8
Gastroenteric Viruses

Miren Iturriza-Gómara, Chris I. Gallimore, and Jim Gray

1. INTRODUCTION

In recent years, viruses have been recognized increasingly as an important cause of foodborne infections. More than 160 enteric viruses are excreted in the feces of infected individuals, and some may also be present in the vomitus. Food and water are directly contaminated with fecal material, through the use of sewage sludge in agriculture, sewage pollution of shellfish culture beds, or may be contaminated by infected food-handlers.

Several groups of viruses cause gastroenteritis. The most common etiological agents are rotaviruses (RVs), human caliciviruses, which include noroviruses (NVs) and sapoviruses (SVs), astroviruses (ASVs), and enteric adenoviruses (ADVs, types 40 and 41).

Among the human caliciviruses, NVs are a leading cause of acute viral gastroenteritis worldwide and are responsible for sporadic cases and outbreaks of gastroenteritis affecting all age groups. Outbreaks in semiclosed environments such as hospitals, cruise ships, and homes of elderly persons *(1,2)* are frequent. As with all enteric viruses, transmission is predominantly person-to-person, but transmission via contaminated food, water, or the environment has often been demonstrated.

RVs are the most common cause of endemic acute infantile gastroenteritis. Mostly in the developing world, they are responsible for approx 600,000–800,000 deaths every year in children aged below 5 yr *(3,4)*, and in developed countries they remain the most common cause of pediatric hospitalization in children aged below 2 yr *(4)*. Outbreaks involving other age groups, in particular the elderly, are frequent in semiclose environments such as hospitals and nursing homes. Sporadic cases in young adults are usually resulting from the contact with infected children. Foodborne transmission has been implicated in rotavirus outbreaks *(5)*.

SVs, ASVs, and ADVs are mostly associated with sporadic cases of gastroenteritis in children aged below 5 yr. Outbreaks of gastroenteritis associated with these viruses can also occur in nurseries, schools, and pediatric hospital wards, and occasionally may involve adults in residential and nursing homes. Foodborne transmission has not been well documented for these viruses.

Viruses such as coronaviruses (CoVs) and toroviruses (ToVs) have been described but their role in acute gastroenteritis is not fully understood *(6–9)*. The severe acute respiratory syndrome (SARS)-CoV was also associated with enteric symptoms and is excreted in the feces of infected patients *(10)*. Picobirnaviruses and Aichi viruses have been found in the feces of individuals with gastroenteritis *(11–16)*, but their significance

From: *Infectious Disease: Foodborne Diseases*
Edited by: S. Simjee © Humana Press Inc., Totowa, NJ

as causative agents of gastroenteritis in humans and their role in foodborne diseases remain unclear.

Other enteric viruses, not associated with gastroenteritis, such as hepatitis A virus, hepatitis E virus, and enteroviruses, including polioviruses are excreted in the feces of infected individuals and are also transmitted via contaminated food and water.

2. CLASSIFICATION AND IDENTIFICATION

2.1. Human Caliciviruses

Human caliciviruses, noroviruses (NV; formerly known as Norwalk-like or small round-structured viruses), and sapoviruses (SV; formerly known as Sapporo-like viruses) are members of the Caliciviridae family, which are nonenveloped viruses with a genome of positive-sense ssRNA. NVs and SVs can be distinguished morphologically and this allowed the first classification scheme for these viruses *(17)*. Both are approx 30–35 nm in diameter, but NVs have an amorphous structure with a ragged outer edge, and the SVs or “classical” caliciviruses display the characteristic cup-shaped structures from which the Caliciviridae family derives their name (*calix* = cup in latin).

NVs are currently classified into two and possibly three genogroups: GI, GII and GIII, based on the sequence diversity within the capsid *(18)*. Within GI, seven genotypes have been identified to date, including GI-1 (Norwalk/1968/US; accession number M87661), which is the prototype strain for the NV genus. Eight different genotypes have been identified to date within GII, and a single genotype constitutes GIII (Table 1) *(19,20–21)*.

Among SVs, three genogroups have been proposed *(22–24)*; genogroup 1 is represented by the Sapporo/1982/JP strain (accession number U65427), genogroup 2 by the London/1992/UK (accession number U95645) strain, and genogroup 3 by the Houston/1990/US strain (accession number U95644).

2.2. Rotaviruses

RVs are members of the Reoviridae family, and are nonenveloped triple-layered viruses, which possess a segmented genome consisting of 11 dsRNA segments. By EM, particles are approx 75 nm in diameter with a wheel-like structure from which they derive their name.

RVs are classified into groups A–E based on the antigenic differences of the viral middle layer *(25)*. Group A RVs are the most common cause of human gastroenteritis, but groups B and C RVs also infect humans. Group A RVs are further classified into subgroups (SG) based on the immunological reactivities of the middle layer protein VP6, and into G and P types according to the diversity of the outer layer proteins VP7 (*Glycoprotein*) and VP4 (*Protease-sensitive protein*), respectively *(25)*. Four different SGs (I, II, I+II and nonI/nonII), 14 or 15 G types (G1–G15) and 20 P types (P[1]–P[20]) have been identified to date among Group A RVs *(25)*.

2.3. Astroviruses

ASVs are members of the Astroviridae family. They are nonenveloped viruses with a genome of positive-sense ssRNA. By EM, they appear as spherical particles of 35–40 nm in diameter with the characteristic 5–6 pointed Star of David configuration which gives these viruses their name.

Table 1
Classification of Noroviruses

Genogroup	Genotype	Reference virus	Examples
I	1	Norwalk/1968/US	KY/89/JP
	2	Southampton/1991/UK	White Rose, Crawley
	3	Desert Shield 395/1990/SA	Birmingham 291, Potsdam
	4	Chiba 407/1987/JP	Thistle Hall, Valetta, Malta
	5	Musgrove/1989/UK	Butlins
	6	Hesse 3/1997/GE	Sindlesham, Mikkeli, Lord Harris
	7	Winchester/1994/UK	Lwymontley
II	1	Hawaii/1971/US	Wortley, Girlington, Port Canaveral 1994, Richmond 1994, Westover 1994, Honolulu 1994, Miami 1986, Stepping Hill, Pfaffenhofen
	2	Melksham/1994/UK	Snow Mountain, Melksham
	3	Toronto 24/1991/CA	Mexico, Auckland, Rotterdam, New Orleans 279
	4	Bristol/1993/UK	Lordsdale, Camberwell, Pilgrim, SymGreen, Grimsby, Burwash Landing 1995, Miami Beach 1995, Withybush
	5	Hillingdon/1990/UK	White River, Welterhof, New Orleans 1994
	6	Seacroft/1990/UK	Florida 1993, Baltimore 1993
	7	Leeds/1990/UK	Gwynedd, Venlo, Creche, Bridlington, HCALV
	8	Amsterdam/1998/NL	
III	1	Alphatron/1998/NL	Fort Lauderdale, Saint Cloud

Modified from ref. *21*

ASVs are classified into eight serotypes *(26)* based on the serological tests using type-specific antibodies. Phylogenetic analysis of sequences from a region of the ORF2 has shown that genotypes correlate serotypes *(27)*.

2.4. Adenoviruses

Human ADVs are members of the Adenoviridae family. They are nonenveloped icosahedral particles of 80 nm in diameter and possess a genome of dsDNA. ADVs are classified into six different subgroups or species (A–F) and within these subdivided into 51 distinct serotypes according to immunological, biochemical, and biological differences *(28)*. Among these, ADVs of subgroup F, serotypes 40 and 41 have been associated with gastroenteritis, and these are termed enteric or fastidious ADVs *(29,30)*.

2.5. Toroviruses, Coronaviruses, Picobirnaviruses, Aichi Viruses, and Small Round Viruses

CoVs and ToVs are two genus within the Coronaviridae family. CoVs are enveloped particles of 120–160 nm in diameter with an internal icosahedral core of approx 65 nm in diameter and a helical nucleocapsid. They have large surface projections with stem and globular portions which give them their characteristic appearance from which their name derives (*corona* is the latin word for crown) *(28)*.

Table 2
Quantity of Virus Excreted in Feces at the Peak of Infection and the Probability of Detection by EM

Viruses	Quantity in feces (per g)	Probability of detection
Rotaviruses Group A	$10^{8–12}$	++++
Rotaviruses Group C	$10^{5–7}$	+++
Noroviruses	$\leq 10^{7–8}$	+/–
Sapoviruses	$\leq 10^{7–8}$	+/–
Astroviruses	$10^{7–8}$	+
Adenoviruses	$10^{7–10}$	++
Enteroviruses	$<10^{6}$	–

CoVs are classified into three genogroups, of which genogroups 1 and 2 have human CoVs representatives (229E in genogroup 1 and OC43 in genogroup 2), and the SARS CoVs which initially appeared not to belong to any of the three known genotypes may possibly be classified within genogroup 2 *(31)*.

ToVs differ morphologically from CoVs in that their nucleocapsid has a tubular appearance and the particles may be disk-, kidney-, or rod-like shaped. ToVs are grouped in a single genogroup, which contains bovine, equine, porcine, and human viruses *(27)*.

Picobirnaviruses are a new genus of the Birnaviridae family *(28)*. These are nonenveloped round viruses of 24–41 nm diameter with a bi-segmented dsRNA genome. Picobirnaviruses have been found in human and animal feces *(12–15)*.

Aichi virus is a member of the Picornaviridae family, recently included in a separate genus, kobuvirus, which also includes a bovine virus (32,33). They are small nonenveloped viruses of 22–30 nm diameter with a genome of positive-sense ssRNA.

Small round viruses or parvo-like virus particles found in human feces *(34,35)* are DNA viruses of approx 22 nm diameter.

3. DIAGNOSIS

Although EM has traditionally been used for the detection of enteric viruses in the feces of infected individuals, this is a labor-intensive and relatively insensitive method, as detection requires approx 10^6/g virus particles in feces. This is a problem particularly for the detection of caliciviruses (Table 2). Immune EM, which increases sensitivity and allows virus characterization when type-specific antibodies are used, has also been used for the detection of enteric viruses. Serological methods have been developed for the detection of some of these viruses. Enzyme immunosorbent assays (EIA) and passive particle agglutination tests (PPAT), some of which are available commercially, provide sensitivity comparable to, or better than, EM for the detection of RVs, NVs, ASVs, and ADVs. More recently, molecular methods, reverse-transcription polymerase chain reaction (RT-PCR), PCR, or nucleic acid-based sequence amplification (NASBA) assays have been developed for the detection of enteric viruses. These methods provide improved sensitivity for the detection of all enteric viruses, but have had a major impact on the detection of human caliciviruses (Table 3). Viruses do not replicate in food or water, and

Table 3
Detection of Enteric Viruses By EM Compared With PCR/RT-PCR

Virus	Number detected by EM	Percent	Number detected by PCR/RT-PCR[a]	Percent change (PCR-EM/EM) × 100
Rotavirus	70	25.8	86	+22.9
Norovirus	6	2.2	46	+666.7
Adenovirus	12	4.4	40	+233.4
Sapovirus	1[b]	0.4	8	+700
Astrovirus	3	1.1	7	+133.3
Virus detected	92	33.9	187	+103.3
No virus detected	179	66.1	111	–38.0
Total	**271**	**100.0**	**298**	

[a]Includes detection of dual and triple infections. EM detected one dual infection (rotavirus and adenovirus).
[b]Appearance of "classical calicivirus."
From ref. *64*

the concentration of virus particles in contaminated products is likely to be very small and not distributed homogeneously throughout the foodstuff. Testing for the presence of viruses in food, water, or environmental samples has only been possible since the development of very sensitive molecular methods, which include virus elution from the foodstuff, followed by concentration *(36)* efficient nucleic acid extraction methods for the removal of inhibitors of amplification.

One frequent source of foodborne enteric virus infections is shellfish. The development of a method for dissecting the stomach and digestive diverticula of shellfish *(37)* followed by nucleic acid extraction and DNA amplification-based methods *(37–41)* allows reliable and sensitive detection of enteric viruses in contaminated shellfish.

3.1. Human Caliciviruses

EM has been used for first-line diagnosis of NVs and SVs in clinical samples *(17,42, 43)*, however in recent years, EM has been replaced in many laboratories with in-house or commercial EIAs for the detection of NVs *(44–46)*. Molecular diagnosis using nucleic-acid extraction and RT-PCR assays has been introduced into many laboratories for the detection of NVs and SVs *(23,47–51)*. With the development of sensitive nested PCR assays, detection of NVs contamination of foodstuffs has recently been feasible. In particular the detection of NVs in oysters and other shellfish has been widely reported *(40,52–57)*. Some foods, including raspberries, are contaminated with NVs *(58)*. The detection of NVs in other foodstuffs is still in its developmental stage although a few studies have been undertaken *(59–61)*.

3.2. Rotaviruses

Because the number of RVs particles that are excreted at the peak of infection may be as high as 10^{12}/g in feces, diagnosis can be made using EM. EM will not however distinguish between RVs of different groups, and for this immune EM can be used. Most laboratories use EIA or PPAT, which use broadly reacting capture antibodies directed against epitopes of Group A RV VP6, for the routine diagnosis of RV infections. Commercially available assays have a sensitivity for detection comparable to EM, but are more prone to nonspecific reactions (reviewed in ref. *[62]*). The use of RT-PCR

for the detection and characterization of RVs provides increased sensitivity and specificity *(62–64)*. Molecular methods for the detection of RVs are not routinely used in diagnostic laboratories, but their increased sensitivity make them useful for detecting low viral loads in asymptomatic infections, virus in samples that have been collected late after the onset of symptoms, or virus in environmental or food and water samples *(40,65–68)*.

3.3. Astroviruses

ASVs were first detected by EM *(69,70)*. Initially, characterization of ASVs was carried out by immune EM, but the development of EIA and RT-PCR assays for the detection and typing of ASV has made the detection of ASV available to diagnostic laboratories *(27, 71–73)*. In addition to increased sensitivity, the RT-PCR, used in conjunction with DNA sequencing, provides genotyping data which are vital for molecular epidemiological studies, and could be used for outbreak tracking, whether foodborne or otherwise.

3.4. Enteric Adenoviruses

ADVs were first identified by EM in the feces of children with gastroenteritis *(74)*. These viruses induced typical cytopathic effects in cell culture, but could not be passaged or typed *(75)*, for this reason, these viruses were designated fastidious ADVs. Later, the cell line 293 was shown to support the propagation of enteric ADVs, and permitted the development of neutralization assays *(76)*. This method is, however, time consuming and relatively insensitive, and most diagnostic laboratories have a number of rapid serological tests available (immunofluorescence, EIA, and PPAT assays) many with a sensitivity of detection comparable to EM (reviewed in *[77]*). In recent years, broadly reactive PCR methods have been developed for detecting ADVs, and in conjunction with restriction endonuclease analysis provide a sensitive tool for ADV characterization *(78)*. PCRs which use primers specific to a region of the genome (the long-fiber gene), highly conserved between ADV 40 and 41 but significantly divergent between these and other human ADVs have also been developed for the specific detection of enteric ADVs *(79)*.

3.5. Aichi Virus

EM is inappropriate for the detection and identification of these viruses in clinical samples. Aichi viruses cannot be differentiated from other enteroviruses excreted in feces. An enzyme-linked immunosorbent assay was developed for the detection of antibody responses to Aichi virus infection and RT-PCR assays have also been developed to detect the RNA genome of the virus in fecal and oyster samples *(80,81)*.

3.6. Toroviruses, Coronaviruses, Picobirnaviruses, and Small Round Viruses

Many novel gastroenteric viruses in humans were first discovered by EM including ToVs *(82,83)*, CoVs *(6)*, and small round viruses *(34)*. The picobirnaviruses were first detected using polyacrylamide gel electrophoresis (PAGE) of RNA derived from rat and human feces *(15,84)*. Molecular methods for the detection of these viruses have been developed *(85,86)*, although further studies are required.

4. RESERVOIRS

Humans are the principal reservoir of many of the enteric viruses, and person-to-person spread is the major route of transmission. The members of many virus families

Table 4
Enteric Virus Families and Genera That Infect Humans and Also Other Animal Species

Virus family	Genus	Animal host species	Evidence for zoonotic transmission
Caliciviridae	Norovirus	Bovine, porcine	No
	Sapovirus	None	
Reoviridae	Rotavirus A	Avian, bovine, canine, equine, feline, lapine, murine, ovine, porcine, simian	Yes
	Rotavirus B	Bovine, porcine	No
	Rotavirus C	Bovine, porcine	No
Adenoviridae	Adenovirus	Bovine, canine, equine, murine, ovine, porcine	No
Astroviridae	Astrovirus	Bovine, duck, feline, ovine, porcine, turkey	No
Coronaviridae	Coronavirus	Bovine, canine, feline, murine, porcine, turkey	Yes
	Torovirus	Bovine, equine, porcine	No
Picornaviridae	Kobuvirus	Bovine	No
Birnaviridae		Bovine, lapine, rat	No

infect animal species (Table 4), although zoonotic transmission is rare *(87)* with the exception of RVs *(88)*. Evidence of interspecies transmission of RVs has been obtained through comparative analysis of the genes derived from RV isolates from humans or animals either by whole-genome hybridization methods *(89)* or by gene sequencing and phylogenetic analysis (reviewed in ref. *[90]*), and many RV genotypes are shared among different species (Table 5).

The recent SARS epidemic is thought to have originated through transmission of the SARS CoV from an animal reservoir *(91)*, highlighting the potential for CoVs to cross the species barrier.

The lack of evidence for other viruses crossing the species barrier and of recombination/ reassortment between animal and human pathogens needs to be addressed. Concomitant studies of disease in humans and animals in the same geographical locations are required.

5. FOODBORNE OUTBREAKS

Foods that are consumed raw or minimally processed, such as fruit, vegetables, and shellfish, are typically implicated as vehicles for the transmission of enteric viruses. However, a wide variety of foods have been implicated in foodborne viral gastroenteritis outbreaks (Table 6).

Enteric viruses can be present in foodstuffs through direct contamination with untreated sewage-sludge used in agriculture or sewage polluting shellfish culture beds. Food can also become contaminated during processing either by the use of polluted water in the preparation process or by infected food-handlers. Food-handlers have been shown to contaminate food during presymptomatic, symptomatic, and postsymptomatic infections *(92–96)*.

Table 5
Rotavirus Genotypes Found in Human and Commonly Found in Other Animal Species

Genotype found in humans	Other host animal species
G3	Cats, dogs, monkeys, goats
G5	Pigs, horses
G6	Calves
G8	Calves
G9	Lams
G10	Calves
P[6]	Pigs
P[9]	Cats
P[11]	Calves, horses
P[14]	Pigs
P[19]	Pigs

Modified from ref. *97*

The majority of food- or waterborne outbreaks in which a virus is identified are caused by NVs *(58,97–107)*. RVs, and possibly ASVs, have also been implicated in food- or waterborne outbreaks *(5,105,106)*, but much less frequently. Aichi virus was first isolated in 1989 in BS-C1 cells from patients in outbreaks of oyster-associated gastroenteritis, in Japan *(16)*. Later studies have also showed a link between oyster-associated gastroenteritis and the acquisition of Aichi virus-specific antibodies in some patients *(110)*.

SVs and ADVs have yet to be confirmed as the cause of any food- or waterborne gastroenteritis outbreaks. Recently, outbreaks suspected of being foodborne have been detected among passengers on cruise ships. Multiple enteric viruses, SVs, ADVs, NVs, and RVs were detected in symptomatic patients suggesting the ingestion of fecally contaminated food or water (unpublished data).

6. PATHOGENICITY

Gastroenteritis viruses infect mainly the epithelial cells of the proximal part of the small intestine. The intestinal lumen is lined with a layer of polarized epithelial cells (enterocytes), which cover the villi and crypts. The enterocytes lining the villi are non-dividing absorptive cells, and those lining the crypts are undifferentiated proliferative cells that differentiate in order to renew the absorptive enterocytes of the villi. Some enteric viruses, RVs, ADVs, and ASVs infect the mature enterocytes exclusively, CoVs and ToVs infect the crypt and basal villus enterocytes, and enteric parvoviruses infect the crypt cells in the animal models (reviewed in *[109]*).

Viral diarrhea is caused by several factors:

- Primary malabsorption that originates from decreased absorption due to mature enterocyte cell death, which results in shortening of the villi, and also induces loss of enzymes leading to the accumulation of undigested carbohydrates and proteins.
- Reactive crypt hyperplasia which leads to increased secretion into the intestinal lumen.
- Decreased intestinal motility induced by the autonomous central nervous system.

In RV diarrhea, the symptoms precede the appearance of any histological changes *(112)*. This suggested that, in RV infection at least, other mechanisms in addition to the

Table 6
Foods Implicated in the Transmission of Gastroentetritis (Additional Data Obtained From http://www.cdc.gov/foodborneoutbreaks/)

Food category	Product	Virus identified
Vegetables	Green salad	NV, RV
	Green beans	NV
	Brocoli	NV
Dairy	Ice cream	NV
	Cheese	NV
	Cream	NV
Meat	Chicken	NV
	Beef	NV
	Hamburger	NV
Fish	Oyster[a]	NV, RV
	Shrimp	NV
Confectionary	Cheesecake	NV
	Lemonade	NV
	Slush drink	NV
Bakery products	Sandwich	NV, RV
	Donought	NV
Fruit	Berries	NV
	Melon	NV
	Pinaple	NV
	Grapes	NV
	Grapefruit	NV
Other	Eggs	NV
	Pasta	NV
	Rice	NV
	Pizza	NV

[a]Multiple NV genotypes have often been associated with outbreaks following the consumption of oysters.

ones described above must exist. One of the RV nonstructural proteins (NSP4), and a short peptide derived from it (aa 114–135) were shown to induce diarrhea in a dose-dependent manner in the neonatal mouse model *(113)*. NSP4 is the first identified viral enterotoxin, which has the capacity to mobilize intracellular calcium and increase the cellular membrane chloride permeability *(114,115)*, and NSP4 is also secreted from the infected enterocytes in early infection *(116)*. Recently, it has been observed that RV evokes intestinal fluid and electrolyte secretion by activation of the enteric nervous system *(117)*.

7. CLINICAL CHARACTERISTICS

7.1. Caliciviruses

The incubation period for NVs is 24–48 h, and the mean duration of illness is 12–60 h. The clinical manifestations of NV are characterized by nausea, projectile vomiting, diarrhea, and abdominal cramps. Fever, chills, and lethargy can also occur *(29)*. Vomiting is usually more common in children, and diarrhea is the main symptom in adults *(118,119)*.

The incubation period for SVs is 24–36 h, with illness lasting for 1–4 d. Symptoms include diarrhea (95% cases) and vomiting (60%), as well as fever and abdominal pain *(120)*.

7.2. Rotaviruses

The incubation period is usually 2 d, with the illness lasting for an average of 3–8 d *(29)*. Vomiting and watery diarrhea are the predominant symptoms, and fever and abdominal pain are also frequent. Extraintestinal spread of RVs has also been reported on numerous occasions, and may be associated to neurological disease *(121,122)*.

7.3. Astroviruses

The incubation period is between 24 and 36 h, with illness lasting for 1–4 d. Symptoms include vomiting, diarrhea, fever, and abdominal pain *(29)*.

7.4. Enteric Adenoviruses

The incubation period can vary between 3 and 10 d, with illness often lasting for more than 1 wk. Diarhea is more prominent than vomiting or fever *(29)*.

7.5. Aichi Virus

With Aichi virus gastroenteritis, diarrhea has been demonstrated in 58% of patients, and others include abdominal pain (92%), vomiting (71%), and fever (58%) *(81)*. However, these viruses have not been identified as a cause of gastroenteritis outside Japan, and their importance and spread remain unclear.

8. CHOICE OF TREATMENT

Viral gastroenteritis is usually self-limiting and its symptoms resolve without significant sequelae. In the cases of prolonged diarrhea, especially in infantile RV gastroenteritis, rehydration therapy with oral rehydration salt (ORS) solution (WHO formula; http://www.who.int/medicines/organization/par/edl/expcom13/ors.doc) or in severe cases, intravenous rehydration is indicated.

9. SUMMARY AND CONCLUSIONS

Enteric viruses are transmitted mainly from person-to-person. However, as these viruses are excreted in the feces of infected individuals, food and water can become contaminated with fecal material directly (sewage pollution) or indirectly by infected food-handlers.

The transmission of foodborne enteric virus infection by food-handlers can be prevented through the instigation of good hygiene practices. Symptomatic food-handlers should remain away from work for 48 h after the last episode of vomiting or diarrhea and should not prepare food for others during this period.

Procedures should be in place to address an incident of vomiting in the workplace. Exposed food and food that has been handled by an infected person should be destroyed. All contaminated areas, including vertical surfaces, must be thoroughly cleaned and attention to hand-washing procedures should be emphasized.

The extent of foodborne infection is not fully known *(123,124)*, a study conducted in Sweden estimated the annual incidence of foodborne illness at 38–79 per 1000 inhabitants

(125). More alarming estimates from the United States attribute 76 million illnesses, 325,000 hospitalizations, and 5000 deaths to foodborne infections *(126)*. Mead et al. estimate that 34% of the hospitalizations attributable to foodborne transmission have a viral etiology.

To date, most foodborne viral gastroenteritis outbreaks have been associated with NV infections. However, it is likely that the other enteric viruses will also be transmitted via contaminated food and water with similar frequencies. A study assessing viral contamination of several shellfish beds in France that lasted more than 3 yr detected ASV in 17% of the samples, NV in 23%, enterovirus in 19%, and RV in 27% *(40)*. Similarly, in another study ADV contaminated shellfish was found in 47% of the samples tested and included sampling in areas considered unpolluted according to current methods for the determination of microbiological quality based on coliform counts *(127)*.

There may be several confounding factors that affect the detection of foodborne viral gastroenteritis. They include:

- Many laboratories will only investigate for the presence of NVs in suspected foodborne outbreaks.
- NVs are an extremely diverse group of viruses *(128)*, and although several broadly reactive NV-specific assays are available, there is no single test that will detect all NVs with the same efficiency. Also, geographical differences detected among NV genotypes have been observed (unpublished data). Contaminated foodstuffs can be sourced from all over the world, and it is possible that the methods available in any one country, although being suitable for the locally endemic strains, may not be efficient for the detection of variants from other geographical regions.
- Immunity to NV is short-lived (~6 mo) and the number of individuals susceptible to symptomatic illness is constantly high. However, other enteric viruses (RV, ADV, and ASV) induce long-lasting immunity, which does not prevent reinfections but protects most adults from illness. Therefore, identifying a foodborne outbreaks caused by RV or ADV may be difficult as most people will not show symptoms or these will be very mild, and may not even give rise to the suspicion of a foodborne outbreak.

It is clear that further work is required in order to properly estimate the burden of food- and waterborne viral diseases. The advent of molecular methods of exquisite sensitivity provides the tools for the examination of food and water and may provide data on the zoonotic transmission of animal viruses into the human population via the food chain.

REFERENCES

1. Gallimore, C. I., Cubitt, D., du Plessis, N., and Gray, J. J. (2004) Asymptomatic and symptomatic excretion of noroviruses during a hospital outbreak of gastroenteritis. *J. Clin. Microbiol.* **42,** 2271–2274.
2. Gallimore, C. I., Richards, A., and Gray, J. J. (2003) Molecular diversity of noroviruses associated with outbreaks on cruise ships: comparisons with strains circulating in the UK. *Comm. Dis. Pub. Health* **6,** 285–293.
3. Parashar, U. D., Bresee, J. S., Gentsch, J. R., and Glass, R. I. (1998) Rotavirus. *Emerg. Infect. Dis.* **4,** 561–570.
4. Parashar, U. D., Hummelman, E. G., Bresee, J. S., Miller, M. A., and Glass, R. I. (2003) Global illness and deaths caused by rotavirus disease in children. *Emerg. Infect. Dis.* **9,** 565–572.
5. MMWR. (2000) Foodborne outbreak of Group A rotavirus gastroenteritis among college students–District of Columbia, March–April. *Morb. Mort. Wkly Rep.* **49,** 1131–1133.

6. Caul, E. O., Paver, W. K., and Clarke, S. K. R. (1975) Coronavirus particles in faeces from patients with gastroenteritis. *Lancet* **1,** 1192.
7. Clarke, S. K. R., Caul, E. O., and Egglestone, S. I. (1979) The human enteric coronaviruses. *Postgrad. Med. J.* **55,** 135–142.
8. Koopmans, M. and Horzinek, M. C. (1994) Toroviruses of animals and humans: a review. *Adv. Virus. Res.* **43,** 233–273.
9. Koopmans, M. P., Goosen, E. S., Lima, A. A., et al. (1997) Association of torovirus with acute and persistent diarrhea in children. *Pediatr. Infect. Dis. J.* **16,** 504–507.
10. Leung, W. K., To, K. F., Chan, P. K., et al. (2003) Enteric involvement of severe acute respiratory syndrome-associated coronavirus infection. *Gastroenterology* **125,** 1011–1017.
11. Ludert, J. E. and Liprandi, F. (1993) Identification of viruses with bi- and trisegmented double-stranded RNA genome in faeces of children with gastroenteritis. *Res. Virol.* **144,** 219–224.
12. Gallimore, C. I., Appleton, H., Lewis, D., Green, J., and Brown, D. W. G. (1995) Detection and characterisation of bisegmented dsRNA viruses (picobirnaviruses) in human faeces. *J. Med. Virol.* **45,** 135–140.
13. Gallimore, C. I., Lewis, D., and Brown, D. W. G. (1993) Detection and characterization of a novel bisegmented double-stranded RNA virus (picobirnavirus) from rabbit faeces. *Arch. Virol.* **133,** 63–73.
14. Grohmann, G. S., Glass, R. I., Pereira, H. G., et al. (1993) Enteric viruses and diarrhea in HIV-infected patients. *N. Engl. J. Med.* **329,** 14–20.
15. Pereira, H. G., Fialho, A. M., Flewett, T. H., Teixeira, J. M. S., and Andrade, Z. P. (1988) Novel viruses in human faeces. *Lancet* **2,** 103–104.
16. Yamashita, T., Kobayashi, S., Sakae, K., et al. (1991) Isolation of cytopathic small round viruses with BC-C-1 cells from patients with gastroenteritis. *J. Infect. Dis.* **164,** 954–957.
17. Caul, E. O. and Appleton, H. (1982) The electron microscopical and physical characteristics of small round structured fecal viruses: an interim scheme for classification. *J. Med. Virol.* **9,** 257–265.
18. Koopmans, M., van Strien, E., and Vennema, H. (2003). Molecular epidemiology of human caliciviruses. In: *Viral Gastroenteritis. Prespectives in Medical Virology* (Desselberger, U. and Gray, J. J., eds.), Elsevier, Amsterdam, pp. 523–554.
19. Green, K. Y., Ando, T., Balayan, M. S., et al. (2000) Taxonomy of the caliciviruses. *J. Infect. Dis.* **181,** S322–S330.
20. Mayo, M. A. (2002) A summary of taxonomic changes recently approved by ICTV. *Arch. Virol.* **147,** 1655–1663.
21. Green, K., Chanock, R., and Kapilian, A. (2001) Human caliciviruses. In: *Fields Virology* (Knipe, D. M., Howeley, M. M., et al., eds.), 4th edn. Lippincott Williams and Wilkins, Philadelphia:841–874.
22. Berke, T., Golding, B., Jiang, X., et al. (1997) Phylogenetic analysis of the caliciviruses. *J. Med. Virol.* **52,** 419–424.
23. Jiang, X., Cubitt, W. D., Berke, T., et al. (1997) Sapporo-like human caliciviruses are genetically and antigenically diverse. *Arch. Virol.* **142,** 1813–1827.
24. Noel, J. S., Liu, B. L., Humphrey, C. D., et al. (1997) Parkville virus: a novel genetic variant of human calicivirus in the Sapporo virus clade, associated with an outbreak of gastroenteritis in adults. *J. Med. Virol.* **52,** 173–178.
25. Estes, M. (2001) Rotaviruses and their replication. In: *Fields Virology* (Knipe, D. M., Howley, P. M., et al., eds.), 4th edn, Lippincott Williams & Wilkins, Philadelphia, pp. 1747–1785.
26. Lee, T. W. and Kurtz, J. B. (1994) Prevalence of human astrovirus serotypes in the Oxford region 1976–92, with evidence for two new serotypes. *Epidemiol. Infect.* **112,** 187–193.
27. Noel, J. S., Lee, T. W., Kurtz, J. B., Glass, R. I., and Monroe, S. S. (1995) Typing of human astroviruses from clinical isolates by enzyme immunoassay and nucleotide sequencing. *J. Clin. Microbiol.* **33,** 797–801.

28. van Regenmortel, M. H. V., Fauquet, C. M., Bishop, D. H. L., et al. (2000) *Virus Taxonomy, Seventh Report of the International Committee on Taxonomy of Viruses*, Academic, San Diego, CA.
29. Desselberger, U. and Gray, J. (2004) Viruses associated with acute diarrhoeal disease. In: *Principles and Practice of Clinical Virology* (Zuckerman, A. J., Banatvala J. E., Pattison, J. R., Griffiths, P., and Schoub B., eds.), 5th edn, Wiley, Chichester, UK, pp. 249–270.
30. Echevarria, M. (2004) Adenovirus. In: *Principles and Practice of Clinical Virology* (Zuckerman, A. J., Banatvala J. E., Pattison, J. R., Griffiths, P., and Schoub B., eds.), 5th edn, Wiley, Chichester, UK, pp. 249–270.
31. Zhu, G. and Chen, H. W. (2004) Monophyletic relationship between severe acute respiratory syndrome coronavirus and group 2 coronaviruses. *J. Infect. Dis.* **189,** 1676–1678.
32. Yamashita, T., Sakae, K., Tsuzuki, H., et al. (1998) Complete nucleotide sequence and genetic organization of Aichi virus, a distinct member of the Picornaviridae associated with acute gastroenteritis in humans. *J. Virol.* **72,** 8408–8412.
33. Yamashita, T., Ito, M., Kabashima, Y., Tsuzuki, H., Fujiura, A., and Sakae, K. (2003) Isolation and characterization of a new species of kobuvirus associated with cattle. *J. Gen. Virol.* **84,** 3069–3077.
34. Paver, W. K., Ashley, C. R., Caul, E. O., and Clarke, S. K. R. (1973) A small virus in human faeces. *Lancet* **1,** 237–239.
35. Paver, W. K. and Clarke, S. K. R. (1976) Comparison of human fecal and serum parvo-like viruses. *J. Clin. Microbiol.* **4,** 67–70.
36. Dubois, E., Agier, C., Traore, O., et al. (2002) Modified concentration method for the detection of enteric viruses on fruits and vegetables by reverse transcriptase-polymerase chain reaction or cell culture. *J. Food. Prot.* **65,** 1962–1969.
37. Atmar, R. L., Neill, F. H., Romalde, J. L., et al. (1995) Detection of Norwalk virus and hepatitis A virus in shellfish tissues with the PCR. *Appl. Environ. Microbiol.* **61,** 3014–3018.
38. Atmar, R. L., Metcalf, T. G., Neill, F. H., and Estes, M. K. (1993) Detection of enteric viruses in oysters by using the polymerase chain reaction. *Appl. Environ. Microbiol.* **59,** 631–635.
39. Atmar, R. L., Neill, F. H., Woodley, C. M., et al. (1996) Collaborative evaluation of a method for the detection of Norwalk virus in shellfish tissues by PCR. *Appl. Environ. Microbiol.* **62,** 254–258.
40. Le Guyader, F., Haugarreau, L., Miossec, L., Dubois, E., and Pommepuy, M. (2000) Three-year study to assess human enteric viruses in shellfish. *Appl. Environ. Microbiol.* **66,** 3241–3248.
41. Schwab, K. J., Neill, F. H., Fankhauser, R. L., et al. (2000) Development of methods to detect "Norwalk-like viruses" (NLVs) and hepatitis A virus in delicatessen foods: application to a food-borne NLV outbreak. *Appl. Environ. Microbiol.* **66,** 213–218.
42. Curry, A., Bryden, A., Morgan-Capner, P., et al. (1999) A rationalised virological electron microscope specimen testing policy. PHLS North West Viral Gastroenteritis and Electron Microscopy Subcommittee. *J. Clin. Path.* **52,** 471–474.
43. Lewis, D., Ando, T., Humphrey, C. D., Monroe, S. S., and Glass, R. I. (1995) Use of solid-phase immune electron microscopy for classification of Norwalk-like viruses into six antigenic groups from 10 outbreaks of gastroenteritis in the United States. *J. Clin. Microbiol.* **33,** 501–504.
44. Hale, A. D., Crawford, S. E., Ciarlet, M., et al. (1999) Expression and self-assembly of Grimsby virus: antigenic relationship to Norwalk and Mexico virus. *Clin. Diag. Lab. Immunol.* **6,** 142–145.
45. Richards, A. F., Lopman, B. A., Gunn, A., et al. (2003) Evaluation of a commercial ELISA for detecting Norwalk-like virus antigen in faeces. *J. Clin. Virol.* **26,** 109–115.
46. Vipond, I. B., Pelosi, E., Williams, J., et al. (2000) A diagnostic EIA for detection of the prevalent SRSV strain in United Kingdom outbreaks of gastroenteritis. *J. Med. Virol.* **61,** 132–137.

47. Ando, T., Monroe, S. S., Gentsch, J. R., et al. (1995) Detection and differentiation of antigenically distinct small round structured viruses (Norwalk-like viruses) by reverse transcription PCR and Southern hybridization. *J. Clin. Microbiol.* **33,** 64–71.
48. Green, J., Gallimore, C. I., Norcott, J. P., Lewis, D., and Brown, D. W. G. (1995) Broadly reactive reverse transcriptase polymerase chain reaction (RT-PCR) for the diagnosis of SRSV-associated gastroenteritis. *J. Med. Virol.* **47,** 392–398.
49. Green, S. M., Lambden, P. R., Deng, Y., et al. (1995) Polymerase chain reaction detection of small round-structured viruses from two related hospital outbreaks of gastroenteritis using inosine-containing primers. *J. Med. Virol.* **45,** 197–202.
50. Matson, D. O., Zhong, W., Nakata, S., et al. (1995) Molecular characterisation of a human calicivirus with sequence relationships closer to animal caliciviruses than other known human caliciviruses. *J. Med. Virol.* 4**5,** 215–222.
51. Vinje, J., Deijl, H., van der Heide, R., et al. (2000) Molecular detection and epidemiology of Sapporo-like viruses. *J. Clin. Microbiol.* **44,** 113–118.
52. Green, J., Henshilwood, K., Gallimore, C. I., Brown, D. W. G., and Lees, D. N. (1998) A nested reverse transcriptase PCR assay for detection of small round-structured viruses in environmentally contaminated molluscan shellfish. *Appl. Environ. Microbiol.* **64,** 858–863.
53. Henshilwood, K., Green, J., Gallimore, C. I., Brown, D. W. G., and Lees, D. N. (1998) The development of polymerase chain reaction assays for detection of small round structured and other human enteric viruses in molluscan shellfish. *J. Shellfish Res.* **17,** 1675–1678.
54. Le Guyader, F. S., Neill, F. H., Dubois, E., et al. (2003) A semiquantitative approach to estimate Norwalk-like virus contamination of oysters implicated in an outbreak. *Int. J. Food Microbiol.* **87,** 107–112.
55. Lees, D. (2000) Viruses and bivalve shellfish. *Int. J. Food Microbiol.* **59,** 81–116.
56. Lees, D. N., Henshilwood, K., Gallimore, C. I., Green, J., and Brown, D. W. G. (1995) Detection of small round structured viruses in shellfish by RT-PCR. *Appl. Environ. Microbiol.* **61,** 4418–4424.
57. Nishida, T., Kimura, H., Saitoh, M., et al. (2003) Detection, quantitation, and phylogenetic analysis of noroviruses in Japanese oysters. *Appl. Environ. Microbiol.* **69,** 5782–5786.
58. Ponka, A., Maunula, L., von Bonsdorff, C. H., and Lyytikainen, O. (1999) An outbreak of calicivirus associated with consumption of frozen raspberries. *Epidemiol. Infect.* **123,** 469–474.
59. Gouvea, V., Santos, N., do Carmo Timenetsky, M., and Estes, M. K. (1994) Identification of Norwalk virus in artificially seeded shellfish and selected foods. *J. Virol. Meth.* **48,** 177–187.
60. Sair, A. I., D'Souza, D. H., Moe, C. L., and Jaykus, L. A. (2002) Improved detection of human enteric viruses in foods by RT-PCR. *J. Virol. Meth.* **100,** 57–69.
61. Schwab, K., J., Neill, F. H., Le Guyader, F., Estes, M. K., and Atmar, R. L. (2001) Development of a reverse transcription-PCR-DNA enzyme immunoassay for detection of "Norwalk-like" viruses and hepatitis A virus in stool and shellfish. *Appl. Environ. Microbiol.* **67,** 742–749.
62. Iturriza-Gómara, M., Green, J., and Gray, J. J. (2000) Methods of rotavirus detection, sero-and genotyping, sequencing and phylogenetic analysis. In: *Rotaviruses: Methods and Protocols. Methods in Molecular Medicine* (Gray, J. J. and Desselberger, U., eds.), Humana, Totowa, NJ, pp. 189–217.
63. Iturriza-Gómara, M., Wong, C., Blome, S., Desselberger, U., and Gray, J. (2002) Molecular characterisation of VP6 genes of human rotavirus isolates: correlation of genogroups with subgroups and evidence of independent segregation. *J. Virol.* **76,** 6596–6601.
64. Simpson, R., Aliyu, S., Iturriza-Gómara, M., Desselberger, U., and Gray, J. (2003) Infantile viral gastroenteritis: on the way to closing the diagnostic gap. *J. Med. Virol.* **70,** 258–262.
65. Kang, G., Iturriza-Gómara, M., Wheeler, J. G., et al. (2004) Quantitation of Group A rotavirus RNA by real time reverse-transcription polymerase chain reaction: correlation with clinical severity in children in South India. *J. Med. Virol.* **73,** 118–122.

66. Muniain-Mujika, I., Girones, R., and Lucena, F. (2000) Viral contamination of shellfish: evaluation of methods and analysis of bacteriophages and human viruses. *J. Virol. Meth.* **89,** 109–118.
67. Villena, C., El-Senousy, W. M., Abad, F. X., Pinto, R. M., and Bosch, A. (2003) Group A rotavirus in sewage samples from Barcelona and Cairo: emergence of unusual genotypes. *Appl. Environ. Microbiol.* **69,** 3919–3923.
68. Jean, J., Blais, B., Darveau, A., and Fliss, I. (2002). Rapid detection of human rotavirus using colorimetric nucleic acid sequence-based amplification (NASBA)-enzyme-linked immunosorbent assay in sewage treatment effluent. *FEMS Microbiol. Lett.* **210,** 143–147.
69. Appleton, H., Buckley, M., Thom, B. T., Cotton, J. L., and Henderson, S. (1977) Virus-like particles in winter vomiting disease. *Lancet* **19,** 409–411.
70. Madeley, C. R. and Cosgrove, B. P. (1975) 28 nm particles in faeces in infantile gastroenteritis. *Lancet* **6,** 451–452.
71. Herrmann, J. E., Nowak, N. A., Perron-Henry, D. M., Hudson, R. W., Cubitt, W. D., and Blacklow, N. R. (1990) Diagnosis of astrovirus gastroenteritis by antigen detection with monoclonal antibodies. *J. Infect. Dis.* **161,** 226–229.
72. Jonassen, T. O., Monceyron, C., Lee, T. W., Kurtz, J. B., and Grinde, B. (1995) Detection of all serotypes of human astrovirus by the polymerase chain reaction. *J. Virol. Methods.* **52,** 327–334.
73. Saito, K., Ushijima, H., Nishio, O., et al. (1995) Detection of astroviruses from stool samples in Japan using reverse transcription and polymerase chain reaction amplification. *Microbiol. Immunol.* **39,** 825–828.
74. Flewett, T. H., Bryden, A. S., Davies, H. A., and Morris, C. A. (1973) Epidemic viral enteritis in a long-stay children's ward. *Lancet* **1,** 4–5.
75. Madeley, C. R., Cosgrove, B. P., Bell, E. J., and Fallon, R. J. (1977) Stool viruses in babies in Glasgow. I. Hospital admissions with diarrhoea. *J. Hyg.* **78,** 261–273.
76. Takiff, H. E., Straus, S. E., and Garon, C. F. (1981) Propagation and in vitro studies of previously non-cultivable enteral adenoviruses in 293 cells. *Lancet* **17,** 832–834.
77. de Jong, J. C. (2003) Epidemiology of enteric adenoviruses 40 and 41 and other adenoviruses in immunocompetent and immunodeficient individuals. In: *Viral Gastroenteritis. Prespectives in Medical Virology* (Desselberger, U. and Gray, J. J., eds.), Elsevier, Amsterdam, pp. 407–446.
78. Allard, A., Albinsson, B., and Wadell, G. (2001) Rapid typing of human adenoviruses by a general PCR combined with restriction endonuclease analysis. *J. Clin. Microbiol.* **39,** 498–505.
79. Tiemessen, C. T. and Nel, M. J. (1996) Detection and typing of subgroup F adenoviruses using the polymerase chain reaction. *J. Virol. Meth.* **59,** 73–82.
80. Yamashita, T., Sugiyama, M., Tsuzuki, H., Sakae, K., Suzuki, Y., and Miyazaki, Y. (2000) Application of a reverse transcription-PCR for identification and differentiation of Aichi virus, a new member of the Picornavirus family associated with gastroenteritis in humans. *J. Clin. Microbiol.* **38,** 2955–2961.
81. Yamashita, T., Ito, M., Tsuzuki, H., and Sakae, K. (2001) Identification of Aichi virus infection by measurement of immunoglobulin responses in an enzyme-linked immunosorbent assay. *J. Clin. Microbiol.* **39,** 4178–4180.
82. Beards, G. M., Green, J., Hall, C., and Flewett, T. H. (1984) An enveloped virus in stools of children and adults with gastroenteritis that resembles the Breda virus of calves. *Lancet* **1,** 1050–1052.
83. Beards, G. M., Brown, D. W. G., Green, J., and Flewett, T. H. (1986) Preliminary characterisation of torovirus-like particles of humans: comparison with Berne virus of horses and Breda virus of calves. *J. Med. Virol.* **20,** 67–78.
84. Pereira, H. G., Flewett, T. H., Candeias, J. A. N., and Barth, O. M. (1988) A virus with a bisegmented double-stranded RNA genome in rat (*Oryzomys nigripes*) intestines. *J. Gen. Virol.* **69,** 2749–2754.

85. Duckmanton, L., Luan, B., Devenish, J., Tellier, R., and Petric, M. (1997) Characterization of torovirus from human fecal specimens. *Virology* **239,** 158–168.
86. Rosen, B. I., Fang, Z. Y., Glass, R. I., and Monroe, S. S. (2000) Cloning of human picobirnavirus genomic segments and development of an RT-PCR detection assay. *Virology* **277,** 316–329.
87. Oliver, S. L., Dastjerdi, A. M., Wong, S., et al. (2003) Molecular characterization of bovine enteric caliciviruses: a distinct third genogroup of noroviruses (Norwalk-like viruses) unlikely to be of risk to humans. *J. Virol.* **77,** 2789–2798.
88. Cook, N., Bridger, J., Kendall, K., Iturriza-Gómara, M. I., El-Attar, L., and Gray, J. (2004) The zoonotic potential of rotavirus. *J. Infect.* **48,** 289–302.
89. Nakagomi, T. and Nakagomi, O. (2000) Human rotavirus HCR3 possesses a genomic RNA constellation indistinguishable from that of feline and canine rotaviruses. *Arch. Virol.* **145,** 2403–2409.
90. Iturriza-Gómara, M., Desselberger, U., and Gray, J. J. (2003) Molecular epidemiology of rotaviruses: genetic mechanisms associated with diversity. In: *Viral Gastroenteritis. Prespectives in Medical Virology* (Desselberger, U. and Gray, J. J., eds.), Elsevier, Amsterdam, pp. 317–344.
91. Guan, Y., Zheng, B. J., He, Y. Q., et al. (2003) Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. *Science* **302,** 276–278.
92. Gaulin, C., Frigon, M., Poirier, D., and Fournier, C. (1999) Transmission of calicivirus by a foodhandler in the pre-symptomatic phase of illness. *Epidemiol. Infect.* **123,** 475–478.
93. Lo, S. V., Connolly, A. M., Palmer, S. R., Wright, D., Thomas, P. D., and Joynson, D. (1994) The role of pre-symptomatic food handler in a common source of food-borne SRSV gastroenteritis in a group of hospitals. *Epidemiol. Infect.* **113,** 513–521.
94. Parashar, U. D., Dow, L., Fankhauser, R. L., et al. (1998). An outbreak of viral gastroenteritis associated with consumption of sandwiches: implications for the control of transmission by food handlers. *Epidemiol. Infect.* **121,** 615–621.
95. Patterson, T., Hutchings, P., and Palmer, S. (1993) Outbreak of SRSV gastroenteritis at an international conference traced to food handled by a post-symptomatic caterer. *Epidemiol. Infect.* **111,** 157–162.
96. Patterson, W., Haswell, P., Fryers, P. T., and Green, J. (1997) Outbreak of small round structured virus gastroenteritis arose after kitchen assistant vomited. *Comm. Dis. Rep.* **7,** R101–R103.
97. Desselberger, U., Iturriza-Gómara, M., and Gray, J. J. (2001) Rotavirus epidemiology and surveillance. In: Chadwick, D., Goode, J., eds. Novartis Foundation Symposium No. 238. In: Viral Gastroenteritis. Chichester: John Wiley & Sons,: 125–147.
98. Kuritsky, J. N., Osterholm, M. T., Greenberg, H. B., et al. (1984) Norwalk gastroenteritis: a community outbreak associated with bakery product consumption. *Ann. Int. Med.* **100,** 519–521.
99. Fleissner, M. L., Herrman, J. E., Booth, J. W., Blacklow, N. R., and Nowak, N. A. (1989) Role of Norwalk virus in two foodborne outbreaks of gastroenteritis: definitive virus association. *Am. J. Epidemiol.* **129,** 165–172.
100. Herwaldt, B. L., Lew, J. F., Moe, C. L., et al. (1994) Characterization of a variant strain of Norwalk virus from a food-borne outbreak of gastroenteritis on a cruise ship in Hawaii. *J. Clin. Microbiol.* **32,** 861–866.
101. Kilgore, P. E., Belay, E. D., Hamlin, D. M., et al. (1996) A university outbreak of gastroenteritis due to a small round structured virus: application of molecular diagnosis to identify the etiologic agent and patterns of transmission. *J. Infect. Dis.* **173,** 787–793.
102. Christensen, B. F., Lees, D. N., Henshilwood, K., Bjergskov, T., and Green, J. (1998) Human enteric viruses in oysters causing a large outbreak of human foodborne infection in 1996/97. *J. Shellfish Res.* **17,** 1633–1635.

103. Dowell, S. F., Groves, C., Kirkland, K. B., et al. (1995). A multistate outbreak of oyster-associated gastroenteritis: implications for interstate tracing of contaminated shellfish. *J. Infec. Dis.* **171,** 1497–1503.
104. McDonnell, S., Kirkland, K. B., Hlady, W. G., et al. (1997) Failure of cooking to prevent shellfish-associated viral gastroenteritis. *Arch. Int. Med.* **157,** 111–116.
105. Otsu, R. (1999) Outbreaks of gastroenteritis caused by SRSVs from 1987 to 1992 in Kyushu, Japan: four outbreaks associated with oyster consumption. *Eur. J. Epidemiol.* **15,** 175–180.
106. Simmons, G., Greening, G., Gao, W., and Campbell, D. (2001) Raw oyster consumption and outbreaks of viral gastroenteritis in New Zealand: evidence for risk to the public's health. *Aust. N Z J Public Health* **25,** 234–240.
107. Sugieda, M., Nakajima, K., and Nakajima, S. (1996) Outbreaks of Norwalk-like virus associated gastroenteritis traced to shellfish: coexistence of two genotypes in one specimen. *Epidemiol. Infect.* **116,** 339–346.
108. Oishi, I., Yamazaki, K., Kimoto, T., et al. (1994) A large outbreak of acute gastroenteritis associated with astrovirus among students and teachers in Osaka, Japan. *J. Infect. Dis.* **170,** 439–443.
109. Villena, C., Gabrieli, R., Pinto, R. M., et al. (2003) A large infantile gastroenteritis outbreak in Albania caused by multiple emerging rotavirus genotypes. *Epidemiol. Infect.* **131,** 1105–1110.
110. Yamashita, T., Sakae, K., Ishihara, Y., Isomura, S., and Utagawa, E. (1993) Prevalence of newly isolated, cytopathic small round virus (Aichi strain) in Japan. *J. Clin. Microbiol.* **31,** 2938–2943.
111. Michelangeli, F. and Ruiz, M. C. (2003) Physiology and pathophysiology of the gut in relation to viral diarrhoea. In: *Viral Gastroenteritis. Prespectives in Medical Virology* (Desselberger, U. and Gray, J. J., eds.), Elsevier, Ámsterdam, pp. 23–50.
112. Estes, M. K. (2003) The rotavirus NSP4 enterotoxin: current status and challenges. In: *Viral Gastroenteritis. Prespectives in Medical Virology*, (Desselberger, U. and Gray, J. J., eds.), Elsevier, Amsterdam, pp. 207–224.
113. Ball, J. M., Tian, P., Zeng, C. Q., Morris, A. P., and Estes, M. K. (1996) Age-dependent diarrhea induced by a rotaviral nonstructural glycoprotein. *Science* **272,** 101–104.
114. Tian, P., Hu, Y., Schilling, W. P., Lindsay, D. A, Eiden, J., and Estes, M. K. (1994) The nonstructural glycoprotein of rotavirus affects intracellular calcium levels. *J. Virol.* **68,** 251–257.
115. Tian, P., Estes, M. K., Hu, Y., Ball, J. M., Zeng, C. Q., and Schilling, W. P. (1995) The rotavirus nonstructural glycoprotein NSP4 mobilizes Ca^{2+} from the endoplasmic reticulum. *J. Virol.* **69,** 5763–5772.
116. Zhang, M., Zeng, C. Q., Morris, A. P, and Estes, M. K. (2000) A functional NSP4 enterotoxin peptide secreted from rotavirus-infected cells. *J. Virol.* **74,** 11,663–11,670.
117. Lundgren, O., Peregrin, A. T., Persson, K., Kordasti, S., Uhnoo, I., and Svensson, L. (2000) Role of the enteric nervous system in the fluid and electrolyte secretion of rotavirus diarrhea. *Science* **287,** 491–495.
118. Kaplan, J. E., Gary, G. W., Baron, R. C., et al. (1982) Epidemiology of Norwalk gastroenteritis and the role of Norwalk virus in outbreaks of acute nonbacterial gastroenteritis. *Ann. Int. Med.* **96,** 756–761.
119. Kaplan, J. E., Schonberger, L. B., Varano, G., Jackman, N., Bied, J., and Gary, G. W. (1982) An outbreak of acute nonbacterial gastroenteritis in a nursing home. Demonstration of person-to-person transmission by temporal clustering of cases. *Am. J. Epidemiol.* **116,** 940–948.
120. Rockx, B., De Wit, M., Vennema, H., et al. (2002) Natural history of human calicivirus infection: a prospective cohort study. *Clin. Infect. Dis.* **35,** 246–253.
121. Lynch, M., Lee, B., Azimi, P., et al. (2001) Rotavirus and central nervous system symptoms: cause or contaminant? Case reports and review. *Clin. Infect. Dis.* **33,** 932–938.

122. Iturriza-Gómara, M., Auchterlonie, I. A., Zaw, W., Molyneaux, P., Desselberger, U., and Gray, J. (2002) Rotavirus gastroenteritis and CNS infection: detection and characterisation of the VP7 and VP4 genes of rotavirus strains isolated from paired faecal and CSF samples from a child with CNS symptoms. *J. Clin. Microbiol.* **40,** 4797–4799.
123. Koopmans, M., Vennema, H., Heersma, H., et al. (2003). Early identification of common-source foodborne virus outbreaks in Europe. *Emerg. Infect. Dis.* **9,** 1136–1142.
124. Lopman, B., Van Duynhoven, Y., Hanon, F. X., Reacher, M., Koopmans, M., and Brown, D. W. (2002) Laboratory capability in Europe for foodborne viruses. *Euro. Surv.* **7,** 61–65.
125. Lindqvist, R., Andersson, Y., Lindback, J., et al. (2001) A one-year study of foodborne illnesses in the municipality of Uppsala, Sweden. *Emerg. Infect. Dis.* **7,** 588–592.
126. Mead, P. S, Slutsker, L., Dietz, V., et al. (1999) Food-related illness and death in the United States. *Emerg. Infect. Dis.* **5,** 607–625.
127. Muniain-Mujika, I., Calvo, M., Lucena, F., and Girones, R. (2003) Comparative analysis of viral pathogens and potential indicators in shellfish. Int J Food Microbiol. **83,**(1):75–85.
128. Gallimore, C. I., Green, J., Lewis, D., et al. (2004) Diversity of noroviruses cocirculating in the North of England from 1998 to 2001. *J. Clin. Microbiol.* **42,** 1396–1401.