Human and Animal Viruses in Food (Including Taxonomy of Enteric Viruses)

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1.0. INTRODUCTION

In recent years, there has been an increase in the incidence of food-borne diseases worldwide, with viruses now recognized as a major cause of these illnesses. The viruses implicated in food-borne disease are the enteric viruses, which are found in the human gut, excreted in human feces, and transmitted by the fecal-oral route. Many different viruses are found in the gut, but not all are recognized as food-borne pathogens. The enteric viral pathogens found in human feces include noroviruses (previously known as Norwalk-like viruses), enteroviruses, adenoviruses, hepatitis A virus (HAV), hepatitis E virus (HEV), rotaviruses, and astroviruses, most of which have been associated with food-borne disease outbreaks. Noroviruses are the major group identified in food-borne outbreaks of gastroenteritis, but other human-derived and possibly animal-derived viruses can also be transmitted via food.

The diseases caused by enteric viruses fall into three main types: gastroenteritis, enterically transmitted hepatitis, and illnesses that can affect other parts of the body such as the eye, the respiratory system, and the central nervous system including conjunctivitis, poliomyelitis, meningitis, and encephalitis. Four of the enteric viruses—noroviruses, HAV, rotaviruses, and astroviruses—are included in the thirteen major food-borne pathogens identified by the Centers for Disease Control and Prevention (CDC) (Mead et al., 1999). These four viruses are reported to comprise 80% of all food-borne illnesses in the United States, with noroviruses by far the greatest contributor at an estimated 23 million cases per year (Mead et al., 1999).

All enteric viruses except the adenoviruses contain RNA rather than DNA, have a protein capsid protecting the nucleic acid, and are nonenveloped. In the environment and in food, the enteric viruses are inert particles and do not replicate or metabolize because, like all viruses, they are obligate pathogens and require living cells to multiply. Many of the enteric viruses such as astroviruses, enteric adenoviruses, HAV, and rotaviruses are fastidious in their *in vitro* growth requirements but can still be grown in cell cultures. Noroviruses, on the other hand, do not grow *in vitro*, and no animal model exists for the human noroviruses yet. For many years, the lack of a culture system limited investigations focusing on the role of noroviruses in food-borne disease, although progress is now being made after the *in vitro* culture of a mouse norovirus (Wobus et al., 2004). Cell cultures are

generally used for the analysis of culturable viruses. Using culture methods, infectious viruses can be identified through their ability to produce changes in inoculated cells (cytopathic effects or CPE) or through expression of viral antigens that may be detected serologically. The advantage of culture-based methodology is that it can be either quantitative or qualitative and produces unambiguous results with respect to virus presence and infectivity.

Until the introduction of molecular methods, enteric viruses were mainly identified by electron microscopy (EM) including solid-phase immune electron microscopy (SPIEM). The SPIEM is more sensitive than direct EM because, in the presence of specific antibodies, the virus particles are coated with specific antibody and aggregated together, making them more easily distinguishable from the background matrix. Many of the "small round viruses," which include astroviruses, noroviruses, sapoviruses, and parvoviruses, were first discovered through the use of EM.

Molecular methods are now the most commonly used techniques for the identification of enteric viruses in foods, but other methods are also available for virus detection in human specimens. Identification of enteric viruses can also be carried out by enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and, for the culturable viruses, culture-PCR, which is a combination of cell culture and polymerase chain reaction (PCR) methods. The latter technique detects only the infectious virus and is preferable to direct PCR, which currently detects both infectious and noninfectious viruses.

Enteric viruses are generally resistant to environmental stressors, including heat and acid. Most resist freezing and drying and are stable in the presence of lipid solvents. It is not clear whether pasteurization at 60°C for 30 min inactivates all enteric viruses. Many enteric viruses show resistance to ultrahigh hydrostatic pressure, which is now being widely used as a novel food-processing treatment for shellfish, jams, jellies, and dairy products (Wilkinson et al., 2001; Kingsley et al., 2002). The resistance of enteric viruses to environmental stressors allows them to resist both the acidic environment of the mammalian gut and also the proteolytic and alkaline activity of the duodenum so that they are able to pass through these regions and colonize the lower digestive tract. These properties also allow survival of enteric viruses in acidic, marinated, and pickled foods; frozen foods; and lightly cooked foods such as shellfish. Most enteric viruses are believed to have a low infectious dose of 10–100 particles or possibly even less. Hence, although they do not multiply in food, enough infectious virions may survive in food, be consumed, and cause disease.

Enteric viruses have been shown to retain infectivity in shellfish and in fresh, estuarine, and marine waters for several weeks at 4°C (Jaykus et al., 1994; Scientific Committee on Veterinary Measures relating to Public Health, 2002). The length of virus survival appears to be temperature dependent and is inversely related to increased temperature. The enteric viruses may survive longer if attached to particulate matter or sediments, where they can present a greater potential risk to human health (Jaykus et al., 1994).

Most viruses causing food-borne disease are of human origin, and the source of viral contamination generally originates from human fecal material. Viral contamination of foods can occur pre- or postharvest at any stage in the food harvesting, processing, and distribution chain. The key factors influencing the risk of contamination of fresh produce are water quality, field-worker hygiene, and food-handler hygiene. Thus, sewage contamination and poor hygiene practices play a major role in the contamination process.

The globalization of the food supply means that the source of fresh produce may not always be known and the quality may not always be controlled. Although it is presumed that fresh produce is "clean, green, and healthy," it may not be so, especially when it is imported from countries where general hygiene practices do not meet international standards. This knowledge, combined with a number of outbreaks associated with contaminated fresh produce, has led to consumer suspicion of imported foods in many countries.

The opportunities for both pre- and postharvest viral contamination are numerous. The quality of the growing waters is important for shellfish quality. Preharvest virus contamination occurs when filter-feeding bivalve shellfish grow in waters contaminated with sewage or fecal material. Shellfish filter between 4 and 20 L of water every hour, sieving out and accumulating food particles, including bacteria, viruses, and heavy metals. Feeding rates depend on water temperature and salinity and availability of food and particulate matter. Bacteria and viruses become trapped in the mucus of the gills, which is then pushed into the digestive gland where viruses appear to concentrate. Shellfish can accumulate high concentrations of viruses within a few hours when surrounding waters contain sufficient levels of virus, so that concentrations in shellfish may be 100 to 1,000 times greater than the surrounding waters.

Virus uptake varies between shellfish species and also between individuals. In winter, the shellfish are physiologically less active and so do not accumulate or remove viruses as fast as in the warmer seasons. In clean waters, shellfish depurate or cleanse themselves of bacteria and particulate matter. However, some studies have shown that depuration does not remove viruses efficiently, and there is no correlation between the removal of bacteria and viruses (Lees, 2000). This was demonstrated in a large hepatitis A outbreak in Australia where oysters were depurated for 36 hr before consumption but still retained infectious HAV (Conaty et al., 2000).

Fresh produce may have been irrigated or washed in water containing human fecal material or handled by field workers or food handlers with poor hygiene practices. In such situations, the produce may be contaminated with disease-causing enteric viruses. Foods at the greatest risk of virus contamination at the preharvest stage are shellfish, soft berry fruits, herbs, and salad greens. Foods at risk from contamination by food handlers include a wide range of foods that are subjected to much handling and are subsequently consumed cold or uncooked. These include bread and bakery goods, lightly cooked or raw shellfish, sandwiches, salads, herbs, fresh fruits, cold meats, and

cold desserts. It is probable that the current trend for the consumption of raw or lightly cooked ready-to-eat (RTE) foods, especially salads and sandwiches, has increased the risk of food-borne viral disease. Poor food handling was shown to be a key risk factor in the transmission of noroviruses and rotaviruses in The Netherlands (de Wit et al., 2003).

All food-borne viruses are transmitted by the fecal-oral route and are generally host specific for humans, although animal strains of the same virus may also exist. Viruses are frequently host specific, preferring to grow in the tissue of one species rather than a range of species. Both animal and human strains exist in all of the enteric viral genera. A key question still to be answered is whether animal viruses can infect humans and vice versa. The pathogenic strains of astrovirus, adenovirus, and enterovirus that infect animals appear to be distinct from those that infect humans. Thus, although noroviruses have been isolated from animal feces, so far they have not been implicated in human disease (Sugeida et al., 1998; van der Poel et al., 2000; Oliver et al., 2003).

Zoonotic infections are generally not transmitted by food. However, the risk of zoonotic viral disease from meat products contaminated with animal viruses has been identified in some countries; tick-borne encephalitis virus (TBE) and hepatitis E virus (HEV) being two examples. HEV is possibly the first virus reported to cause zoonotic food-borne viral disease (Tei et al., 2003). Nonviral infectious proteinaceous agents, or prions, that cause diseases such as bovine spongiform encephalopathy (BSE), scrapie, and Creutzfeldt-Jakob disease, also transmit disease from animals to man via the food-borne route but are not discussed in this chapter.

As a result of the advances in methodology for detection of viruses in foods, the extent and role of viruses in food borne viruses have been clarified in recent years. The development of new molecular methods, including real-time PCR–based methods, for the detection of nonculturable or difficult to culture viruses has shown their frequent presence in the environment and in foods, especially shellfish. These methods have also allowed investigation of virus responses to environmental stressors and have contributed to increased knowledge of enteric virus behavior in foods and in the environment.

2.0. HEPATITIS A VIRUS

2.1. Distribution and Transmission

Several different viruses cause hepatitis but only two, HAV and HEV, are transmitted by the fecal-oral route and are listed as "Severe Hazards" in Appendix V of the U.S. Food and Drug Administration's Food Code (Cliver, 1997). The hepatitis viruses are so named because they infect the liver, rather than sharing phylogenetic or morphological similarities, and each of the five different hepatitis viruses is classified in a distinct viral family. HAV causes hepatitis A, a severe food and waterborne disease that was formerly known

as infectious hepatitis or jaundice. The virus is primarily transmitted by the fecal-oral route but can also be transmitted by person-to-person contact. Hepatitis A infection occurs worldwide and is especially common in developing countries where more than 90% of children have been reported to be infected by 6 years of age (Cliver, 1997; Cromeans et al., 2001). The infection is often asymptomatic in children.

In recent years, the incidence of hepatitis A infection in many countries has decreased as sewage treatment and hygiene practices have improved, but this has also led to an overall lowering of immunity in these populations with consequent increase in susceptibility to the disease. As a result, there is an increasing risk of contracting hepatitis A infection from fresh foods imported from regions of the world where HAV is endemic and general hygiene standards are poor. Hepatitis A is a serious food-borne infection and hence is a notifiable disease in most of the developed countries. This means that accurate data on its occurrence are recorded in these countries. In the United States, hepatitis A is reported as the most common cause of hepatitis with a reported death rate of 0.3%. However, the actual incidence of hepatitis A is assumed to be 10 times that of the reported cases. Between 1980 and 2001, the CDC was notified of an average of 25,000 cases/year, but when corrections were made to the data, the average case numbers were estimated to be approximately 260,000 per year (Fiore, 2004).

No seasonal distribution of HAV has been observed, with infection occurring throughout the year, but the disease has been reported to have a cyclic occurrence in endemic areas. This cyclic pattern has been observed in the United States, particularly among low socioeconomic, Native American, and Hispanic populations, with large increases in hepatitis A infections occurring approximately every 10 years. However, the main transmission route is probably from person to person rather than being food-borne (Cromeans et al., 2001; Fiore, 2004).

2.2. Taxonomy and Morphology

HAV is a 27- to 32-nm, nonenveloped, positive-sense, single-stranded RNA virus with a 7.5-kb genome, icosahedral capsid symmetry, and a buoyant density in cesium chloride of 1.33–1.34 g/ml. The virus is classified in the Picornaviridae family in its own distinct genus, Hepatovirus (Table 2.1) but was formerly classified in the Enterovirus genus as Enterovirus 72. It has a structure similar to that of other picornaviruses. There is one species, HAV, with two strains or biotypes: human HAV and simian HAV. These two distinct strains are phylogenetically distinct and have different preferred hosts. Human HAV infects all species of primates including humans, chimpanzees, owl monkeys, and marmosets, whereas simian HAV infects green monkeys and cynomolgus monkeys. Seven genotypes have been recognized, of which four infect humans and the remaining three infect nonhuman primates.

Unlike many RNA viruses, the genome of HAV is highly conserved, with an average variation of only 1–4%, but there are two groups within the genus

Table 2.1 Characteristics of Food-borne Viruses

Virus Genus or Species	Family	Nucleic Acid Type	Envelope	Morphology/ Symmetry	Size of Virion (nm)	Culturable[a]	Genome Size (kb)	Disease
Adenovirus	Adenoviridae	dsDNA	N	Icosahedral	70–90	Y[a]	28–45	Respiratory, eye, and gastroenteritis infection
Astrovirus	Astroviridae	(+) ssRNA	N	Icosahedral	28–30	Y[a]	7–8	Gastroenteritis
Norovirus	Caliciviridae	(+) ssRNA	N	Icosahedral	28–35	N	7.4–7.7	Epidemic gastroenteritis
Sapovirus	Caliciviridae	(+) ssRNA	N	Icosahedral	28–35	N	7.4–7.7	Gastroenteritis
Hepatovirus: hepatitis A virus	Picornaviridae	(+) ssRNA	N	Icosahedral	27–32	Y[a]	7.5	Inflammation of liver, hepatitis
Hepevirus: hepatitis E virus	Hepeviridae	(+) ssRNA	N	Icosahedral	32–34	N	7.2	Inflammation of liver, hepatitis
Rotavirus	Reoviridae	dsRNA	N	Icosahedral	60–80	Y	16–27	Gastroenteritis
Enterovirus	Picornaviridae	(+) ssRNA	N	Icosahedral	28–30	Y[a]	7.2–8.4	Poliomyelitis, meningitis, encephalitis
Parvovirus	Parvoviridae	ssDNA	N	Icosahedral	20–30	N	5	Gastroenteritis
Tick-borne encephalitis virus	Flaviviridae	(+) ssRNA	N	Icosahedral	45–60		9.5–12.5	Tick-borne encephalitis via milk
Coronavirus	Coronaviridae	(+) ssRNA	Y	Helical	80–220	Y[a]	20–30	Gastroenteritis, respiratory infections
Torovirus	Toroviridae	(+) ssRNA	Y	Helical	100–150	Y	20–25	Gastroenteritis in animals and? humans
Picobirnavirus	Birnaviridae	(+) ssRNA	N	Icosahedral	35			Gastroenteritis in animals and? humans

[a] Not all strains within the genus are culturable; wild-type strains are often difficult to culture.

that show diversity of 10% and up to 25% (Cromeans et al., 2001). HAV has been classified into seven genotypes based on sequence analysis of the VP1 and VP3 genes that code for surface proteins (Robertson et al., 1991, 1992). Characterization of these genotypes has been useful in outbreak investigations for tracing infection sources, and strains within these genotypes have shown more than 85% genetic similarity (Niu et al., 1992; Cromeans et al., 2001).

2.3. Growth and Biological Properties

HAV can be cultured in several different primate cell lines including African green monkey kidney cells (BSC-1), fetal rhesus monkey kidney cells (FRhK-4 and FRhK-6), and human fibroblasts (HF), but wild-type strains are difficult to culture and generally do not produce CPE in cell cultures. Immunofluorescence is often used for detection of HAV antigen in infected cells because of the lack of CPE. The virus is usually slow-growing and the yield in cell cultures is lower as compared with most other picornaviruses. Consequently, it is difficult to identify the virus in clinical, food, or environmental sources by culture alone. Under normal conditions, the virus requires 3 weeks for *in vitro* growth. Laboratory-adapted strains such as HM 175 are able to produce CPE and so have been used extensively in research studies. These viruses require less time for *in vitro* growth and produce visible CPE or plaques. Molecular techniques, including culture-PCR, have become the method of choice for detection of virus in nonhuman samples, whereas clinical diagnosis is usually based on the patient's immune response. HAV antigens are conserved and antibodies are generated against a single antigenic site composed of amino acid residues of VP3 and VP1 proteins on the virus surface.

HAV is very stable, showing high resistance to chemical and physical agents such as drying, heat, low pH, and solvents, and has been shown to survive in the environment, including seawater and marine sediments, for more than 3 months (Sobsey et al., 1988). The virus retains integrity and infectivity after 60-min incubation at 60°C and is only partially inactivated after 10–12 hr at 56°C. The heat resistance of HAV is reported to be greater in foods and shellfish. After heating in a can for 19 min at 60°C, HAV inoculated into oysters was not fully inactivated. Under refrigeration and freezing conditions, the virus remains intact and infectious for several years. It is also resistant to drying, remaining infectious for more than 1 month at 25°C and 42% humidity, and shows even greater resistance at low humidity and low temperatures.

Although HAV infectivity decreased by 2 to 5 $\log_{10}$ after exposure to 70% alcohol for 3 min and 60 min at 25°C, it was resistant to several preservatives and solvents including chloroform, Freon, Arklone, and 20% ether and was not inactivated by 300 mg/L perchloroacetic acid or 1 g/L chloramine at 20°C for 15 min (Hollinger and Emerson, 2001) . The virus is stable at pH 1.0 and survives acid marination at pH 3.75 in mussels for at least 4 weeks (Hollinger and Emerson, 2001; Hewitt and Greening, 2004). Gamma irradiation is not

effective for inactivation of HAV on fresh fruits and vegetables, but the virus does appear to be inactivated by high hydrostatic pressure. Hydrostatic pressure, now used as an isothermal preservation method for perishable foods, inactivated HAV after 5-min exposure at 450 MPa (Kingsley et al., 2002). Overall HAV exhibits greater resistance to stressors than other picornaviruses.

2.4. Infection and Disease

HAV infects the epithelial cells of small intestine and hepatocytes, causing elevation of liver enzymes and inflammation of the liver. The cytotoxic T-cell immune response destroys infected liver cells, releasing the virus particles into the bile duct from where they are excreted in the feces. The virus is believed to initially enter the liver via the bloodstream, and it is not clear if intestinal replication occurs.

The virus has an incubation period of 2 to 6 weeks with an average of 28 days. Initially the symptoms are nonspecific and include fever, headache, fatigue, anorexia, dark urine, light stools, and nausea and vomiting with occasional diarrhea. One to 2 weeks later, characteristic symptoms of hepatitis such as viremia and jaundice appear. Peak infectivity occurs in 2 weeks preceding the onset of jaundice, and the virus is present in the blood at 2 to 4 weeks. The HAV is shed in large numbers ($>10^6$ particles/g) in feces from the latter 2 weeks of the incubation period for up to 5 weeks. Jaundice is usually evident from week 4 to 7 and virus shedding generally continues throughout this period. Diagnosis is based on the detection of anti-HAV IgM antibody, which can be detected before the onset of symptoms and becomes undetectable within 6 months of recovery. Acute hepatitis is usually self-limiting, but overall debility lasting several weeks is common and relapses may occur.

The HAV has not been associated with development of chronic liver disease, but on rare occasions fulminant disease that results in death may occur. Because the onset of symptoms occurs several weeks after infection, it is rare to have the suspected food available for analysis. A killed vaccine that provides long-lasting immunity has been commercially available since 1995 and is commonly given to travelers at high risk. This vaccine could be used in the food industry to immunize food workers to reduce the risk of food contamination by workers.

2.5. Food-borne Disease

HAV has been associated with many outbreaks of food-borne disease. Contamination generally occurs either preharvest or during food handling. There are a number of documented outbreaks of disease resulting from consumption of HAV-contaminated shellfish, the largest of which occurred in China in 1988 when approximately 300,000 people were infected after consumption of partially cooked, HAV-contaminated clams harvested from a growing area impacted by raw sewage (Halliday et al., 1991). A few of the shellfish-associated outbreaks include oysters in Australia (Conaty et al., 2000), oysters in Brazil (Coelho et al., 2003), mussels in Italy (Croci et al., 2000),

and clams in Spain (Bosch et al., 2001). In most of these outbreaks, sewage was generally the source of pollution.

Contamination of shellfish with HAV is still common in Italy, Spain, and other European countries. Preharvest contamination of fruits and vegetables, including strawberries (Niu et al., 1992), raspberries (Reid and Robinson, 1987; Ramsay and Upton, 1989), blueberries (Calder et al., 2003), lettuce (Pebody et al., 1998), and green onions (CDC, 2003) has also been reported and has resulted in outbreaks of disease in countries such as Finland and New Zealand, where populations have low or no immunity to the disease (Pebody et al., 1998; Calder et al., 2003). The source of contamination in these outbreaks was reported to be either infected fruit-pickers or contaminated irrigation waters.

The other main source of HAV infection is from food handlers and food processors. Because HAV is shed before symptoms become apparent and $>10^6$ infectious virus particles can be excreted per gram of feces, HAV-infected produce harvesters and food handlers, without knowing, can become a source of contamination. In areas with poor hygiene practices, this can present a risk to human health. Food-borne outbreaks of HAV are relatively uncommon in developing countries where there are high levels of immunity in the local population, but tourists in these regions can be susceptible if they are not vaccinated.

3.0. HEPATITIS E VIRUS

3.1. Distribution and Transmission

HEV is believed to be a major etiologic agent of enterically transmitted non-A, non-B hepatitis in humans worldwide (Emerson and Purcell, 2003). The virus is transmitted by the fecal-oral route and occurs widely in Asia, northern Africa, and Latin America, including Mexico, where waterborne outbreaks are common. Although originally it was believed that HEV did not occur in industrialized countries, in recent years it has been identified in Europe, Australasia, and the United States. However, it rarely is a cause of overt disease in these countries (Clemente-Casares et al., 2003; Emerson and Purcell, 2003). The virus has been isolated from raw sewage in Spain, France, Greece, Italy, Austria, and the United States (Jothikumar et al., 1993; Pina et al., 1998). Transmission is generally via fecally contaminated water, and evidence for food-borne transmission has not been definitively documented. Epidemics and sporadic cases of HEV are responsible for the majority of enterically transmitted acute hepatitis in regions where HEV is considered endemic. Antibodies to HEV have been detected in many animal species, which has led to a discussion on the possible zoonotic aspect of HEV.

3.2. Taxonomy and Morphology

HEV was first isolated and identified by Balayan et al. (1983) in acute and convalescent specimens collected from a case of non-A, non-B hepatitis. It

is a 32- to 34-nm, nonenveloped, positive-sense, single-stranded RNA virus with a linear genome of 7.2 kb (Table 2.1). The capsid symmetry is icosahedral, and the buoyant density in potassium tartrate–glycerol gradient is 1.29 g/ml. HEV was originally classified in the Caliciviridae because of similarities in structural morphology and genome organization. The virus was then reclassified in the family Togaviridae because of similarities between the replicative enzymes of HEV and the togaviruses. However, the current International Committee on Taxonomy of Viruses (ICTV) classification places HEV under a new family, Hepeviridae, genus Hepevirus (van Regenmortel et al., 2000). Two HEV serotypes and four major HEV genotypes have been identified based on nucleotide and protein sequencing. Genotype 1 includes Asian and African strains, genotype 2 includes a Mexican strain, genotype 3 includes United States swine and human strains, and genotype 4 includes strains from China, Japan, and Taiwan (Emerson and Purcell, 2003).

3.3. Growth and Biological Properties

Although there are reports describing culture of HEV, none have shown sustained replication with production of virus particles, and there is no recognized culture system for the virus. HEV is generally identified by molecular methods. The inability to grow the virus has hampered research on the ability of this virus to survive in the environment.

3.4. Infection and Disease

As is the case with HAV, HEV produces an acute disease with generally mild symptoms. Although the disease can be quite severe in some cases, it is usually self-limiting and does not progress to a carrier or chronic state. The virus infects the liver and produces symptoms of hepatitis after a 22–60 day incubation period. Symptoms may include viremia, nausea, dark urine, and general malaise. The virus is excreted in bile and feces from 2 weeks before the elevation of liver enzymes and continues until the enzyme levels return to normal. Identification and diagnosis is generally by detection of IgM and IgG responses in patients' sera to recombinant HEV protein antigens or by molecular assays to identify the virus in feces or sera. In general, the mortality rate from hepatitis E infections is about 1% but may reach as high as 17–30% in pregnant women (Cromeans et al., 2001; Emerson and Purcell, 2003). The major mode of transmission appears to be contaminated water. Secondary person-to-person transmission has been estimated at 0.7–8.0% and is relatively uncommon (Cromeans et al., 2001).

3.5. Food-borne Disease

Food-borne outbreaks of HEV are the most common in developing countries with inadequate environmental sanitation. Large waterborne outbreaks have also been reported in Asian countries (Cromeans et al., 2001; Emerson and Purcell, 2003). HEV was not thought to be endemic in developed countries, and the first reported human cases of acute hepatitis E in the United

States were attributed to travel in HEV-endemic countries. In 1997, however, HEV was isolated from a U.S. resident with hepatitis with no history of travel. Simultaneously, the virus was also identified in domestic swine (Meng et al., 1997; Schlauder et al., 1998) and has now been documented in humans and swine in many other countries including Argentina, Australia, Austria, Canada, Germany, Greece, Japan, Korea, The Netherlands, New Zealand, Spain, and Taiwan (Clemente-Casares et al., 2003). The waterborne transmission route has been proved, and there have been reports of possible foodborne outbreaks in China, but corroborating evidence is lacking. There has been no evidence of HEV transmission via seafood.

3.6. Zoonotic Transmission

The HEV has been isolated from swine in several countries where hepatitis E in humans is rare, including Spain, New Zealand, The Netherlands, Japan, and Canada (Emerson and Purcell, 2003). The reservoirs of infection for HEV are unknown, but the virus has been isolated from the feces of a wide range of domestic animals. Recent reports from Japan show that the virus may be transmitted to humans by close contact with infected swine or from the consumption of contaminated raw or undercooked pork, wild boar liver, and deer meat (Tei et al., 2003; Yazaki et al., 2003). The most convincing evidence of zoonotic transmission to date is a recent report in which consumption of raw deer meat by a Japanese family was implicated in the transmission of HEV (Tei et al., 2003). In another study, Tei et al. (2004) investigated the risks associated with consumption of uncooked deer meat in a case control study and found that, in the area studied, eating uncooked deer meat was a risk factor.

Meng et al. (2002) found that veterinarians and people working with pigs were more likely to have antibodies to HEV. The isolation of a swine HEV that cross-reacts with an antibody to the capsid antigen of human HEV provides additional evidence for a zoonotic transmission route (Meng et al., 1997). Nonhuman primates can also be infected by swine HEV; inoculation of rhesus monkeys with swine HEV led to seroconversion, fecal shedding of virus, viremia, and development of a mild acute hepatitis with slight elevation in liver enzymes. Similar studies in chimpanzees also resulted in seroconversion and fecal shedding of virus but not viremia or hepatitis (Meng et al., 1998). These findings suggest that swine HEV may infect humans and that swine could be a zoonotic reservoir for HEV.

HEV is shed in the feces and bile of swine for 3–5 weeks after infection (Halbur et al., 2001). The excretion of HEV in feces of infected pigs could lead to the spread of HEV in the environment and increase the potential for zoonotic transmission. Similarly, fecal contamination of runoff waters from pig farms or from lands on which untreated pig manure has been spread could contaminate irrigation and surface waters with subsequent HEV contamination of fruits, vegetables, and shellfish. Although there is increasing evidence of the zoonotic transmission of HEV, the risk factors are still largely unknown.

4.0. NOROVIRUS AND SAPOVIRUS

4.1. Distribution and Transmission

Noroviruses, previously known as small round structured viruses (SRSVs) and Norwalk-like viruses (NLVs), are now the most widely recognized viral agents associated with food-borne and waterborne outbreaks of nonbacterial gastroenteritis and probably the most common cause of food-borne disease worldwide. These viruses cause epidemic viral gastroenteritis resulting in large outbreaks. The prototype norovirus, Norwalk virus, was first discovered by Kapikian et al. (1972) after an outbreak of gastroenteritis in a school in Norwalk, Ohio. Immune electron microscopy was used to examine feces from volunteers who consumed fecal filtrates from infected cases (Dolin et al., 1971; Kapikian et al., 1972). At that time, most cases of gastroenteritis that could not be attributed to a bacterial agent were termed *acute nonbacterial gastroenteritis*. The discovery of Norwalk virus provided the first evidence of a viral etiology for human diarrheal disease. Despite this discovery, noroviruses remained largely unrecognized until about 15 years ago because their detection was technically difficult and because the illness is generally mild and short-lived and is not reportable to public health authorities.

Noroviruses are primarily transmitted through the fecal-oral route, by consumption of fecally contaminated food or water, or by direct person-to-person spread. Secondary spread may also occur by airborne transmission. Outbreaks commonly occur in closed community situations such as rest homes, schools, camps, hospitals, resorts, and cruise ships and where the food and water sources are shared. Because norovirus infections are not notifiable, the total burden of disease is not known and is generally grossly under-reported (Mead et al., 1999; Wheeler et al., 1999). However, some of the disease burden is recorded through the notification of gastroenteritis outbreaks to the public health disease surveillance systems in many developed countries. It has been estimated that noroviruses are responsible for approximately 60% of food-borne disease in the United States, including more than 9 million cases, 33% of hospitalizations, and 7% of deaths related to food-borne disease each year (Mead et al., 1999). Fankhauser et al. (2002) found that 93% of 284 nonbacterial gastroenteritis outbreaks in the United States were due to norovirus, and in 57% of these, contaminated food was the vehicle of infection. The majority of viral gastroenteritis outbreaks in Europe have been attributed to noroviruses, where they were reported to be responsible for more than 85% of nonbacterial gastroenteritis outbreaks between 1995 and 2000 (Lopman et al., 2003).

The sapoviruses, formerly described as the "Sapporo-like viruses," or SLVs, also belong to the Caliciviridae family and cause gastroenteritis among both children and adults, although association with food-borne transmission is rare. The sapoviruses are most commonly associated with pediatric disease in infants, and transmission is more likely to be from person to person.
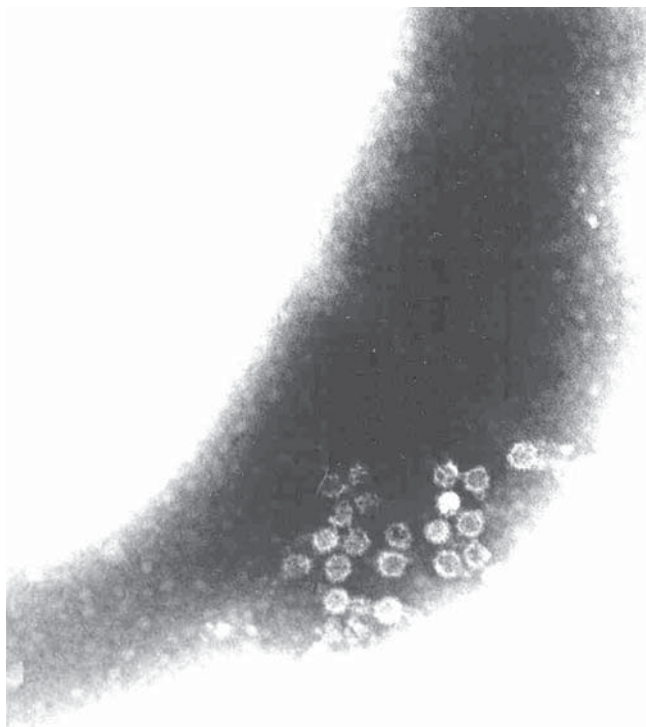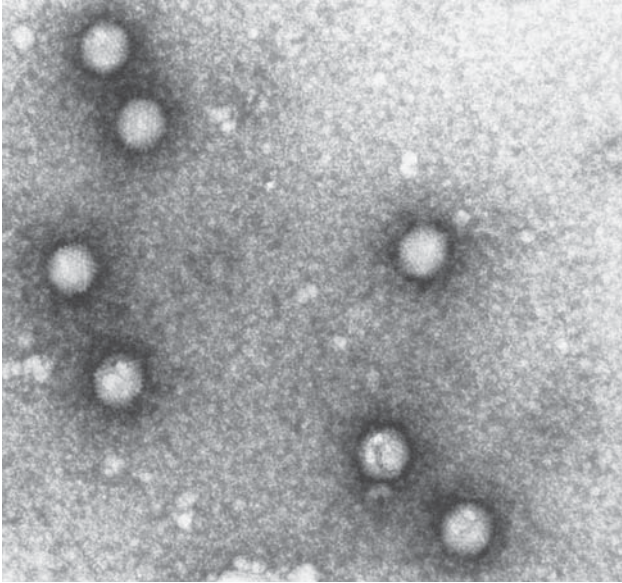
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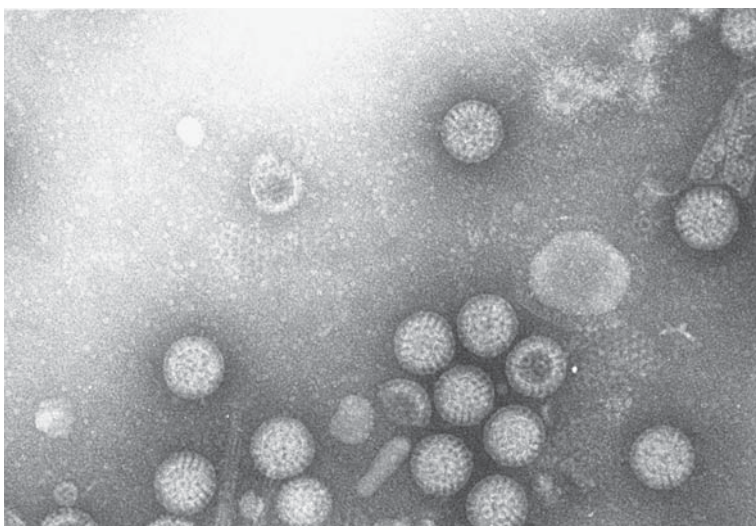
b

Figure 2.1. Electron micrographs of human enteric viruses. Negative staining.
(a) Baculovirus-expressed recombinant Norwalk virus-like particles (VLPs);
(b) rotavirus; (c) adenovirus.

Figure 2.1. *Continued*

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4.2. Taxonomy and Morphology

There are four genera in the Caliciviridae family: Norovirus and Sapovirus, which are both human pathogens, and Lagovirus and Vesivirus, which infect animals and are not known to be pathogenic for humans. The Norwalk-like viruses and the Sapporo-like viruses were renamed as Norovirus and Sapovirus in August 2002 by the ICTV (van Regenmortel et al., 2000). The noroviruses do not show the characteristic cup-shaped morphology of caliciviruses but instead show a "fuzzy" or ragged appearance by direct electron microscopy, which is why they were classified as a distinct group until 1995 (Fig. 2.1a). Sapoviruses have a morphological appearance more typical of the caliciviruses, with distinct cup-shaped indentations on the surface of the virions.

The noroviruses are 28- to 35-nm, nonenveloped, linear, positive-sense, single-stranded RNA viruses with a genome of approximately 7.6 kb and icosahedral capsid symmetry (Table 2.1). The buoyant density in cesium chloride gradient is 1.36–1.41 g/ml. The genome is composed of three open reading frames (ORFs), which code for the nonstructural proteins including the RNA polymerase (ORF1), the capsid protein (ORF2), and a minor structural protein (ORF3).

There is a single species, norovirus, which has seven designated strains: Norwalk virus, Snow Mountain virus, Hawaii virus, Southampton virus, Lordsdale virus, Mexico virus, Desert Shield virus, and one tentative species, swine calicivirus, listed in the ICTV database. Noroviruses have a defined nomenclature whereby strains are named after the geographic location of the outbreak from which they were first identified. A number of distinct

genogroups and genotypes have been characterized based on DNA sequencing of PCR products from the RNA polymerase region in ORF1 (Ando et al., 1995). Sequencing of the genetically variable capsid gene (ORF2) has produced further strain discrimination and recognition of additional genotypes (Fankhauser et al., 2002; Green et al., 2000; Vinje et al., 2004). Currently, four norovirus genogroups (GI, GII, GIII, GIV, and GV) have been identified, and these are subdivided into at least 15 genetic clusters (Ando et al., 2000). Genogroup III includes the bovine enteric caliciviruses, including the Jena and Newbury agents, which are genetically closer to noroviruses than other known caliciviruses (Fig. 2.2). Genogroup V includes the recently identified murine norovirus, MNV-1.

The sapoviruses are 28- to 35-nm, nonenveloped, positive-sense, single-stranded RNA viruses with a genome of approximately 7.6 kb and icosahedral capsid symmetry (Table 2.1) and exhibit the properties of the Caliciviridae. They are small round viruses with a morphology similar to that of noroviruses by EM. The ICTV (van Regenmortel et al., 2000) lists six species of Sapovirus all named according to their first identification: Houston/86, Houston/90, London 29845, Manchester virus, Parkville virus, and Sapporo virus. The sapoviruses are genetically more similar to the members of the Lagovirus genus (rabbit calicivirus) than to those in the
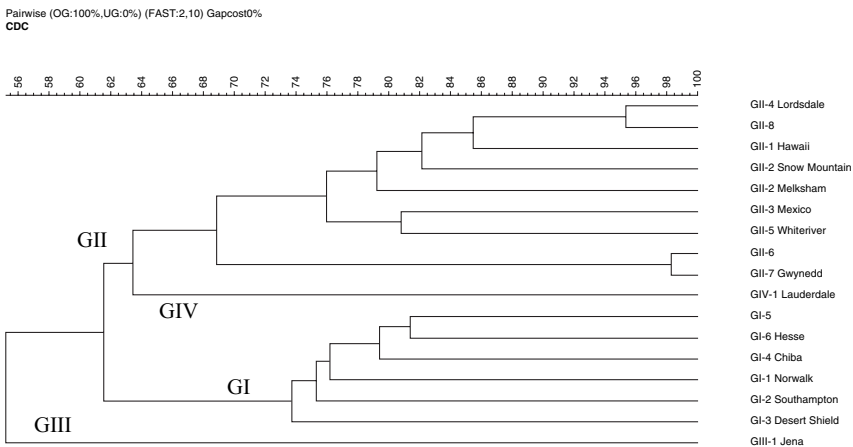

Figure 2.2. Dendrogram showing genetic relationships of norovirus sequences in a 172-bp region of the polymerase gene (region B) of the genome. References sequences from Genbank and CDC Calicinet database. Norovirus strains represented are Norwalk virus M87661, Chiba virus AB022679, Southampton virus L07418, Desert Shield virus U04469, Hesse virus AF093797, Jena virus AJ011099, Hawaii virus U07611, Snow Mountain virus L23831, Melksham virus X818879, Mexico virus U22498, Lordsdale virus X86557, White river virus AF414423, Gwynedd virus AF414409, Ft. Lauderdale virus AF414426, GI/5 AF414406, GII/6 AF414407, and GII/8 AY054299. Dendrogram created by unweighted pair-group method using arithmetic averages (UPGMA).

Norovirus genus. Three genogroups (I, II, and III) have been identified based on sequence analysis (van Regenmortel et al., 2000, Schuffenecker et al., 2001). These viruses are reported to be genetically more similar to animal caliciviruses than the noroviruses (Matson et al., 1995).

4.3. Growth and Biological Properties

Most information on the biology and properties of noroviruses has been obtained through human volunteer studies in the 1970s (Dolin et al., 1971, 1972; Green et al., 2001). Human noroviruses are nonculturable, and until recently no animal model had been identified. Inoculation of chimpanzees with Norwalk virus elicited immune responses but no symptoms developed and no virus was shed in feces (Wyatt et al., 1978). Sustained attempts have been made to culture human noroviruses over the past 10–15 years but without success. More than 26 different cell lines combined with many varied cell culture supplements and growth conditions have been evaluated, but no norovirus-induced CPE or replicating norovirus was obtained (Duizer et al., 2004). Noroviruses have now been identified that infect animals, including pigs, cattle, and mice, and progress in this field is now being made with the growth of a mouse norovirus in artificial culture (Wobus et al., 2004). This culture system will help to discover more about human noroviruses and their mechanisms of pathogenicity. The infected mice develop gastroenteritis, and so this discovery holds potential as a future model for human norovirus disease.

Prior to the development of molecular methods, there was limited knowledge about these viruses because their identification was difficult. The inability to culture noroviruses coupled with the problems associated with identification of the virus by EM restricted their detection for many years. Noroviruses are difficult to identify by direct EM in fecal samples and foods because of their small size and the nature of the background matrices. Immune electron microscopy (IEM) is frequently used to improve the sensitivity of detection, but the antibody coating can mask the appearance of the virus. The development of assays such as reverse transcription-polymerase chain reaction (RT-PCR) has facilitated the detection and identification of these viruses, and consequently the role of noroviruses in gastroenteritis outbreaks has been clarified. Noroviruses show great genetic diversity, which has complicated their identification by molecular assays. To date, none of the numerous norovirus primer sets designed have been able to detect 100% of known norovirus strains, but some sets have been found to be more sensitive and have a broader detection range than others (Vinje et al., 2003).

The lack of a culture system for noroviruses has hindered the development of traditional immunological and serological detection assays because it is not possible to cultivate sufficient noroviruses *in vitro* to generate antigen for antibody production. Recently, advances in routine detection of noroviruses have been made with the development of commercially available ELISA assays. These assays use monoclonal antibodies prepared against

recombinant norovirus capsid proteins generated in a baculovirus expression system. The norovirus capsid proteins are also known as virus-like particles, or VLPs, and are essentially empty viral protein coats without the nucleic acid. The noninfectious VLPs are highly immunogenic, making them suitable for antiserum and vaccine production (Estes et al., 2000). However, commercially available ELISA assays are not able to detect all norovirus strains and are reported to have limited sensitivity and specificity (Richards et al., 2003; Burton-Mcleod et al., 2004).

Because norovirus is nonculturable, its infectivity can only be assessed in human dose-response experiments, hence there is little information on its survival characteristics. Studies using human volunteers showed that norovirus retains infectivity when heated to 60°C for 30 min and therefore is not inactivated by pasteurization treatment. The virus also retains infectivity after exposure to pH 2.7 for 3 hr at room temperature (Dolin et al., 1972; Green et al., 2001). Further evidence of its resistance to low pH was shown when norovirus was exposed to heat treatment and subsequent marination at pH 3.75 in mussels for 1 month. No decrease in norovirus titer was observed by real-time RT-PCR (Hewitt and Greening, 2004). There are anecdotal reports of people developing gastroenteritis after eating pickled shellfish. Norovirus, like other enteric viruses, remains infectious under refrigeration and freezing conditions, appears to survive well in the environment, and is resistant to drying. This was demonstrated when carpet layers became ill after lifting carpet that had become contaminated 12 days earlier in a rest-home outbreak (Cheesbrough et al., 2000).

Fecal pollution from sewage discharges, septic tank leachates, and boat discharges has caused contamination of shellfish beds, recreational water, irrigation water, and drinking water. It is probable that noroviruses persist in these environments for extended periods of time. In live oysters, noroviruses were still detectable after 4–6 weeks in natural growing conditions (Greening et al., 2003). These viruses are also resistant to treatment with 3.75 to 6.25 mg chlorine/L, which is equivalent to free residual chlorine of 0.5 to 1.0 mg/ml, a level of free chlorine consistent with that generally present in a chlorinated drinking water supply. However, the viruses were inactivated after treatment with 10 mg/L of chlorine, which is the concentration applied to water supplies after a contamination event (Green et al., 2001). Norovirus also retained infectivity after exposure to 20% ether at 4°C for 18 hr (Dolin et al., 1972).

As with noroviruses, sapoviruses have also not been cultured *in vitro* yet. In addition, sapoviruses have not been studied as intensively as noroviruses, hence little information is available on the biological and physical properties of these viruses. Detection and identification is generally by molecular methods (Jiang et al., 1999; Green et al., 2000; Schuffenecker et al., 2001).

4.4. Infection and Disease

Noroviruses are extremely infectious and cause epidemic gastroenteritis. The infectious dose is believed to be as low as 10–100 virus particles (Caul, 1996).

Recent dose-response studies show that both the infective dose and host susceptibility may vary according to the infecting norovirus strain (Moe et al., 2004; Lindesmith et al., 2005). The mechanism of pathogenicity of noroviruses is still not clearly understood because of the inability to propagate these viruses, but information is being obtained from the *in vitro* culture of a mouse norovirus (Wobus et al., 2004).

It is known that the mature enterocyte cells in the small intestine become infected and that malabsorption of fats, D-xylose, and lactose occurs for up to 2 weeks. Unusually, gastric emptying is also delayed, and this may explain the nausea and characteristic projectile vomiting associated with norovirus infection. Large numbers of noroviruses are excreted in feces from the onset of symptoms and continue to be shed in decreasing numbers for up to 2 weeks after infection. Animals infected with the Newbury agent, bovine caliciviruses assigned to Norovirus genogroup III, show similar symptoms, pathological changes and processes as seen in humans (Appleton, 2001).

In the absence of reliable laboratory tests for norovirus, Kaplan et al. (1982) developed epidemiological and clinical criteria for the diagnosis of noroviral gastroenteritis outbreaks. These criteria were stools negative for bacterial pathogens, a mean or median duration of illness of 12–60 hr, vomiting in ≥50% of cases, and a mean or median incubation period of 24–48 hr. These criteria are still widely used. The symptoms of acute-onset projectile vomiting, watery nonbloody diarrhea with abdominal cramps, and nausea may develop within 12 hr of exposure, and low-grade fever also occurs occasionally. Dehydration is a common complication that can particularly affect the young and elderly, necessitating rehydration therapy. There is no evidence of any long-term sequelae after norovirus infection. The symptoms associated with Sapovirus infection are similar to those of noroviral gastroenteritis, but the sapoviruses do not cause epidemic gastroenteritis.

The mechanism of immunity to norovirus infection is not clear. Infection normally stimulates production of both gut and serum antibody, and although immunity to the infecting norovirus strain may develop, it is generally short-lived, strain-specific, and does not confer protection against future infection. Reinfection with a different strain can occur soon after the initial infection. Thus, given the genetic variability of noroviruses, people are likely to be reinfected many times during their lifetimes. Recent research has suggested that there may be a genetic determinant involved in susceptibility to norovirus infection, with people belonging to histo-blood group O being at greater risk for severe infection (Hutson et al., 2002, 2004).

Projectile vomiting is a characteristic symptom that can contribute to secondary spread through droplet infection, where droplets containing virus may contaminate surfaces or be swallowed. Evidence that norovirus transmission occurs through aerosolization of vomit was clearly demonstrated at a U.K. hotel. During a meal, a guest vomited at the table, and norovirus infection spread in a radial pattern through the restaurant, progressively decreasing from 91% attack rate among those seated at the same table to an attack rate of 25% in those patrons who were seated the farthest distance away

from the guest who vomited (Marks et al., 2000). Norovirus infection characteristically has an attack rate of 50–70% or even higher in some situations. This high attack rate combined with a low infectious dose, prolonged virus excretion, short-term immunity, and the environmental stability of the noroviruses contributes to the epidemic nature of noroviral gastroenteritis.

Norovirus infection was termed *winter vomiting disease* because outbreaks occurred most frequently in the winter months, especially in rest homes and institutions. This seasonality is no longer apparent as norovirus outbreaks are now reported to occur throughout the year.

4.5. Food-borne Disease

Noroviruses are the main cause of food-borne viral gastroenteritis worldwide with food-borne transmission accounting for a large proportion of norovirus outbreaks in many countries. Food-borne norovirus outbreaks resulting from preharvest contamination of foods such as shellfish and postharvest contamination through food handling have been reported worldwide. Among these are several outbreaks resulting from consumption of norovirus-contaminated shellfish (Dowell et al., 1995; Christensen et al., 1998; Berg et al., 2000; Simmons et al., 2001), bakery products (Kuritsky et al., 1984), delicatessen meats (Schwab et al., 2000), sandwiches (Parashar et al., 1998; Daniels et al., 2000), raspberries (Ponka et al., 1999), water and ice (Beller et al., 1997; Brugha et al., 1999; Beuret et al., 2002). Presymptomatic infection in food handlers has also been shown to cause outbreaks of food-borne norovirus infection (Lo et al., 1994; Gaulin et al., 1999).

Among the 284 outbreaks of norovirus illness reported to CDC from July 1997 to June 2000, the cause of transmission was not determined in 42, or 24% of outbreaks (Fankhauser et al., 2002). Determination of the original source of the virus is often problematic because several modes of transmission frequently operate during norovirus gastroenteritis outbreaks. Although the initial transmission route may be through consumption of contaminated foods, secondary transmission via direct contamination of the environment or person-to-person contact also often occurs. This results in wide dissemination where infection quickly spreads through institutions, schools, camps, resorts, and cruise ships and causes large-scale epidemics with more than 50% attack rates.

The use of DNA sequencing techniques for genotyping of noroviruses has greatly assisted the epidemiologic investigation of gastroenteritis outbreaks. The comparison of noroviral sequences from fecal specimens and contaminated foods, such as oysters, can clearly indicate if it is a common source outbreak or if individual cases are somehow related. In 1993, 23 gastroenteritis outbreaks across 6 states in the United States were shown to be related to consumption of oysters harvested from a single area and contaminated with the same norovirus strain (Dowell et al., 1995).

There are few reports of Sapovirus infection directly resulting from consumption of food. An outbreak of viral gastroenteritis among adults at a

school in Parkville, Maryland, in 1997 was determined to be food-related. The causal agent was a Sapovirus later designated as the Parkville virus (Noel et al., 1997).

4.6. Zoonotic Transmission

Research in Japan, The Netherlands, and the United Kingdom has demonstrated calicivirus-like particles and calicivirus RNA sequences in the cecum of pigs and in fecal samples from calves (Sugieda et al., 1998; Dastjerdi et al., 1999; van der Poel et al., 2000). Molecular analysis of these enteric caliciviruses, now termed the *Jena* and *Newbury agents*, shows that they are genetically more closely associated with human noroviruses than with other known caliciviruses and they are now assigned to Norovirus genogroup III. Although the discovery of these noroviruses prompted concerns that calves and pigs may be a reservoir of infection for human noroviral disease, there is no documented evidence of transmission to humans (Oliver et al., 2003). Similarly, there are no reports of zoonotic transmission of sapoviruses.

5.0. ROTAVIRUS

5.1. Distribution and Transmission

Rotaviruses are the major cause of severe diarrhea and gastroenteritis in infants and young children. It is estimated that rotaviruses cause more than 130 million cases of diarrhea in children under 5 years of age annually worldwide (Glass and Kilgore, 1997). Rotaviral infection is a particularly serious problem in developing countries where up to 600,000 deaths occur annually among children. In the United States, rotaviruses are estimated to cause about 4 million infections per year resulting in almost 70,000 hospitalizations and more than 100 deaths annually (Kapikian et al., 2001; Sattar et al., 2001). Although the disease occurs in all age groups, it is generally considered to be a mild infection in adults, hence the true extent of adult infections is not known.

Rotaviruses are transmitted by the fecal-oral route and cause disease in both humans and animals, especially domestic animals, with subsequent serious economic loss. Although the animal and human strains are usually distinct, some human strains are closely related to animal strains, and cross-species infections do occur (Sattar et al., 2001). Infection is not generally recognized as food-borne, but outbreaks associated with food and water have been reported in a number of countries (Sattar et al., 2001).

5.2. Taxonomy and Morphology

Rotaviruses are classified in the genus Rotavirus in the family Reoviridae, a large family composed of nine genera. Electron micrographs of rotaviruses show a characteristic wheel-like appearance, hence the name rotavirus, derived from the Latin meaning "wheel" (Fig. 2.1b). These viruses are distinct in that they have a complex segmented genome that undergoes reassortment during replication. There are five species of Rotavirus, designated

Rotavirus A (simian rotavirus) through Rotavirus E (porcine rotavirus). Two possible species, Rotavirus F (avian) and Rotavirus G (avian) are also listed but they differ in their ability to reassort the genome segments. Most human infections are caused by Rotavirus A, B, and C, but all rotaviral species can infect a range of vertebrates, including primates, ruminants, rodents and birds (van Regenmortel et al., 2000; Sattar et al., 2001).

Rotaviruses are 60- to 80-nm, nonenveloped, linear segmented double-stranded RNA viruses with icosahedral capsid symmetry (Table 2.1). The 16- to 27-kb, genome is enclosed by a triple-layered capsid composed of a double protein shell and an inner core. Eleven segments of DNA code for six struc-tural and five nonstructural proteins. Two of the structural proteins, VP7 (gly-coprotein) and VP4 (protease or P protein), comprise the outer shell of the capsid and are important in virus infectivity. These two proteins are used to define the rotavirus serotype; there are 14 VP7 serotypes and 11 VP4 serotypes within the Rotavirus A species. The VP6 protein located on the inner capsid layer is designated the group-specific antigen and is the major target of rotavirus diagnostic assays. This protein is believed to play a role in the development of protective immunity. Genomic reassortment of the rotaviral RNA segments may occur during replication, particularly when there is coinfection with more than one strain. In the replication phase, the immature virus particles acquire a transient lipid envelope as they develop in the endoplasmic reticulum of the host cell.

5.3. Growth and Biological Properties

Although many rotaviruses can be grown in cell cultures, they have proved difficult to cultivate *in vitro*, and growth is restricted to a few cell lines derived mainly from monkey kidneys. Addition of trypsin to the culture medium is required to enhance viral growth in cell cultures. Rotaviruses do not show the same tolerance to extreme conditions as other enteric viruses, although they are stable in the environment and can be stored for several months at 4°C or even 20°C. They are resistant to drying and may survive on fomites and surfaces. Heating at 50°C for 30 min reduces their infectivity by 99%, and infectivity is rapidly lost at pH <3.0 and >10.0. Repeated cycles of freeze-thaw can also destroy infectivity. The viruses are resistant to solvents such as ether and chloroform and to non-ionic detergents such as deoxycholate. Chelating agents such as EDTA disrupt the outer shell and inactivate rotaviruses. Treatment with disinfectants such as chlorine, phenol, formalin, and 95% ethanol is also effective against rotavirus (Kapikian et al., 2001). Normal cooking temperatures are usually sufficient to inactivate rotaviruses. The viruses are found in water and sewage, are resistant to chlorine levels present in drinking water, and are persistent in the environment. Human rotavirus can survive for several weeks in river water at 4°C and 20°C.

5.4. Infection and Disease

The incubation period for rotavirus infection is 1 to 2 days. The characteris-tic symptoms of vomiting and watery diarrhea develop quickly and persist for 3 to 8 days, frequently accompanied by fever and abdominal pain. Dehy-

dration is a key factor that contributes to the high infant death rate from rotavirus disease, especially in developing countries where rehydration therapy is often not readily available. Virus is shed in feces for 5 to 7 days. The main transmission route is fecal-oral. Because rotaviruses most often infect young children, the major route of transmission is believed to be person-to-person through care-givers and the general adult population. Rotaviruses can also infect adults and have also been occasionally associated with food and water borne outbreaks. In particular, Rotavirus B strains have caused large epidemics in human adults in China. Group C rotavirus causes sporadic outbreaks in children (Glass and Kilgore, 1997; Sattar et al., 2001). Rotavirus disease is more common during the winter months in countries with a temperate climate. In tropical regions, outbreaks can occur both in the cooler and drier months and throughout the year especially where transmission is related to contaminated water supplies and where no sewage treatment systems exist (Cook et al., 1990; Ansari et al., 1991).

Some immunity develops after infection although it does not give complete protection from future infections. However, repeat infections are often less severe than the original infection. An oral rotavirus vaccine was developed in the late 1980s, but distribution was delayed after lengthy investigations into possible complications associated with the vaccine. This vaccine has recently been approved for commercial global distribution.

5.5. Food-borne Disease

The virus is stable in the environment, hence infection can occur through consumption of contaminated water or food and contact with contaminated surfaces. Eleven food-borne outbreaks consisting of 460 cases of rotaviral gastroenteritis were reported in New York between 1985 and 1990. Seven outbreaks were associated with food-service premises, and the implicated foods included salad, cold foods, shepherd's pie, and water or ice (Sattar et al., 2001). In a recent study in the Netherlands, lack of food handling hygiene was identified as one of the main risk factors for rotavirus infection (de Wit et al., 2003).

Large-scale outbreaks of rotaviral gastroenteritis have been reported in Japanese primary schools with more than 3,000 cases recorded for one outbreak (Hara et al., 1978; Matsumoto et al., 1989). School lunches prepared at a central facility were suspected as the vehicle of infection, but no rotavirus was isolated from food or water. In Costa Rica, market lettuce was found to be contaminated with rotavirus and HAV at a time when there was a high incidence of rotaviral diarrhea in the community (Hernandez et al., 1997). Waterborne rotaviral outbreaks have been reported in many countries, including China, Germany, Israel, Sweden, Russia, and the United States (Ansari et al., 1991; Sattar et al., 2001). Large numbers of rotaviral particles are excreted in feces after infection, and calves infected with rotavirus are known to shed 10^{10} particles per gram of feces. Contamination of water supplies by animals could therefore be a source of waterborne disease. Links have been reported between human and animal rotaviral disease, and it is possible that zoonotic transmission of rotavirus may also occur.

6.0. ASTROVIRUS

6.1. Distribution and Transmission

Astroviruses are distributed worldwide and have been isolated from birds, cats, dogs, pigs, sheep, cows, and man. The main feature of astrovirus infection in both humans and animals is a self-limiting gastroenteritis. The astroviruses are a common cause of human gastroenteritis, with most cases of infection detected in young children under 1 year of age (Bresee and Glass, 1999; Appleton, 2001). A surveillance study in the United Kingdom reported that astroviruses were the most common viral cause of infectious gastrointestinal disease (Roderick et al., 1995). Although astroviruses cause a mild infection in adults, they have been associated with gastroenteritis in immunocompromised adults. Transmission is through the fecal-oral route via food, water, and person-to-person contact. Asymptomatic excretion occurs in 5–20% of neonates and young children and is a significant source of infection, especially in nurseries, childcare centers, and hospitals (Caul, 1996; Bresee and Glass, 1999; Appleton, 2001).

6.2. Taxonomy and Morphology

The astroviruses were first recognized in 1975 (Madeley and Cosgrove, 1975) and were named according to their star-like appearance under the electron microscope. They belong to the family Astroviridae, and human astrovirus is the single type species in the genus Mamastrovirus. Astroviruses are 28- to 30-nm, spherical, nonenveloped, positive-sense, single-stranded RNA viruses with a genome of about 6.8–8 kb and a buoyant density of 1.32 g/ml in potassium tartrate–glycerol gradient (Table 2.1). Because only 10% of astroviruses exhibit the typical 5- or 6-pointed star-like morphology by direct EM, the efficiency of detection was restricted until the introduction of molecular detection methods and improved culture techniques. At least eight human serotypes, two bovine serotypes, and one serotype of each of feline, ovine, and porcine astrovirus are recognized. The human strains are all antigenically distinct from the bovine and ovine strains. A second genus, Avastrovirus, contains the type species turkey astrovirus, of which there are two serotypes. This virus infects birds, including turkeys and ducks (van Regenmortel et al., 2000).

6.3. Growth and Biological Properties

Astroviruses have been isolated in cell cultures but are fastidious viruses to grow *in vitro*. Although human, bovine, feline, and porcine astroviruses have been isolated in primary embryonic kidney cell lines such as human embryonic kidney cells (HEK), only human and porcine astroviruses have been adapted to grow in established cell lines, and trypsin is required in the growth medium to boost infectivity. Although CaCo-2 continuous cell line has proved to be useful for the propagation of astroviruses (Willcocks et al., 1990), virus detection is carried out mainly by EM of stool specimens, molecular assays, or by combined culture-PCR methods. Astroviruses are resistant

to extreme environmental conditions. Their heat tolerance allows them to survive 50°C for 1 hr. At 60°C, the virus titer falls by 3 $\log_{10}$ and 6 $\log_{10}$ after 5 and 15 min, respectively. The virus is also stable at pH 3.0 and is resistant to chemicals, including chloroform, lipid solvents, and alcohols and to non-ionic, anionic, and zwitterionic detergents (Appleton, 2001).

6.4. Infection and Disease
Clinically, astroviruses cause symptoms similar to those of caliciviruses after an incubation period of 3–4 days. Symptoms include diarrhea, fever, nausea, and general malaise with occasional vomiting. Normally, diarrhea persists for only 2–3 days but can be prolonged for up to 14 days with virus excretion in feces. Outbreaks commonly occur in institutional settings, especially pediatric wards. In temperate climates, a seasonal peak in winter and spring occurs, but infections may occur throughout the year.

6.5. Food-borne Disease
Epidemiological evidence of transmission by foods is limited, but infections via contaminated shellfish and water have been reported (Oishi et al., 1994; Appleton, 2001). In 1991, a large outbreak of acute gastroenteritis occurred in Japan involving thousands of children and adults from 14 different schools (Oishi et al., 1994). The outbreak was traced to food prepared by a common supplier for school lunches. Astrovirus type 6 was identified by immune electron microscopy and confirmed by molecular and culture methods. There are several Japanese reports of astrovirus genomes identified in shellfish, and there is evidence that astroviruses appear to contribute to food borne outbreaks of gastroenteritis mainly through the consumption of contaminated oysters (Kitahashi et al., 1999).

7.0. ADENOVIRUS

7.1. Distribution and Transmission
The adenoviruses are widespread in nature infecting birds and mammals including man. They commonly cause respiratory disease but may also be involved in other illnesses such as gastroenteritis and conjunctivitis. In particular, the enteric adenoviruses cause gastroenteritis and are the second most important cause, after rotaviruses, of acute gastroenteritis in children under 4 years of age (Allard et al., 1990, Bresee and Glass, 1999). Adenoviruses can be transmitted from person-to-person by direct contact or via fecal-oral, respiratory or environmental routes.

7.2. Taxonomy and Morphology
Adenoviruses belong to the Adenoviridae family and are classified into two genera: the Mastadenovirus, which infects mammals, and the Aviadenovirus, which infects birds. More than 100 members of the Adenoviridae have been isolated from humans and animals, including birds and amphibians. Adenoviruses are 80- to 110-nm, nonenveloped, linear double-stranded DNA

viruses with icosahedral symmetry and a genome of 28–45 kb (Table 2.1; Fig. 2.1c). The buoyant density in cesium chloride is 1.32–1.35 g/ml. Six species of human adenoviruses (HAdV-A to HAdV-F) have been identified according to DNA homology (van Regenmortel et al., 2000). Between 50% and 90% DNA homology exists within these species, but only 5–20% homology exists between the species. To date, 51 human adenovirus serotypes have been recognized, including serotypes 40 and 41, the *enteric adenoviruses*, which comprise the HAdV-F species.

7.3. Growth and Biological Properties

Serotypes 40 and 41 of enteric adenoviruses are difficult to grow in cell cultures, whereas most of the nonfecal types are culturable. Adenoviruses are slow-growing compared with a majority of enteroviruses and can be quickly overgrown in some cell lines. The A549 and 293 cell lines have been successfully used for the isolation of adenoviruses from food and environmental samples. Adenoviruses are resistant to various chemical and physical agents including lipid solvents and to adverse pH conditions (Enriquez et al., 1995; Thurston-Enriquez et al., 2003a, 2003b). They can withstand freeze-thawing several times without a significant decrease in titer but are inactivated after heating at 56°C for more than 10 min. The adenoviruses are capable of prolonged survival in the environment and are considered to be more stable than enteroviruses in many environmental situations.

7.4. Infection and Disease

Most human adenovirus infections in normally healthy individuals are mild or subclinical but can be associated with respiratory, ocular, and gastrointestinal disease. In most cases of clinical infection, the symptoms are relatively mild. Of the many types of adenoviruses, only HAdV serotypes 40 and 41 are generally associated with fecal-oral spread and cause gastroenteritis, although all serotypes are shed enterically in feces. HAdV types 40 and 41 can be detected in large numbers in the feces of young children with acute gastroenteritis. In immunocompromised individuals, infection with types 40 and 41 may cause chronic diarrhea. Although rare, some deaths have been reported in immunocompromised children (Bresee and Glass, 1999). Adenoviruses can cause persistent asymptomatic infections and may become established in tonsils, adenoids, and intestines of infected hosts. It is not known whether they are capable of reactivation causing overt disease.

The virus is shed in large numbers in feces and respiratory secretions, often for months or years after infection. The main transmission routes are the fecal-oral route for the enteric adenoviruses and aerosols or direct contact for the nonenteric serotypes. Waterborne transmission of adenovirus has been associated with conjunctivitis in children. Enteric adenovirus infections are common all year round, whereas outbreaks of adenovirus-associated respiratory disease normally occur from late winter to early summer.

7.5. Food-borne Disease

Adenoviruses have been identified in a variety of environmental samples, including wastewater, sludge, shellfish, and in marine, surface and drinking waters. No food-borne or waterborne outbreaks associated with the enteric adenoviruses have been reported, but, as these viruses are common in the environment, it is possible that disease has occurred but the source of infection has not been recognized. There is no documented evidence for food-borne transmission or disease resulting from consumption of adenovirus-contaminated shellfish.

8.0. ENTEROVIRUSES

8.1. Distribution and Transmission

Enteroviruses include polioviruses, coxsackie A and B viruses, and echoviruses, many of which are culturable. They are transmitted by the fecal-oral route and are excreted in feces but do not generally cause gastroenteritis. Polioviruses were the first viruses to be shown to be food-borne, but because of the mass immunization campaigns, virulent wild-type strains are now rarely seen. Outbreaks of food-borne illness associated with coxsackieviruses and echoviruses have been reported (Cliver, 1997; Sattar and Tetro, 2001).

8.2. Taxonomy and Morphology

The enteroviruses are 28- to 30-nm, nonenveloped, positive-sense, single-stranded RNA viruses with icosahedral symmetry and a genome of 7.2–8.4 kb (Table 2.1). They are classified in the large Picornaviridae family, and seven species have been designated within the Enterovirus genus, namely bovine enterovirus, human enterovirus A, human enterovirus B, human enterovirus C, human enterovirus D, poliovirus, porcine enterovirus A, and porcine enterovirus B. Within these different species, numerous serotypes have been reported. The enteroviruses belong to the human enterovirus A (Enterovirus 71) and human enterovirus D (Enterovirus 68 and 70) species. Coxsackie A viruses belong to human enterovirus A, human enterovirus B, and human enterovirus C species. All of the coxsackie B viruses and echoviruses are members of the human enterovirus B species. The Poliovirus species is composed of three distinct serotypes. There are five unassigned tentative species and 22 serotypes within the genus Enterovirus, including two coxsackie A viruses (types CV-A4 and CV-A60) (van Regenmortel et al., 2000).

8.3. Growth and Biological Properties

Many of the enteroviruses are culturable, including all serotypes of poliovirus, echoviruses, and coxsackie B viruses. The enteroviruses are resistant to environmental stressors including heat, adverse pH, and chemicals. Because they are easily cultured *in vitro* and are stable in the environment, live attenuated vaccine strains of poliovirus have been used as indicator

Table 2.2 Classification of Enteroviruses

Family Picornaviridae; Genus Enterovirus

Species
Bovine enterovirus, BEV (2 serotypes)
Human enterovirus A, HEV-A (10 serotypes)
Human enterovirus (1 serotype)
Coxsackie A virus (9 serotypes)
Human enterovirus B, HEV-B (36 serotypes)
Coxsackie A virus (1 serotype)
Coxsackie B virus (6 serotypes)
Echovirus (28 serotypes)
Human enterovirus (1 serotype)
Human enterovirus C, HEV-C (11 serotypes)
Coxsackie A virus (11 serotypes)
Human enterovirus D, HEV-D (2 serotypes)
Human enterovirus (2 serotypes)
Poliovirus, PV (3 serotypes)
Porcine enterovirus, PEV-A (1 serotype)
Porcine enterovirus B, PEV-B (2 serotypes)

viruses for the presence of other virulent enteric viruses in food and water. They have also been used extensively in environmental and food virology research for methods development and to gather information on virus recovery, persistence, and behavior in these settings.

8.4. Infection and Disease

Enteroviruses cause a range of diseases, including viral meningitis and poliomyelitis. They are mainly spread by either the fecal-oral route or direct contact with respiratory secretions of an infected person. The virus is spread through the fecal-oral route mainly among small children who are not yet toilet trained, by adults changing the diapers of an infected infant, and through consumption of fecally contaminated food or water. The enteroviruses multiply mainly in the gastrointestinal tract but can also multiply in other tissues such as nerve and muscle, as does the poliovirus. The incubation period is usually between 3 and 7 days with virus transmission to others occurring from 3 to 10 days after symptoms develop. Enteroviral infection is most common in summer and early autumn, and many infections are asymptomatic. Only a few people (approximately 0.001%) develop aseptic or viral meningitis, and no long-term complications normally follow the mild illnesses or aseptic meningitis. On rare occasions, a person may develop myocarditis or encephalitis.

8.5. Food-borne Disease

The first recorded outbreak associated with food-borne viruses was an outbreak of poliomyelitis linked to consumption of raw milk in 1914 (Jubb, 1915). A further 10 outbreaks associated with raw milk consumption were reported in the United States and United Kingdom over the following 35

years (Sattar and Tetro, 2001). The widespread introduction of pasteurized milk in the 1950s decreased transmission by this route. There have been very few recorded food-borne outbreaks associated with enterovirus infection despite the regular occurrence of enteroviruses in the environment. Enteroviruses, including echoviruses and coxsackie A and B viruses, have been isolated from sewage, raw and digested sludge, marine and fresh waters, and shellfish. In two reported food-borne outbreaks associated with echoviruses in the United States, the source of the virus was not identified (Cliver, 1997). No outbreaks associated with the consumption of shellfish have been reported.

9.0. OTHER VIRUSES WITH POTENTIAL FOR FOOD-BORNE TRANSMISSION

Other viruses transmitted by the fecal-oral route and found in feces of humans and animals include the parvoviruses, coronaviruses, toroviruses, picobirnaviruses, and the tick-borne encephalitis virus (Table 2.1). The ability of many of these viruses to cause gastroenteritis in humans and or animals is still unproven, and there is little evidence to link them with food-borne disease (Glass, 1995; Cliver, 1997; Bresee and Glass, 1999).

9.1. Parvovirus

Parvoviruses have been proposed as causal agents of human gastroenteritis. Their role in viral gastroenteritis of some animal species has been well documented. Parvoviruses are single-stranded DNA viruses and are among the smallest known viruses at 18–26 nm in diameter. They have a smooth surface with no discernable features and were included with "small round viruses" before definitive classification of these viruses was completed. Three possible serotypes known as the Parramatta agent, the cockle agent, and the Wollan/Ditchling group have been identified by IEM. There is limited evidence of parvovirus association with food-borne disease, but it has been linked with consumption of contaminated shellfish (Appleton and Pereira, 1977; Appleton, 2001). The "cockle agent" parvovirus was implicated in a large U.K. outbreak related to consumption of contaminated cockles (Appleton, 2001). More than 800 confirmed cases of gastroenteritis occurred, and parvovirus was identified in all stools examined from this large gastroenteritis outbreak.

9.2. Coronavirus

Coronaviruses are large (80–220 nm) pleomorphic, enveloped, positive-strand RNA viruses belonging to the Coronaviridae family. They generally cause respiratory infections but can also cause gastroenteritis in animals and are excreted in feces. Their role in human gastroenteritis is unclear, although "coronavirus-like particles" can be identified in human feces (Glass, 1995). There are no reports of food-borne outbreaks, but the 2003 SARS (severe

acute respiratory syndrome) outbreak in Hong Kong was linked to sewage or sewage-contaminated water.

9.3. Torovirus
Toroviruses are 100- to 150-nm, enveloped, positive-sense, single-stranded RNA viruses belonging to the genus Torovirus in the Coronaviridae family. They were first discovered in 1979 and were named Breda viruses (Woode et al., 1982; Glass, 1995). When observed by EM, they have a distinctive pleomorphic appearance with club-shaped projections extending from the capsid. Toroviruses are known to cause gastroenteritis in animals, especially dairy cattle, in which they cause a marked decrease in milk production. Although they have been isolated from feces of children and adults with diarrhea (Koopmans et al., 1991), their exact role in human gastroenteritis and foodborne disease is still unknown.

9.4. Picobirnavirus
The picobirnaviruses are small, 35-nm, positive-sense, single-stranded RNA viruses with a bisegmented genome and are classified in the family Birnaviridae (Glass, 1995). These viruses are known to cause gastroenteritis in a range of domestic animals. They have also been detected in humans with and without diarrhea but have only been associated with gastroenteritis in immunocompromised HIV patients. Little is known about these viruses, although they have been identified in humans from several countries, including Australia, Brazil, England, and the United States. Their role as true human pathogens is unproven, and there is no documented evidence of foodborne transmission.

9.5. Tick-borne Encephalitis Virus
Tick-borne encephalitis viruses (TBE) may also be food-borne (Cliver, 1997). Tick-borne encephalitis is a zoonotic arbovirus infection endemic to Eastern and Central Europe and Russia. However, the distribution of these viruses can extend to Northern Europe, China, Japan, and Korea. The TBE viruses and the other closely related arboviruses causing yellow fever, Japanese encephalitis, and dengue are all members of the Flaviviridae. Three subtypes of TBE virus cause tick-borne encephalitis: the Eastern European subtype, the Western European subtype, and the Siberian subtype. Most cases occur in spring and summer after bites of different species of *Ixodes* tick. Food-borne transmission is less common but can occur after consumption of unpasteurized dairy products from infected cattle and goats. The disease is serious and can result in long-term neurological sequelae or death. Increased tourism to the endemic areas has extended the risk of travellers acquiring TBE. Vaccines are now available in some countries.

9.6. Other Food-borne Routes of Virus Transmission
Transmission of viral disease can occur through human breastfeeding. Human breast milk is a transmission route for some blood-borne viruses.

There are reports of human immunodeficiency virus (HIV), human lymphotrophic virus-1 (HTLV-1), and cytomegalovirus (CMV) being transmitted to infants in milk from infected mothers during breastfeeding (Sattar and Tetro, 2001). This can present serious problems in less developed countries with a high incidence of HIV and few alternative options available to infected mothers.

10.0. SUMMARY AND CONCLUSIONS

Food-borne disease is increasing worldwide and has become a major public health problem. The majority of food-borne viral disease is caused by noroviruses and HAV. Food-borne transmission of other enteric viruses is less common. HEV is the only virus that appears to be a likely candidate for direct zoonotic transmission from animals to man, and to date there is little evidence to support its transmission by this route.

The overall contribution of viruses to global food-borne disease burden is unknown because accurate data on the prevalence of food-borne viral disease are not available for all countries. National epidemiological surveillance systems vary from country to country, and a large proportion of viral infections are not notifiable and therefore not reported. However, from epidemiological data that have been collected in Europe, the United States, and other countries, it is apparent that viruses play a significant role and that the economic burden of food-borne viral gastrointestinal disease can be substantial in terms of staff illness, time away from employment, and disruption to services.

Over the past 15 years, the introduction of molecular methods for detection and identification of enteric viruses, many of which are not culturable or are difficult to grow in culture, has greatly increased our understanding of their role in food-borne disease. The development of molecular techniques, such as PCR and real-time quantitative PCR, is rapidly increasing the knowledge base by facilitating studies on the behavior and persistence of these viruses in food matrices. However, it is important to recognize the limitations of these techniques. PCR-based methods currently detect both infectious and noninfectious viruses and are not able to determine viral infectivity, which is the key factor when assessing human health risks from food-borne pathogens. It is important that data generated solely from molecular-based assays is judiciously interpreted when studying these viruses. Use of cell culture combined with PCR methods (culture-PCR) can overcome some of these problems for those viruses that are difficult to grow. Unfortunately, the infectivity status of the main food-borne viral pathogen, norovirus, still cannot be determined by *in vitro* methods. This has limited our knowledge of the natural history and biological properties of this pathogen and has also slowed progress in the development of effective control and intervention strategies.

11.0. REFERENCES

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