon and others 1990; Kobayashi and others 1991). Norwalk-like virus infection typically presents with rapid onset (24 to 48 h) of acute vomiting, diarrhea, nausea, and abdominal cramps, and typically lasts between 24 to 48 h (McCarthy and others 2000). Viral shedding in stool can occur as early as 15 h after exposure, with prolonged excretion for 7 to 14 d (Graham and others 1994; Okhuysen and others 1995). Virus can also be shed in vomitus, providing a further mode of NLV transmission (Keswick and others 1985; Patterson and others 1997; Marks and others 2000). Immunity to infection with the NLVs has been shown to be transient, and those who do become infected may be subject to reinfection within 6 mo to a y (Greenberg and Matsui 1992; Graham and others 1994; Cliver 1997). Consequently, much of the adult population appears susceptible to NLV infection throughout life (Lees 2000). While efforts are underway to develop an edible vaccine for the HuCVs (Estes and others 2000; Tacket and others 2000), currently, one does not exist.

Other foodborne enteric viruses

While many other groups of human enteric viruses have been identified, foodborne transmission of these agents is considered rare. Rotaviruses most often infect young children worldwide, with nearly all children acquiring serum antibodies to group A rotaviruses by 5 y of age (Cukor and Blacklow 1984). While there have been many waterborne disease outbreaks linked to rotaviruses (Gerba 1988; Mehnert and Stewien 1993), as well as the demonstration of the presence of these viruses in bivalve mollusks (for example clams, cockles, mussels, and oysters) harvested from fecally-contaminated waters, there has been no documented disease following seafood consumption (Lees 2000). Astroviruses, also associated with gastroenteritis in young children, have been detected in naturally grown oysters (Kitahashi and others 1999), and there have been a few outbreaks associated with their consumption (Caul 1987; Oishi and others 1994). A group of small round viruses (SRV) that are not serologically related to NV or HAV have reportedly caused several outbreaks of shellfish-associated gastroenteritis (Appleton 1994; Jaykus 2000b). While these viruses possess characteristics similar to those of the parvoviruses, they may represent more than 1 virus type and may include members of the HuCVs (Appleton 1987). The hepatitis E virus is transmitted predominantly by sewage-contaminated water and person-to-person contact (Cromeans and others 1994; Cliver 1997), however, only a few foodborne cases have been reported to date (Chan 1995; Stolle and Sperner 1997).

Transmission of the Human Enteric Viruses

Three major routes for viral contamination of foods have been recognized and include: (1) shellfish contaminated by fecally polluted marine waters; (2) human sewage pollution of drinking and irrigation waters; and (3) ready-to-eat (RTE) and prepared foods contaminated as a result of poor personal hygiene of infected food handlers (Jaykus 2000a). In addition, the NLVs have been shown to be spread by aerosolization of vomitus and through fomites (Patterson and others 1997; Marks and others 2000). Significant outbreaks of viral foodborne disease are detailed in Table 2.

Shellfish harvesting waters

Bivalve molluskan shellfish are filter feeders capable of concentrating viruses and other pathogens, and, in so doing, they act as passive vehicles for enteric disease transmission. Human sewage pollution is the ultimate source of viruses contaminating shellfish harvesting waters, usually arising from illegal overboard waste discharge from boaters, from failing septic systems along the shoreline, or from treated and untreated municipal wastewater and sludge discharges, which occur mostly during heavy rainfall periods (Jaykus and others 1994; Shieh and others 2000). Infected individuals may shed as many as 10^6 to 10^{10} infectious viruses per gram of feces, and raw sewage can contain anywhere from 10^3 to 10^5 infectious virus particles per liter (Rodgers 1981; Jaykus and others 1994). Common treatment processes, including chlorination, have not been shown to completely eliminate enteric viruses in sewage (De Leon and Jaykus 1997). The lack of correlation between the fecal coliform indicators and the presence of enteric viruses in shellfish and their harvesting waters is, at least in part, due to the increased resistance and persistence of the viruses when compared to the less hardy Gram-negative indicator bacteria (Gerba 1988; Lees 2000). Although over 100 different types of human enteric viruses can be excreted in human feces, only a few (HAV, NLVs, astroviruses, SRVs) have been epidemiologically linked to shellfish-associated viral disease, and, of those, HAV and the NLVs are the ones most commonly implicated in these outbreaks.

Infectious hepatitis caused by HAV is probably the most serious viral infection linked to shellfish consumption (Richards 1985). It has been demonstrated that actively feeding bivalve mollusks can filter up to 10 gallons of water per h (Winn 1999) and concentrate HAV 100-fold (Enriquez and others 1992). Hepatitis A virus can remain infectious for at least 2 wk in feces (Cromeans and others 1994), and persist in contaminated shellfish for up to 6 wk after concentration (Glass and others 1996). It has also been shown to survive in marine waters longer than other human enteric viruses (Bosch and Shields 1987). Hepatitis A virus might not be eliminated as readily as enteroviruses during depuration, helping to explain why depurated shellfish have been linked to outbreaks of HAV infection (Richards 1985). Indeed, investigators have demonstrated differences in the relative patterns and rates of elimination by depuration when comparing the *E. coli* fecal indicators to model viruses, such as poliovirus and representative coliphage. In general, the elimination rates for enteric bacteria during depuration are 1 to 2 orders of magnitude higher than the virus removal rates (Power and Collins 1989, 1990).

The 1st documented outbreaks of shellfish-associated hepatitis in the United States occurred in the early 1960s, and since then many more cases have been reported (Richards 1985; De Leon and Jaykus 1997). A major outbreak of hepatitis A in Shanghai, China in 1988, in which 300000 cases were linked to the consumption of shellfish harvested from a site impacted by human sewage pollution, currently ranks as the largest foodborne disease outbreak ever reported (Halliday and others 1991). In Italy, shellfish consumption is a major risk factor for HAV infection (Mele and others 1997; Leoni and others 1998), and it has been estimated that 7% of all worldwide HAV cases may be associated with the consumption of contaminated bivalve mollusks (Cliver and others 1983).

Norwalk-like viruses are most frequently identified as the causative agents in viral gastroenteritis outbreaks associated with bivalve mollusk consumption (Lees 2000). The 1st epidemiological linkage between the NLVs and shellfish-associated gastroenteritis was made in the U.K. in 1976, where the consumption of sewage-contaminated oysters was the suspected cause of disease (Ap-

Table 2–Recent epidemiological investigations linking viruses in food commodities to foodborne disease outbreaks

Agent	Food	Samples Tested	Methods	Conclusions	Reference
HAV	Strawberries	Outbreak samples (clinical only)	RT-PCR (Single and nested) Sequencing	Identical nucleotide sequences of amplicons from patients in MI, WI, LA, AZ, and TN	Hutin and others 1999
NLV (G I / G II)	Oysters	Outbreak samples (clinical only)	EM RT-PCR Sequencing EIA	12/12 samples positive by EM and/or RT-PCR Identical nucleotide sequences of amplicons from 7/7 stool samples tested 11/14 serum pairs had a • 4-fold increase in NV Antibody	Kohn and others 1995
NLV	Shellfish	Outbreak samples (food only)	RT-PCR (Single and nested) Sequencing	4/4 outbreak samples Positive	Lees and others 1995
NLV	Oysters	Outbreak samples (clinical and food)	RT-PCR (nested) Sequencing	Co-existence of 2 different NLV genogroups in single oyster specimen by RT-PCR and sequencing	Sugieda and others 1996
NLV (G II)	Oysters	Outbreak samples (food only)	RT-PCR Sequencing	2/3 recalled outbreak oysters samples positive	Shieh and others 1999
NLV (G II)	Deli meats	Outbreak samples (clinical and food)	RT-PCR (nested) Sequencing	Identical nucleotide sequences of amplicons from food and clinical Samples	Schwab and others 2000
NLV	Raspberries	Outbreak samples (clinical and food)	RT-PCR Southern Hyb. Sequencing	Identical nucleotide sequences of amplicons from food and clinical Samples	Gaulin and others 1999b
NLV (G II)	Salad	Outbreak samples (clinical only)	EM RT-PCR	6/6 clinical samples Positive Evidence of transmission by an asymptomatic food handler	Gaulin and others 1999a
NLV (G II)	Sandwiches	Outbreak samples (clinical only)	EM RT-PCR Sequencing	9/20 positive samples by EM 7/20 positive samples by RT-PCR Identical nucleotide sequences of amplicons from asymptomatic food handler and infected company employees	Parashar and others 1998
NLV (G II)	Potato salad	Outbreak samples (clinical only)	EM RT-PCR Sequencing	Airborne transmission of NLV infection Identical nucleotide sequences of amplicons from food handler and infected guests	Patterson and others 1997
NLV (G I)	Box lunches	Outbreak samples (clinical and food)	EM RT-PCR Sequencing	4/4 positive samples by EM 5/6 positive samples by RT-PCR No virus detected in food samples from box lunches	Becker and others 2000
NLV (G II)	Raspberries	Outbreak samples (clinical and food)	RT-PCR Sequencing	4/9 positive samples by RT-PCR No virus detected from the raspberries	Ponka and others 1999

pleton and Pereira 1977). During the summer of 1978, a large oyster-associated gastroenteritis outbreak, involving approximately 2000 persons in Australia (Murphy and others 1979), was also linked to the Norwalk agent. In the U.S.A., the 1st documented outbreak of NLV-associated gastroenteritis following raw oyster consumption occurred in 1980 (Gunn and others 1982). Since then, outbreaks have continued to occur (Cliver 1995; Stafford and others 1997; Otsu 1999), and in Louisiana alone, 3 large out-

breaks in the early 1990s illustrated the role of improper human sewage discharge in propagating NLV disease (Kohn and others 1995; CDC 1997; Berg and others 2000).

Drinking and irrigation waters

Although waterborne transmission of human enteric viruses is well documented, drinking water is only one route by which humans become exposed to waterborne pathogens. Human populations may also be exposed by the consumption of crop materials that had been grown in fields irrigated with wastewater, or fertilized and conditioned with inadequately decontaminated sewage sludge (Metcalf and others 1995). For instance, Katzenelson and others (1976) demonstrated that Israeli communities using wastewater effluents for irrigation had an increased incidence of infectious hepatitis as compared with other communities. Strawberries linked to a recent HAV outbreak may have been contaminated during irrigation, or by the use of human feces as fertilizer (Niu and others 1992; Hutin and others 1999). Unfortunately, there are few published studies that report on the stability of viruses in sewage that is composted and used on agricultural lands. A 1984 study found that sludged plots compared favorably to control plots with respect to the occurrence and survival of enteroviruses (Wallis and others 1984). We do know that adsorption is a major factor in virus removal and persistence in soils, with adsorptive capabilities being dependent upon both virus and soil type (Mesche and Sobsey 1998). Although there is little quantitative data available to estimate the relative importance of contaminated water and fertilizer in the propagation of foodborne viral disease, there remains a high probability that this is a significant mode of contamination and subsequent disease transmission.

Ready-to-eat and prepared foods

The vehicle of virus transmission is not identified in many foodborne disease outbreaks, and infected food handlers who practice poor personal hygiene appear to be an extremely common source of contamination (Hedberg and Osterholm 1993). For instance, a recent review suggested that HAV and the NLVs accounted for over 60% of foodborne outbreaks, and ill food workers were identified as the most common source of contamination (Guzewich and Ross 1999). Most of these workers were ill either prior to or at the time of food preparation, but, in a few cases, asymptomatic workers were believed to be the source of infection. In a study conducted by Bean and others (1990), poor personal hygiene was found to be the contributing factor most commonly implicated in outbreaks of hepatitis A infection (96%) and NLV (78%) gastroenteritis. Given that the NLVs can be shed in the feces for over 14 d postinfection (Estes and Leparc-Goffart 1999), and that fecally-associated HAV remains infectious for at least 2 wk (Cromeans and others 1994), there is a significant opportunity for the recovering, asymptomatic food handler to continue to contaminate product during food preparation.

Most foods involved in outbreaks for which human handling is a factor are the ready-to-eat (RTE) foods, defined as "food that is edible without washing, cooking, or additional preparation by the consumer or by the food establishment and that is reasonably expected to be consumed in that manner" (U.S. Public Health Service 1999). The contamination of RTE and prepared foods most frequently comes from poor hand-washing practices of food handlers after toilet use, as fecal material can be left on hands or even under nails, which then can come in contact with food products (Jaykus 2000a). Handling cooked products with bare hands has been identified as a major factor for pathogen transfer to RTE foods (Bryan 1995), and there is presumed to be a direct correlation between the number of pathogenic organisms on a food employee's hands and the probability of microbial transfer from hands to cooked food products (Restaino and Wind 1990).

Hepatitis A virus outbreaks linked to poor personal hygiene of infected food handlers have been reported for lettuce, salads, sandwiches, hamburgers, spaghetti, and bakery products (Rosenblum and others 1990; Battegay and others 1995; Feinstone 1996; Cliver 1997). In 1992, up to 5000 people may have been exposed to HAV following the consumption of a variety of gourmet foods prepared by an infected food handler in Denver, Colorado (Dalton and others 1996). A recent study investigating the transfer of HAV from fingers to food demonstrated a 9.2% rate of transfer (Bidawid and others 2000a). Treating contaminated fingerpads with water, medicated or nonmedicated topical disinfectants, or alcohol reduced the amount of infectious virus transferred to lettuce to between 0.3 and 0.6%, depending on the topical agent used (Bidawid and others 2000a). These results suggest that the risk of HAV contamination of foods could be significantly reduced through proper hand-washing and decontamination procedures, although compliance is always an issue (Guzewich and Ross 1999). While an effective, economical HAV vaccine is available, the cost effectiveness of vaccinating food service workers has been questioned, and there is no current recommendation for vaccinating this population (Jacobs and others 2000).

The predominant mode of transmission of the NLVs is now recognized as the consumption of contaminated foods (Tauxe 1997; Mead and others 1999). The NLVs have been associated with many outbreaks caused by poor food handler hygiene, including the consumption of contaminated chicken, turkey, tuna salad, and cafeteria sandwiches (Pether and Caul 1983; Gross and others 1989; Lo and others 1994), bakery products (Kuritsky and others 1984; Andersen and others 1996), hamburgers and french fries (Guest and others 1987; Parashar and others 1998), and potato, fruit, and tossed salads (White and others 1986). In recent nonbacterial gastroenteritis outbreaks, combined molecular and immunological methods have been used to support epidemiological evidence, indicating a common food source for virus transmission. For instance, Parashar and others (1998) used reverse transcription-polymerase chain reaction (RT-PCR) and IEM of clinical specimens from both patients and an asymptomatic food handler to establish causality in a genogroup II NLV outbreak. Gaulin and others (1999a) confirmed the importance of the asymptomatic food handler by reporting NLV transmission by a food handler in the pre-symptomatic phase of illness. In a particularly interesting case, a genogroup I NLV was found to be the etiological agent in an outbreak of foodborne viral gastroenteritis among athletes from 2 college football teams (Becker and others 2000). Although the primary source of infection was sandwiches served to the 1st team in a box lunch, the 2nd team developed symptoms after contact on the field with the ill players of the 1st team. This particular case demonstrated the role of the infected food handler in the initiation of a propagated viral foodborne disease outbreak.

Vomitus

A major factor in the above outbreak scenario included exposure to vomitus. Soon after the discovery of NV in 1972, Greenberg and others (1979) hypothesized that transmission of the virus might not only occur via the fecal-oral route, but also by a "vomitus-oral" route. Their hypothesis was based on the detection of NV antigen in vomitus samples from experimentally infected volunteers using radioimmunoassay (RIA). Other, more recent outbreak investigations provide further evidence that vomiting is an important transmission factor. For instance, Patterson and others (1997) reported on the role of a kitchen assistant who vomited into a sink used to prepare a potato salad that was subsequently identified as the vehicle in a NLV outbreak. Even though a chlorine-based disinfectant was used to clean the sink prior to the potato salad preparation, these investigators concluded that that routine surface chlorination alone could not be relied upon to inacti-

vate NV (Keswick and others 1985). Marks and others (2000) reported on an outbreak of NLV gastroenteritis following a meal in a large hotel, during which one of the diners vomited. In this case, attack rates were inversely related to the distance between secondary cases and the primary case who vomited, consistent with airborne spread of the virus and infection by inhalation and subsequent ingestion of virus particles. The vomitus-oral route of transmission is likely to explain how NLVs are spread so rapidly and why propagated epidemics frequently occur.

Environmental persistence of human enteric viruses

Studies on the persistence of human enteric viruses have demonstrated that environmental surfaces also play an important role in the spread of these infectious agents. Factors influencing the environmental stability of viruses include relative humidity (RH), temperature, and the type of surface contaminated (Sattar and others 1986). For instance, the survival of rotavirus and HAV on nonporous surfaces has been shown to be inversely proportional to the level of relative humidity and temperature (Sattar and others 1986; Mbithi and others 1991). Likewise, the half-life of HAV was greater than 7 d at 20 °C and RH of 25%, while only 2 d at 20 °C and RH of 95% (Mbithi and others 1991). Similar results have been observed for rotavirus, which demonstrates a half-life of approximately 10 d at 22 °C and 25% RH, but only 12 h at 22 °C and RH of 85% (Sattar and others 1986). When 3 model enteroviruses (poliovirus, echovirus, and coxsackievirus) were added to uncooked vegetables and stored under household conditions, infectivity was retained for as long as 15 d (Pirtle and Beran 1991). Similarly, after poliovirus type 1 and coxsackieviruses were added to foods and held at room temperature and 10 °C, the viruses were still infectious after 1 wk and 1 mo, respectively (Pirtle and Beran 1991). Recently, investigators have used the cultivable FCVs as a surrogate for NLVs in environmental persistence studies. The FCVs were shown to persist at 4 °C for up to 60 d with less than 50% reduction in infectivity (Doultree and others 1999). These same viruses were more readily inactivated at higher temperatures (21 °C and 37 °C), with complete loss of virus infectivity after 14 to 28 d at room temperature, and after 1 to 10 d at 37 °C. Environmental stability of the NLVs has been further elucidated by Green and others (1998b), who were able to detect NLV RNA in 11 out of 36 (31%) environmental swabs taken 3 d into a 1994 hospital outbreak of NLV infection. All of these samples were collected from areas in the affected ward, including lockers, sinks, curtains, and commodes. In another large institutional NLV outbreak, Green and others (1999) reported detection of NLV RNA from environmental surfaces such as toilet rims and seats (72% samples positive), carpets (70%), horizontal surfaces (41%), and other frequently handled objects.

Human hands and fomites have also been shown to play an important role in the direct, as well as the indirect, spread of certain types of viruses (Mbithi and others 1992, 1993). In 1998, ten cases of HAV were linked to a bartender who had chronic diarrhea and had served drinks while incubating HAV (Sundkvist and others 2000). After an epidemiological investigation, fomite transmission via the drinking glasses was determined to be the most likely route of transmission. Mbithi and others (1992) conducted experiments to determine HAV survival on human hands and its subsequent transfer to inanimate surfaces. After placing a fecal suspension of HAV on the fingerpads of 5 volunteers, 16 to 30% of the initially recoverable virus remained detectable after 4 h; after 20 min of drying, 27% of the virus was transferable, while after 4 h of drying, only 1.6% of the surviving virus could be transferred. While the degree of virus transfer decreased with drying, residual moisture on hands after hand washing has been shown to facilitate the transfer of residual viruses (Larson 1985; Springthorpe and Sattar 1998). Similar studies conducted using rotavirus demonstrated survival on human hands for up to 4 h and transfer of 16.1 and 1.8% of infectious rotavirus to inanimate objects at 20 and 60 min after inoculation, respectively (Ansari and others 1988).

Virus persistence in food processing and storage

Interest in the persistence of human enteric viruses after heating peaked after several documented outbreaks of HAV and viral gastroenteritis were linked to the consumption of cooked shellfish (Appleton and Pereira 1977; Sockett and others 1985; Morse and others 1986). Standards set for commercial shellfish cooking operations in the U.K. are based on research demonstrating a 4 $\log_{10}$ inactivation of HAV in shellfish after holding at an internal temperature of 85 to 95 °C for 1 min (Lees 2000). In contrast to the U.K. standards, a recent study suggested that heat treatments of 60 °C for 30 min, 80 °C for 10 min, and 100 °C for 1 min were insufficient to completely eliminate HAV in contaminated mussels (Croci and others 1999); only after a treatment of 100 °C for 2 min was the virus completely inactivated. Unfortunately, the tradeoff between food safety and organoleptic product quality impacts consumer acceptance under conditions of more severe heat treatment.

While standard thermal inactivation studies for NLVs in shellfish are not possible because of the inability to cultivate the viruses *in vitro*, Slomka and Appleton (1998) used a model feline calicivirus (FCV) to demonstrate that this virus family was less resistant to heat than was HAV. These investigators demonstrated the complete inactivation of FCVs in shellfish tissues when heated to an internal temperature of 78 °C or higher. Doultree and others (1999) confirmed these results, finding that a model FCV was only partially inactivated after exposure to 70 °C for 1 to 3 min, but that no virus could be recovered after exposure to 70 °C for 5 min or boiling (100 °C) for 1 min. With respect to persistence during depuration, Schwab and others (1998) compared the efficacy of depuration on the elimination of NV and the fecal indicator *E. coli* in oysters, finding a 95% reduction in bacterial levels but only a 7% reduction in NV concentration after 48 h. The high frequency of shellfish-associated NLV outbreaks is in part attributable to the environmental stability of these viruses (Schwab and others 1998).

Considerably less work has been done to evaluate thermal inactivation of enteric viruses in other food commodities. Studies in the late 1970s indicated a resistant fraction of the population when viruses were heated in liquid suspension. This fraction was seen as a tail in thermal inactivation curves, and was presumed to be either an experimental artifact associated with inconsistent heat penetration or else due a resistant fraction of infectious RNA liberated from virions ruptured during the heating process (Tierney and Larkin 1978; Larkin and Fassolitis 1979). However, more recent work has confirmed biphasic inactivation kinetics when heat was applied to 3 different dairy products inoculated with HAV (Bidawid and others 2000b). In general, these investigators concluded that < 0.5 min at 85 °C was sufficient to cause a 5-log inactivation of HAV titer in skim milk, homogenized milk, and cream. However, at lower temperatures, increased fat content played a protective role, contributing to the heat stability of the virus.

Unfortunately, viruses remain recalcitrant to many food processing and storage technologies. For instance, gamma irradiation is unacceptable as a means of eliminating viruses in shellfish; at doses of 3.0 kGy, beyond which shellfish tissues begin to show significant organoleptic changes, investigators have reported up to 95% reduction in virus titer (Mallett and others 1991). Investigators have demonstrated no significant reduction in virus titer for oysters artificially inoculated with model human enteroviruses after storage at refrigeration temperatures for over 1 mo (Tierney

and others 1982). Similar results have been documented for refrigerated soft fruit and salad vegetables, on which poliovirus titers remained relatively stable, with D-values never exceeding 14.2 d and frequently insignificant inactivation (Kurdziel and others 2001). Likewise, poliovirus was very stable in whole Pacific oysters held frozen at -17.5 °C for up to 12 wk (DiGirolamo and others 1970). Indeed, most laboratories store enteric virus stock cultures for y at temperatures of -80 °C without appreciable loss in virus infectivity.

Disinfection

Over the y, the kinetics of enteric virus disinfection, particularly with respect to water treatment, have been intensely studied, as reviewed by Letterman (1999). While enteric viruses are more resistant than bacterial pathogens to common sewage treatment processes, chlorine remains a highly effective virucide (U.S. EPA 1999). Virus survival studies have reported contact time (CT) values required to achieve 99.99% inactivation for various enteric viruses to range from 4 mg*min/L to 400 mg*min/L when treated at a free chlorine residual of 0.4 mg/L, pH 7.0, and temperature of 5 °C (AWWA 1979). The U.S. Environmental Protection Agency (EPA) Surface Water Treatment Rules require systems using surface water or ground water under the direct influence of surface water to disinfect (and filter) their water to provide for 99.99% removal/inactivation of viruses (U.S. EPA 2001). As a further precaution and to maintain biological stability during water distribution, the Total Coliform Rule (TCR) requires residual disinfectant of 0.2 mg/L for treated water entering the distribution system. Chlorine, monochloramine, and cholorine dioxide are the compounds most often used to maintain this residual (U.S. EPA 1999).

From the perspective of foodborne illness, hand washing has been shown to help prevent and control the transfer of viruses, but hand washing agents differ in their ability to inactivate viruses (Sattar and Springthorpe 1996). In general, the non-enveloped viruses, such as rotavirus, HAV and the NLVs survive better on skin than do enveloped viruses (Sattar and Springthorpe 1996). Soaps, medicated liquid soaps, and alcohol formulations have all been investigated with regards to microbiocidal/microbiostatic action. Handwashing with plain detergent soap can physically remove microbes; however, antimicrobial soap is needed to kill or inhibit the growth of remaining microorganisms (Larson 1985). Mbithi and others (1993) found a medicated liquid soap containing 0.3% triclosan (Bacti-Stat soap; DEB, Waterford, Ontario, Canada) to be the most effective hand-washing agent to eliminate HAV and poliovirus type 1 (PV1) from finger pads, reducing populations by 92% and 98%, respectively. Although alcohols were not as effective as hand washing agents, Mbithi and others (1993) did find products containing high levels of alcohol to be effective in preventing the transfer of HAV and PV1 from fingerpads to stainless steel surfaces. Alternatively, Ansari and others (1989) reported that products containing 70% alcohol were the most effective in inactivating rotavirus. Doultree and others (1999) demonstrated that high concentrations of hypochlorite (1000 ppm freshly reconstituted granular hypochlorite, or 5000 ppm pre-reconstituted hypochlorite solution), 1% glutaraldehyde, as well as 0.8% iodine were effective in the complete inactivation of FCV (as a surrogate for NLVs). However, quaternary ammonium-based products, anionic detergents and 75% ethanol were relatively ineffective at FCV inactivation. There were similarities between the results of Doultree and others (1999) and those of Mbithi and others (1990) with respect to the efficacy of surface disinfection of HAV. Two percent glutaraldehyde and sodium hypochlorite (> 5000 ppm of free chlorine) have been shown to reduce HAV titers by > 99.9%, while alcohol-based products were considerably less effective. In general, results from all of these studies suggest that selection of hand and surface disinfectants depends not only on the chemical, but also on the particular viral agent whose transmission is to be prevented.

Detection of Human Enteric Viruses in Clinical Specimens

Historical methods for diagnosis of enteric viral infection

Epidemiological criteria have been used in the past to classify suspected human enteric viral illnesses, particularly viral gastroenteritis (Kaplan and others 1982a, 1982b; Jaykus 2000b). Based on data compiled from numerous NLV outbreaks, a provisional diagnosis of viral gastroenteritis can be made if the following criteria are met: (1) bacterial or parasitic agents are not detected in clinical (fecal) specimens; (2) the incubation period is 24 to 48 h; (3) the median duration of illness is 12 to 60 h; and (4) vomiting occurs in at least 50% of ill individuals (Kapikian and others 1996). Historically, the laboratory confirmation of viruses as the cause of food or waterborne illness has been based on the demonstration of a specific immune response to the virus, or, alternatively, the detection of virus particles or antigen in stool (Svensson 2000). In early NV studies, EM of stool specimens was used exclusively for the diagnosis of infection. Complexity of the fecal matrix resulted in later adaptations to include the use of antibody-rich convalescent-phase sera from infected patients to aggregate NV particles for detection by IEM (Kapikian and others 1972; Dolin and others 1982; Lewis and others 1995). These were later replaced by various forms of the more sensitive immunoassay, including RIA (Greenberg and others 1978; Blacklow and others 1979), biotin-avidin immunoassay (Gary and others 1985; Heun and others 1987), immune adherence hemagglutination assay (IAHA) (Kapikian and others 1978), enzyme immunoassay (EIA) (Herrmann and others 1985; Fleissner and others 1989), and enzyme-linked immunosorbent assay (ELISA) (Herrmann and others 1986; Gary and others 1987), all of which have been used for the detection of NV in clinical specimens. It must be noted that all of these clinical detection strategies require the collection of either patient fecal specimens in the 1st few d of illness, or alternatively, acute and convalescent phase sera.

For HAV, early immunological methods, such as immunofluorescence and radioimmunofocus assays (RIFA) were developed to quantitate non-cytopathic virus growth in mammalian cell culture (Lemon and others 1983), with later developments focused on the detection of viruses as antigens in direct immunochemical assays (Jaykus and others 1994). However, in contrast to NV, for which clinical diagnosis is frequently obtained by detection of viral antigen or viral nucleic acid in stool, diagnosis of hepatitis A infection is primarily done by detection of IgM anti-HAV antibodies in serum (Parry and others 1989; Svensson 2000). Because a significant proportion of infected individuals may be asymptomatic and there is a need to screen household contacts, these types of methods have been criticized because they require widespread venepuncture, which is difficult to justify, especially when children are involved. Fortunately, recent methodological developments have facilitated the use of alternative body fluids, such as saliva, to further facilitate the investigation and management of hepatitis A outbreaks (Stuart and others 1992).

Development of new enzyme immunoassays

Recent genetic engineering efforts have resulted in the development of "naked" (lacking RNA) NLV-like particles produced from insect cells infected with a recombinant baculovirus. This has led to the creation of sensitive EIAs for the detection of serum antibodies to the Norwalk (Jiang and others 1992, 1993), Hawaii (Green and others 1997), Snow Mountain (Jiang and others 1995), Toronto (Leite and others 1996), and Sapporo viruses (Numata and others 1997). While initially developed to measure total anti-

NLV immunoglobulin in human sera, these EIAs have been adapted to detect anti-NLV immunoglobulin isotypes IgA, IgG, and IgM (Monroe and others 1993; Treanor and others 1993; Gray and others 1994; Parker and Cubitt 1994; Jiang and others 2000), each having its own benefit. The earliest assays targeted acute and convalescent phase antisera, with infection to HuCVs commonly defined as a > 4-fold increase in virus specific antibody between these 2 samples. However, assays for IgG have been used in serosurveys of HuCV infection in many countries (Numata and others 1994; Parker and others 1994; Jiang and others 2000), while serum IgM EIAs have been used to detect recent infection (Brinker and others 1998). While these NLV EIAs have been used in large-scale epidemiological studies worldwide (Estes and Leparc-Goffart 1999; Honma and others 2000) and continue to be used for some clinical diagnosis, the antigenic differences between the 3 genogroups of the HuCVs has made it difficult to detect exposure to the different HuCV genera using the same antigen or antibody EIAs. Recent efforts by Nakata and others (1998), who used recombinant antigens to all 3 prototypes of HuCVs in 3 different EIA systems to detect HuCV infections in Kenya, demonstrate some progress in the development of more broadly reactive serum EIAs for detection of NLV exposure. Antigen detection EIAs have also been developed to detect viral antigens in clinical (fecal) samples (Graham and others 1994; Okhuysen and others 1995). Using baculovirus-expressed recombinant NV (Graham and others 1994), Mexico (Jiang and others 1995), Grimsby (Hale and others 1999), and Hawaii (Green and others 1997) virus capsid antigens, investigators have been able to raise hyperimmune serum in laboratory animals for development of sandwich-format EIAs.

Molecular methods for virus detection in clinical samples

The polymerase chain reaction (PCR), a sensitive and specific *in-vitro* enzymatic nucleic acid amplification strategy, is currently the most promising technology for the detection of human enteric viruses in foods (Atmar and others 1993; Gouvea and others 1994; Hafliger and others 1997). Theoretically, PCR is capable of amplifying a single specific nucleic acid sequence up to a million-fold, making it an attractive alternative to other pathogen detection methods that require high levels of the target pathogen to achieve detection. The method is particularly attractive for detection of non-culturable infectious agents, since *in vitro* cultural enrichment can be replaced with *in vitro* nucleic acid enrichment.

RT-PCR is now widely used for the detection of human enteric viruses in clinical specimens (De Leon and others 1992; Ando and others 1994; Moe and others 1994). Although these methods have revolutionized detection, 2 major problems continue to hinder the further development of reliable, generic molecular detection methods for clinical, fecal diagnosis: (1) the presence of matrix-associated RT-PCR amplification inhibitors; and (2) the genetic heterogeneity among HuCVs which limits the development of broadly reactive reagents.

A range of techniques have been employed in attempts to remove inhibitory substances from fecal specimens prior to RT-PCR amplification. Many of these protocols require multiple steps and involve the use of reagents such as guanidinium thiocyanate (GTC), polyethylene glycol (PEG), cetyltrimethylammonium bromide (CTAB), phenol-chloroform, and Sephadex (Schwab and others 1997). Other methods that have been tested include the combination of GTC and silica to further purify RNA (Boom and others 1990) and gel chromatography using spin columns (De Leon and others 1992). Hale and others (1996) compared 4 methods for RNA extraction from fecal specimens for detection of SRSVs using RT-PCR, and found the GTC/silica method to be the most efficient in removing inhibitory substances. This has since been confirmed by Svensson (2000).

The choice of primers is another important factor for successful application of RT-PCR, particularly for the detection of the genetically diverse HuCVs (Table 3). Typically, the RNA-dependent RNA polymerase region is the target RT-PCR amplification region for these viruse because it is the most highly conserved among several regions of the NLV genome. The early primers (NV 5′/3′ Pol and NV 36/35) developed for the detection of the NLVs were extremely specific (De Leon and others 1992; Moe and others 1994; Wang and others 1994). Sequencing of an assortment of PCR products obtained using the NV 51-3 primer pair has clearly demonstrated each outbreak strain to be unique with some degree of genetic divergence from the reference 8FIIa Norwalk virus strain.

In an effort to improve primer reactivity, 2nd generation primers that are more broadly reactive have been developed. For instance, the primer set NI/E3, directed at partially conserved regions of the RNA polymerase region of the SRSV genome, detected NLV RNA in 93/101 (91%) fecal samples shown to be SRSV-positive by EM (Green and others 1995a). A disadvantage of the NI/E3 primer set is its low annealing temperature (40 °C) that can lead to nonspecific amplification. Vinje and Koopmans (1996) developed the JV12/JV13 primer set to screen NLV outbreak fecal specimens from the Netherlands. While this primer pair could detect 85% of a panel of an antigenically diverse set of NLV fecal specimens, the low annealing temperature (37 °C) of this primer set is notable. In some ways, the G-1 and G-2 primer sets, developed by Ando and others (1995), are a “gold-standard” with respect to NLV detection and strain discrimination in clinical (fecal) and food samples (Kohn and others 1995; Levett and others 1996; Noel and others 1997; Wolfaardt and others 1997), although the RT-PCR amplicon produced from this set is quite small (123 bp), multiple amplifications are required, as are multiple DNA hybridizations for confirmation of amplicon identity. In short, in a recent review of 9 sets of PCR primers used for the detection of NLVs and SLVs, no single primer pair could be used to detect all NLV or all SLV strains (Honma and others 2000). Investigators are beginning to combine primers, as reported in recent studies which have used the G-1, G-2, and Sapp35/Sapp36 primer sets to detect 95 to 100% of outbreak and sporadic cases of NLV or SLV infection in pediatric settings (Berke and others 1997; Honma and others 2000).

Degenerate primers, a mixture of oligonucleotides varying in nucleotide sequence but having the same number of nucleotides (Bej and others 1991), have also been developed for the detection of HuCVs. The benefit of using degenerate primers lies in the fact that every possible combination of nucleic acid sequence that could code for a given amino acid sequence can be generated and used for PCR amplification. In early work, 2 sets of degenerate primers were used to sequence the polymerase, capsid, and ORF-3 regions of a previously uncharacterized 3rd UK SRSV antigenic type (Green and others 1995b). Le Guyader and others (1996b) reported on the development of a broadly reactive degenerate primer (NVp110) used in conjunction with NV 36 for the amplification of NLV RNA, obtaining positive amplification results from 12 out of 15 fecal specimens representing all 3 NLV genogroups. Others have reported using the NVp110 primer for the detection of NLVs in shellfish (Le Guyader and others 1996a, 2000). Another degenerate primer set (primers 2/4 and primers 5/T25VN) has been used to provide information on genetic variation among the antigenically diverse NLVs, although their use for routine NLV detection in outbreak investigation has not yet been reported in the literature (Monroe 1999).

Currently, a combination of the GI and GII primers, as well as a degenerate set, are routinely used by the CDC in their outbreak investigations (Monroe 1999. A supposedly “universal” primer set that detects both NLVs and SLVs has been reported recently (Jiang and others 1999). Honma and others (2000) confirmed that no single primer pair was able to detect all NLV strains, but the

Table 3–RT-PCR primers for detection of members of the family *Caliciviridae*, including Norwalk-like viruses and Sapporo-like viruses

Primer	Sequence (5'—3') (Polarity)	Location (bp)	Size (bp)	Viruses	Reference
NV-5	CAAATTATGACAGAATCCTTC (+)	4601-4621	260	NV	De Leon and
NV-3	GAGAAATATGACATGGATTGC (-)	4840-4860			others 1992
NV 36	ATAAAAGTTGGCATGAACA (+)	4475-4944	470	NV, UK,	Wang and others 1994
NV 35	CTTGTTGGTTTGAGGCCATAT (-)			SMA, TV	
NV51	GTTGACACAATCTCATCATC (-)	4673-4878	206	NV, SMA,	Moe and others 1994
NV3	GCACCATCTGAGATGGATGT (+)			TV	
NI	GAATTCCATCGCCCACTGGCT (+)	4756-4867	113	SRSV	Green and others 1995a
E3	ATCTCATCATCACCATA (-)			(UK 1, 2, 3, 4)	
JV12	ATACCACTATGATGCAGATTA	Pol region	327	UK1, 2, 3, 4	Vinje and Koopmans 1996
JV13	TCATCATCACCATAGAAAGAG			NET/MX	
Mon381	CCAGAATGTACAATGGTTATGC	5362-5383	322	G2	Shieh and others 2000
Mon383	CAAGAGACTGTGAAGACATCATC	5661-5683			
NVp110	AC(A/T/G)AT(C/T)TCATCATCACCATA (-)	4865-4884	398	UK1, 2,	LeGuyader and others 1996
NVp36	ATAAAAGTTGGCATGAACA	4487-4501		3, 4	others 1996
Sapp 35	GCAGTGGGTTTGAGACCAAAG (-)	4956-4976	470	SV	Honma and others 2000
Sapp 36	GTT GCT GTT GGC ATT AAC A (+)	4487-4505			
SR33	TGTCACGATCTCATCATCACC (-)	4856-4876	123		Ando and others 1995
For negative-strand cDNA sythesis for G1 and G2 primers					
G1					
SR48	GTGAACAGCATAAATCACTGG (+)	4754-4773	123	UK2, NV	Ando and others 1995
SR50	GTGAACAGTATAAACCACTGG (+)	4754-4773			
SR52	GTGAACAGTATAAACCATTGG (+)	4754-4773			
G2					
SR46	TGGAATTCCATCGCCCACTGG (+)	4754-4773	123	UK1, UK3, UK4 (TNA, (HWA, SMA)	Ando and others 1995

NV110/NV36 primer set was the most efficient out of the 9 tested in their study. Even as more sequence information becomes available, it is generally recognized that primer design remains a critical issue for the effective diagnosis of human disease caused by the epidemiologically significant NLV group. There is currently no consensus for the choice of the primer pairs to detect the NLVs and detection may actually change by y and locale (Wolfaardt and others 1997).

Unlike the great diversity of primers reported for detection of the NLV group, primers used for the detection of HAV are fewer in number (Table 4). Many of the primers used for HAV detection target conserved sequences of the viral capsid region. Recently, a nested RT-PCR using primers targeting the VP2 and VP4 capsid region was developed and could detect HAV at concentrations as low as 1 $TCID_{50}$/10 g of mollusc tissue (Croci and others 1999).

Detection of Human Enteric Viruses in Foods

For many y, foods were rarely tested for viral contamination, and, when done, testing was almost entirely limited to shellfish commodities. However, any food that has been handled or is subject to contamination with human feces poses a transmission risk for viral foodborne disease (Cliver 1994a). There are, however, significant impediments to the development of effective virus detection methods targeting food commodities. For instance, since human enteric viruses require live mammalian cells in order to replicate, the traditional food microbiological techniques of cultural enrichment and selective plating, aimed at increasing pathogen numbers while decreasing competitive microflora, cannot be used. It is therefore necessary to separate and concentrate the viruses from the food matrix prior to detection. Historically, detection has been based on the infectivity of the viruses when cultured with live mammalian cells, although more recently, immunological or molecular techniques have also been favored (Jaykus 2000b) (Figure 1).

Virus concentration

Early work in virus concentration and purification from foods was limited to bivalve molluskan shellfish commodities (Jaykus 2000b), with more recent efforts targeting a wider variety of at-risk foods (Leggitt and Jaykus 2000; Schwab and others 2000). Various methods have been developed for the recovery of enteric viruses from shellfish and other seafood, as summarized by Jaykus and others (1994). Two general schemes have proven particularly successful, designated as extraction-concentration and adsorption-elution-concentration, although the adsorption-elution-concentration methods have gained more common use in recent y. The goal in both cases is to separate viruses from shellfish meats, provide a low-volume aqueous solution that is free of cytotoxic material, and recover most of the viruses present in the shellfish sample. Sample manipulations are based on the tendency of the nonenveloped enteric viruses to behave as proteins in solutions, and their ability to remain infectious even after exposure to organic solvents or at extremes of pH (Jaykus 2000b). Both schemes employ conditions favoring the separation of viruses from shellfish tissues, primarily through the use of filtration, precipitation, polyelectrolyte flocculation, and solvent extraction. As investigators have moved toward molecular detection methods, additional virus concentration methods have been reported, including the use of alternative virus precipitation agents such as ProCipitate‰ and Viraffinity‰ (LigoChem, Inc., Fairfield, N.J., U.S.A.) (Jaykus and others 1996; Dix and Jaykus 1998; Leggitt 1998), and immunomagnetic separation methods (Deng and others 1994; Schwab and others 1996; Lopez-Sabater and others 1997). In general, these virus concentration methods result in sample volume reductions of 10 to 1000-fold; virus yields after the application of adsorption-elution-concentration methods have been reported to range from 10 to 90% for shellfish (Sobsey and others 1978; Sob-

Table 4–RT-PCR primers for detection of hepatitis A virus

Primer	Sequence (5'— 3') (Polarity)	Location (bp)	Size (bp)	Reference
H1	GGAAATGTCTCAGGTACTTTCTTTG (-)	2389-2413	247	Le Guyader and others 2000
H2	GTTTTGCTCCTCTTTATCATGCTATG (+)	2167-2192		
H2	GTTTTGCTCCTCTTTATCATGCTATG (+)	2167-2192	210	Le Guyader and others 1994
H3	TCCTCAATTGTTGTGATAGC (-)	2358-2377		
H1	GGAAATGTCTCAGGTACTTTCTTTG (-)	2389-2413	248	Arnal and others 1999
E	GTTTTGCTCCTCTTTATCATGCTATGGATG TTACTACAC (+)	2167-2205		
HAV4	ATTCTACCTGCTTCTCTAATC (-)	6716-6696	412	Hafliger and others 1997
HAV1	TTTGGTTGGATGAAAATGGTT (+)	6305-6325		
Primer 1	CAGACTGTTGGGAGTGG (+)	762 –778	385	Croci and others 1999
Primer 2	TTTATCTGAACTTGAAT (-)	1131-1147		

sey 1987). Limitations of all of these extraction approaches include loss of virus during sample manipulation, the inability to detect the relatively low levels of virus that might be anticipated in naturally-contaminated foods, and the presence of residual toxic or inhibitory compounds that interfere with detection assays (Jaykus and others 1996).

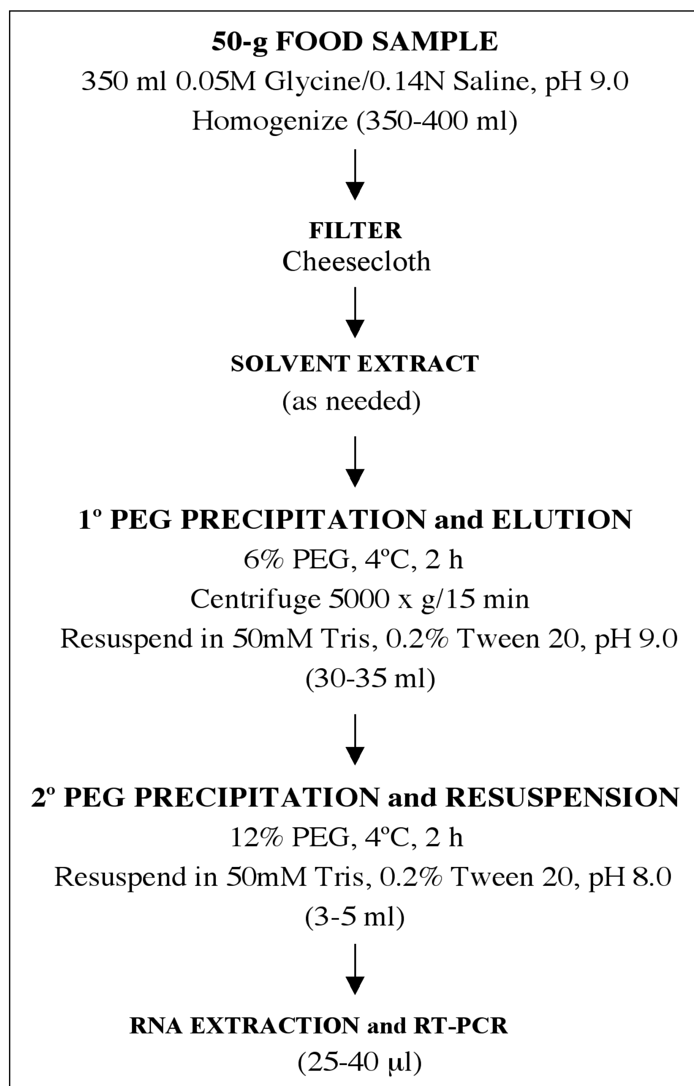

Figure 1–Representation of virus concentration and detection scheme for foods, consisting of sequential steps of filtration, solvent extraction, primary PEG precipitation, secondary PEG precipitation, RNA extraction, and RT-PCR.

Virus detection

Mammalian cell culture infectivity assays. Historically, the detection of human enteric viruses from food concentrates has been based on the infectivity of the viruses for susceptible, live laboratory hosts (Jaykus and others 1994; Jaykus 2000b). Cell lines commonly used for the detection of culturable human enteric viruses include the BGMK (buffalo green monkey kidney-derived) (Sobsey and others 1978; Jaykus and others 1995; De Leon and Jaykus 1997), MA-104 (rhesus monkey kidney-derived, RD (human rhabdomyosarcoma-derived) (Schmidt and others 1978), and HeLa (human cervical carcinoma) cell lines. Due to the ease of propagation of PV1 in mammalian cell culture, it is the most frequently used model virus when developing new detection strategies for food and environmental systems (Jaykus 1997). While the FRhK-4 (fetal rhesus monkey kidney-derived) cell line is used routinely to propagate the HM-175 lab-adapted strain of HAV (Cromeans and others 1987), this cell line remains ineffective for the detection of wild-type HAV (De Leon and Jaykus 1997). Furthermore, there is no susceptible host system for the epidemiologically important HuCVs. While development of host systems for the NLVs remains an active area of research, results to date have been disappointing (White and others 1996).

Immunological methods. Due to the technological limitations of mammalian cell culture infectivity assays, including expense, time to detection, and virus culturability, immunological methods have been proposed, particularly for the detection of those enteric viruses for which no host system exists. Some investigators have adapted immunological methods for the detection of viruses, such as HAV, in shellfish (Sobsey 1985). Furthermore, a number of the clinical immunological methods reported in the literature have indeed been used to detect NV in clinical specimens associated with foodborne disease outbreaks (Heun and others 1987; Hedberg and Osterholm 1993; Lewis and others 1995). However, while shown to be effective for clinical specimens, the general unavailability of reagents and the poor assay detection limits ($> 10^5$ infectious virus particles) of immunulogical methods compromises their practical application for the detection of viral contamination in foods (Parker and others 1993).

Nucleic acid hybridization methods. Gene probes and nucleic acid hybridization methods, both radioactive and nonradioactive, have also been developed for the detection of enteric viruses in clinical, environmental and food samples (Jaykus and others 1994; Bosch and others 1996; Jaykus 2000b). Detection limits for hybridization to genomic viral RNA have been reported at 500 to 1000 infectious units for HAV (Shieh and others 1991), 10^5 physical particles for HuCVs (Kogawa and others 1996), 2.5×10^5 physical particles for rotavirus SA-11 (Dimitrov and others 1985), and 500 to 1000 plaque forming units (PFU) for the coxsackie B3 enterovirus (Jaykus and others 1994). Single-stranded RNA probes

developed to detect HAV in stool and water samples have been shown to be 5 to 8-fold more sensitive than cDNA probes (Jiang and others 1987; Shieh and others 1991). In general, nonradioactive detection systems are 10 times less sensitive than radioactive alternatives (Jaykus and others 1994). Gene probes have been reportedly used for the detection of human enteroviruses (Margolin and others 1989), HAV (Jiang and others 1987), and rotaviruses (Zhou and others 1991) in environmental waters and shellfish (Jaykus 2000b). However, with a desired detection limit of 1 to 10 infectious units, these hybridization methods have significant limitations since assay detection limits usually exceed 10^3 to 10^4 physical particles/sample.

Nucleic acid amplification methods. Several RT-PCR methods have been described for the detection of human enteric viruses from foods, predominantly shellfish (reviewed by Jaykus 2000b). However, despite some limited success in methods development, RT-PCR is still not used routinely to detect viruses in contaminated foods. Certainly, viruses must be concentrated and purified from food matrices before applying detection methods such as RT-PCR, but this is exacerbated by the very small sample volumes (1 to 10 ml) used in nucleic acid amplification. Consequently, food samples present additional challenges due to high sample volumes, low levels of contamination, and the presence of residual food components that can act as enzymatic inhibitors (Rossen and others 1992; Dix and Jaykus 1998; Green and others 1998a; Jaykus 2000b; Shieh and others 1999). In general, 2 different approaches have been used to simultaneously concentrate viruses or viral nucleic acids and perform RT-PCR, and these are: (1) direct nucleic acid extraction RT-PCR; and (2) virion concentration followed by RT-PCR (reviewed by Jaykus 2000b). The direct nucleic acid extraction RT-PCR method involves extraction of total sample RNA, including viral RNA, and is the most widely reported approach for the detection of viruses in environmental and food samples. While the extracted RNA can be of relatively high purity, food-related amplification inhibitors frequently remain and the multiple sample manipulation steps can result in incomplete recovery and/or potential degradation of RNA during the extraction procedure (Drebot and Lee 1997; Jaykus 2000b). In the case of virion concentration, the analyst seeks to concentrate viruses and remove inhibitors prior to application of RNA extraction and RT-PCR amplification. One of these methods, the so-called antibody-capture RT-PCR, involves the direct isolation of viruses from food samples by immunocapture, followed by heat release of the viral RNA and RT-PCR detection (Lopez-Sabater and others 1997). Even though there are fewer sample manipulations involved with this method, the specificity of the approach may be an issue, since only a single virus type can be concentrated by antibody capture. A 2nd virion concentration RT-PCR method relies on further concentration of viruses from the food matrix, frequently to volumes of < 1 ml, and has been applied to virus detection in both artificially (Jaykus and others 1996) and naturally-contaminated (Chung and others 1996) shellfish samples. While this method achieves significant sample volume reductions with the recovery of infectious viruses, substantial viral loss during the extraction steps may reduce overall detection limits (Jaykus 2000b). The reader is referred to Figure 1, which diagrams a representative virus concentration and detection approach for foods.

All of the methodological approaches described above have been applied to artificially-contaminated shellfish species. Unfortunately, only a few methods have been applied to naturally contaminated shellfish (Desenclos and others 1991; Le Guyader and others 1994, 2000; Atmar and others 1995; Lees and others 1995; Chung and others 1996; Haflinger and others 1997; Green and others 1998a; Shieh and others 1999), and on only a few occasions have the methods successfully detected viruses in food samples epidemiologically-linked to disease (Desenclos and others 1991; Lees and others 1995; Le Guyader and others 1996a; Gaulin and others 1999b; Shieh and others 1999; Schwab and others 2000). Only 3 studies have systematically attempted to develop virus detection methods for food products other than shellfish (Gouvea and others 1994; Leggitt and Jaykus 2000; Schwab and others 2000) (Table 3).

Recent progress in polymerase chain reaction detection

Nucleic acid extraction methods.

It is now well recognized that the reliability of nucleic acid amplification methods, for the detection of pathogens in all sorts of samples (clinical, environmental, and foods), depends in large part on the purity of the target template and the number of target molecules (Jiang and others 1992; Deng and others 1994; Jaykus and others 1996). Foods are such complex matrices that an extraction method must be chosen that minimizes the effects of any potentially inhibitory compounds that reduce the PCR amplification efficiency of the intended target (Lampel and others 2000). Because of the high susceptibility of reverse transcriptase to interfering or inhibitory substances (Wilde and others 1990), an efficient RNA extraction step is critical when attempting to detect viruses from complex sample matrices. Two parameters in need of optimization therefore include (1) efficient recovery of the virus and/or viral nucleic acid; and (2) elimination or inactivation of inhibitory substances (Arnal and others 1999). Unfortunately, many of the methods to extract enteric viruses from foods result in the simultaneous co-extraction of inhibitory substances such as acidic polysaccharides, proteins, glycogen, salts, phenolic compounds, and lipids that can in turn inhibit RT-PCR reactions (Beutler and others 1990; Gouvea and others 1990; Demeke and Adams 1992; Wilson 1997; Richards 1999).

A variety of methods have been developed to extract DNA and RNA from foods and other complex samples while reducing the level of inhibitors (reviewed by Wilson 1997). When applied to virus detection in foods, most of these methods have been attempted in the shellfish matrix (Atmar and others 1995; Jaykus and others 1996; Sugieda and others 1996; Dix and Jaykus 1998), and only a few have been reported for other foods (Gouvea and others 1994; Leggitt and Jaykus 2000; Schwab and others 2000). Protocols utilizing Sephadex (De Leon and others 1992), cellulose (Wilde and others 1990), or Chelex (Straub and others 1994) allow salts and small proteins to be effectively eliminated. Methods based on CTAB (Jiang and others 1992; Jaykus and others 1996) and ProCipitate‰ make it possible to eliminate polysaccharides. Shieh and others (1999) found that an acid adsorption-elution step during virus concentration reduced inhibitor carryover, and that further purification of RNA using a silica gel membrane facilitated the removal of additional non-specific RT-PCR inhibitors. Other investigators have used "nested" RT-PCR to improve assay sensitivity, which has been necessary in almost all instances where viruses were detected in naturally contaminated shellfish (Lees and others 1995; Le Guyader and others 1996a; Haflinger and others 1997; Green and others 1998a). Unfortunately, the increased risk of contamination that is associated with extra sample manipulations remains a significant drawback to nested amplification approaches.

Detection of human enteric viruses in non-shellfish food commodities

A recent report has indicated successful detection of NLVs by nested RT-PCR in raspberries epidemiologically implicated in a foodborne outbreak, although the methodological details are sketchy (Gaulin and others 1999b). Gouvea and others (1994) were the first to systematically develop a method to detect NV and rotavirus from representative food commodities other than shellfish, including orange juice, milk, lettuce, and melon. Using

a guanidinium extraction followed by adsorption of RNA to hydroxyapatite and sequential precipitation with CTAB and ethanol, these investigators were able to detect approximately 10^3 genomic copies of rotavirus/10 g of clam meat and 20 to 200 particles of NV/10 g of clam meat, but, again, nested RT-PCR was necessary to achieve detection of low levels of input virus.

Schwab and others (2000) reported the use of TRIzol, a proprietary RNA extraction method, to wash deli meats, including samples artificially contaminated with NV and ones linked to an outbreak of NLV-associated gastroenteritis, for subsequent detection by RT-PCR. By this method, 10 to 100 RT-PCR units (approximately equivalent to 10^2 to 10^3 viral genome copies) of HAV and NV were detected from 20 g deli meat samples. While the TRIzol surface wash method of Schwab and others (2000) was reportedly simple, RT-PCR inhibition persisted unless sample concentrates were diluted 10 to 100-fold, and nested RT-PCR was necessary to further characterize the amplicons from the positive food sample from the outbreak investigation.

Leggitt and Jaykus (2000) developed a prototype method to extract and detect human enteric viruses from lettuce and hamburger samples using an elution-concentration approach followed by a guanidinium RNA extraction and detection by RT-PCR. Poliovirus type 1 and HAV were detected by RT-PCR at initial inoculum levels $\geq 10^2$ and 10^3 PFU/50-g food samples, respectively. Norwalk virus detection in both food products was achieved at inoculum levels $\geq 1.5 \times 10^3$ PCR-amplifiable units/50-g sample. Similar to the TRIzol wash method mentioned above (Schwab and others 2000), their final RNA concentrates were compatible with RT-PCR amplification of viral RNA after a 1 to 2 log dilution for lettuce RNA extracts and 1 log dilution for hamburger RNA extracts (Leggitt and Jaykus 2000).

Although strides have been made in developing virus detection methods for non-shellfish food commodities, barriers still remain. For instance, the need for universal sample extraction methods makes the TRIzol surface wash method impractical for products of complex composition (hamburgers, bakery products) or those of more liquid consistencies (mixed salads) that have to undergo homogenization prior to RNA extraction (Leggitt and Jaykus 2000). An additional problem that is commonly seen in food systems is non-specific amplification due to residual RNA from incomplete ribonuclease activity of reverse transcriptase, and/or food derived DNA or RNA (Jaykus and others 1996). It is well recognized that the effect of residual RT-PCR inhibitors is greater at low copy numbers (Jaykus and others 1996). Furthermore, since the detection target is almost always viral RNA, a clear correlation between detection of RNA and infectivity of the virus must exist. This is currently a hotly debated topic, with some investigators have suggesting that free viral RNA is stable for d to wk and that its detection by RT-PCR is not necessarily indicative of infectious virions (Tsai and others 1995; Richards 1999). Taken together, it is clear that developmental advancements are needed in order to further our ability to detect viral contamination in at-risk food products.

Choice of primers for the detection of human caliciviruses in foods

For food systems, the issue of primer choice is critical. Of course, the danger of choosing primer pairs that are not broadly reactive is the increased probability of obtaining false-negative results. Unfortunately, the more broadly reactive primers either contain a significant degree of degeneracy or else are used under conditions of extremely low annealing temperature. In the case of fecal specimens with high (> 10^6 particles/g) virus levels, such primer degeneracy or low annealing temperature are likely to be less important issues because the amount of viral RNA available to support amplification is quite high, pushing the RT-PCR reaction towards specific amplification. However, for food matrices, where levels of contamination are considerably lower and the food matrix effect is significant, high levels of degeneracy and low annealing temperatures are likely to favor non-specific amplification which has the potential to significantly reduce both assay sensitivity and specificity.

The NV 36/35 primer set has been the most widely used for the detection of NLVs in shellfish and deli meat samples (Atmar and others 1995; Schwab and others 1997, 2000, 2001) (Table 4). Leggitt and Jaykus (2000) used the NV 51-3 primer pair during the development of methods to detect NLVs from seeded hamburger and lettuce samples, while Lees and others (1995) used the NI/E3 primer set for the detection of SRSVs in shellfish associated with 4 separate outbreaks of human gastroenteritis, with all 4 samples yielding positive results. Shieh and others (2000) used both polymerase and capsid primer sets, G2 and Mon381/383, respectively, for nested PCR to identify a NLV G2 strain in 2 oyster samples implicated in a 1998 California outbreak. Hafliger and others (1997) have also reported the use of primers designed to bind to the 3D region of the RNA polymerase gene to detect HAV from shellfish.

Alternative confirmation methods

While PCR will amplify DNA molecules several thousand-fold, detection of the resulting DNA fragments (amplicons) must be done, and, furthermore, amplicon confirmation is necessary to assure that detection is accurate. The most commonly used method for the detection and subsequent confirmation of RT-PCR amplifications is agarose gel electrophoresis followed by Southern hybridization using labeled internal oligonucleotide probes (Hardy and others 1997; Honma and others 2000). Unfortunately, Southern hybridization is a time-consuming and cumbersome method, frequently requiring 2 d before results are obtained. Recently, Schwab and others (2001) reported the development of a DNA EIA (DEIA) to detect RT-PCR-generated amplicons in microtiter wells using virus-specific biotinylated oligoprobes. The DEIA detected low levels of NV RNA RT-PCR amplicons from stool (NV end point of 20 ml of a $10^{-5.5}$ dilution), and both NLV and HAV from bivalve mollusks following bioaccumulation. Assay sensitivity was equal to or better than Southern hybridization, and had the added advantages of ease of interpretation and more rapid (4 h) amplicon confirmation. Other PCR confirmation methods have included specific "nesting" reactions (Gouvea and others 1994; Lees and others 1995; Sugieda and others 1996; Hafliger and others 1997), restriction endonuclease digestion of RT-PCR products (Gouvea and others 1994; Hafliger and others 1997), and direct amplicon sequencing (Lees and others 1995; Le Guyader and others 1996a; Parashar and others 1998; Schwab and others 2000). However, all of these confirmation methods require significant amounts of sample manipulation and remain time-consuming (Jaykus 2000b).

There is a clear need for more rapid endpoint detection and confirmation of PCR amplification products when attempting detection of infectious agents in any sample matrix, including foods. Given this need, investigators have recently focused their efforts on developing alternatives to the classic methods currently in use for the detection and confirmation of PCR products. Significant among these methods is nucleic acid sequence based amplification (NASBA), and the fluorescence resonance energy transfer (FRET) phenomenon (Martell and others 1999).

NASBA (Organon Teknika, Durham, N.C., U.S.A.) is a novel RNA amplification method (Organon Teknika Corp. 1999) that utilizes 3 enzymes (Avian Myeloblastosis Virus Reverse Transcriptase (AMV-RT), RNase H, and T7 RNA polymerase) and 2 specific oligonucleotide primers. The NASBA assay system has several advantages over other nucleic acid amplification methods because it is isothermal, it results in single-stranded RNA, which

can be readily detected by probe hybridization without the need for a denaturation step, and rapid confirmation can be achieved using the automated ECL detection system. NASBA methods are already available as commercial kits for the detection of key bloodborne pathogens such as HIV and cytomegalovirus, and are more recently available as a Basic Kit for use in the development of other diagnostic tests. Greene and others (1999) adapted the NASBA protocol for the detection of NLV RNA, and when compared to RT-PCR, the NASBA was more rapid (6 to 8 h) and demonstrated 10 to 100-fold better detection limits. Although the NASBA is likely to be impacted by many of the same restrictions as RT-PCR (that is, contamination control, sample volume considerations, matrix-associated reaction inhibitors), it is likely to remain an important alternative detection method for foodborne pathogens. Furthermore, since it is isothermal, it may well be more amenable to future biosensor applications.

Other investigators have reported using the FRET phenomenon for the rapid and specific detection of PCR amplicons. Of particular note in recent y is the TaqMan Assay™ (Applied Biosystems, Foster City, Calif., U.S.A.), which has been applied to the detection of other foodborne pathogens including *L. monocytogenes* (Bassler and others 1995; Norton and Batt 1999) and *E. coli* O157:H7 (Witham and others 1996; Chen and others 1998; Oberst and others 1998; Sharma and others 1999; Sharma and Carlson 2000). Other improved and enhanced versions of FRET-based PCR protocols have included molecular beacons (Tyagi and Kramer 1996; McKillip and Drake 2000) and an "asymmetric" 5′ nuclease activity assay (Koo and Jaykus 2000). Although none of these methods has yet been applied for the detection of enteric viruses in clinical, environmental, or food matrices, they offer opportunities that could substantially simplify testing and significantly reduce overall endpoint detection time.

Biosensors and microarray detection

Recently, the sensitive detection of foodborne pathogens in complex environments has been achieved using biosensor methods, that is microchip systems for analyzing the formation of antigen-antibody complexes (Malmqvist 1993; Hirmo and others 1998; Pyun and others 1998; Zhou and others 1998; Mandrell and Wachtel 1999). For most biosensor-based methods, an immunoaffinity step to capture the antigen of interest on beads, a membrane, or a fiber optic probe tip is followed by detection of the antigen by laser excitation of bound antibodies, acoustogravimetric wave transduction, or surface plasmon resonance (Mandrell and Wachtel 1999). These systems report rapid (min) detection along with a reasonably sensitive limit of detection (1×10^3 cfu/ml for bacteria). Recently, biosensors have also been applied to the detection of polioviruses (Kersten and others 1999; McDermott and others 2000). Despite their apparent potential as analytical tools, few biosensors are used routinely and further research is needed to address issues such as advanced sample (pathogen) concentration, elimination of non-specific detection, and improved detection limits before their widespread use in food microbiology.

Recent advances in the detection of sequence-specific nucleic acid hybridization have been achieved using microarrays, high-density microscropic arrays of immobilized nucleic acids (Epstein and Butow 2000). Microarrays can be prepared by synthesizing DNA *in situ* on a glass surface using combinational chemistry (Pease and others 1994), or by robotic microdeposition of cDNAs (0.5- to 2-kb) amplified by PCR (Zammatteo and others 2000). The sample DNA, usually bound to a fluorescent or enzyme label, is exposed to the microarray and hybridizes with the target sequences. The detection of the probe-target hybrid at each spot on the array is achieved either by direct fluorescence scanning or enzyme-mediated detection yielding a semi-quantitative result (de Boer and Beumer 1999). Advantages of DNA microarray technology, as compared to conventional techniques, include the small size of the array allowing for a higher sensitivity, the ability to simultaneously detect diverse individual sequences in complex DNA samples, and the capacity to do comparative analysis of a large number of samples (de Boer and Beumer 1999; van Hal and others 2000). However, as with biosensors, obstacles such as sample size, matrix-associated inhibitors, non-specific binding, and cross hybridization must be overcome before microarrays can be used for the detection and differentiation of pathogens in food samples. Regardless, an oligonucleotide array dot-blot format for NLV strain genotyping has recently been reported (Vinje and Koopmans 2000), and this technology offers promise in the development of more advanced testing strategies for foodborne pathogens.

Conclusions

Epidemiological data clearly demonstrates that foods can act as efficient vehicles for the transmission of human enteric viruses by the fecal oral route. Control strategies that have been proposed include depuration and improved microbial indicators (shellfish), educational initiatives (production agriculture and food handlers), and vaccination (HAV and NLVs). However, all of the strategies require significant resources and none of them are failsafe.

Before the prevalence of viral gastroenteritis in the U.S.A. and worldwide can be fully appreciated, a better understanding of how foods contribute to both outbreak and sporadic cases must be achieved. While recent advances in molecular biology have improved our ability to study foodborne viruses, there are still barriers to overcome. Methodological improvements for the detection of viruses from foods must focus on virus extraction procedures, decreasing the level of matrix-associated RT-PCR amplification inhibitors in the final concentrates, and the development of a universal testing protocol for all foods. Our ability to detect viruses in foods epidemiologically-linked to disease, along with our understanding of the extent to which sporadic cases of viral gastroenteritis are, in fact, associated with foodborne routes of transmission will only improve with further research in this area.

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