Access the complete reference list online at
http://www.expertconsult.com

CHAPTER 62

Calicivirus Infections

Gagandeep Kang • Mary K. Estes • Robert L. Atmar

INTRODUCTION

Until the early 1970s, the cause of most gastroenteritis was unknown. Cases were attributed to the known infectious agents, mainly bacteria, and many other causes, including teething, weaning, diet, old age, drugs, and malnutrition. In 1972, immune electron microscopy of fecal specimens derived from an outbreak at a school in Norwalk, Ohio, USA, resulted in the identification of Norwalk virus, the first time a viral agent was shown to cause gastroenteritis.[1] Subsequently, electron microscopy of stools of young children in the United Kingdom and Japan identified viruses morphologically similar to animal caliciviruses.[2,3] Intensive investigation over the next three decades led to the identification of several viral agents of gastroenteritis and the development of new assays to identify the viral etiology of sporadic episodes and epidemics of gastroenteritis. In 1990, the Norwalk virus genome was cloned and, with the development of reverse transcription–polymerase chain reaction (RT-PCR), many genetically related but antigenically diverse viruses were identified and placed into groups called the "Norwalk-like viruses" and "Sapporo-like viruses."[4,5] In 2001, these groups were given the genus names of *Norovirus* and *Sapovirus*, respectively.

With the identification of new viral agents of gastroenteritis, it has become clear that viruses cause a significant proportion of the enteric illnesses that did not earlier have a defined etiology. Improvements in sanitation and hygiene and better standards of living resulted in a decrease in the proportion of diarrheal disease attributed to bacteria, and an increase in the proportion of cases associated with viral infections. Human caliciviruses are now acknowledged as one of the most frequent causes of gastroenteritis in developed countries and are being increasingly recognized in developing countries and/or tropical regions.[6]

THE AGENT

The name calicivirus is derived from the Latin *calyx*, meaning cup or goblet, and refers to the cup-shaped depressions visible by electron microscopy. These cup-like depressions are more prominent in sapoviruses, earlier called classic human caliciviruses. Viruses now classified as noroviruses often lack the distinct surface depressions and were previously called small, round-structured viruses (***Fig. 62.1***).[7,8] Caliciviruses (family *Caliciviridae*) are nonenveloped, icosahedral 27–40-nm viruses with one major capsid protein enclosing a single-stranded, positive-sense RNA genome. Currently, caliciviruses are classified into four recognized genera, *Norovirus*, *Sapovirus*, *Vesivirus,* and *Lagovirus,* and two other genera that contain bovine viruses (*Nebovirus*) and a rhesus macaque virus (provisionally *Recovirus)* (***Fig. 62.2***). The calicivirus genome is 7.5–7.7 kilobases and has two or three open reading frames (ORFs). In Norwalk virus, the first ORF encodes a nonstructural polyprotein that is processed in infected cells to generate several proteins, including the viral helicase, VPg, protease, and RNA-dependent RNA polymerase. The second ORF encodes VP1, the major capsid protein, and the third encodes VP2, a small, basic protein of unknown function.[9] In sapoviruses, the nonstructural polyprotein and major capsid protein are both encoded in ORF1 and the small basic protein encoded in ORF2.[10] Noroviruses can be genetically classified into five different genogroups (GI, GII, GIII, GIV, and GV), and further divided into different genetic groups or genotypes defined by a minimum amino acid sequence identity over the complete capsid sequence of 85% (***Fig. 62.2***).[11] Genogroup II, the most prevalent human genogroup, presently contains 19 genotypes. Genogroups I, II and IV infect humans, whilst genogroup III is associated with bovine infections and genogroup V infects mice.

A numeric classification system has been proposed based upon numbering genogroups with Roman numerals and genotypes with Arabic numbers.[11,12] For example, the genogroup II norovirus, Lordsdale virus, is a member of genotype 4, and therefore is classified as a GII.4 norovirus. GII.4 viruses currently account for the majority of outbreaks of adult gastroenteritis and the availability of sequencing and modeling techniques has resulted in a better understanding of the evolution and spread of this strain across the globe during the last decade.[13] Sapoviruses are also divided into five genogroups, four of which contain viruses detected in humans in 13 different genotypes.[14] Noroviruses have been found in dogs, lions, and pigs.[15,16] These strains have not been found in human infections, although human strains can infect cows and pigs.[17,18] Serologic data suggest that zoonotic transmission may occur in individuals who have close or prolonged contact with animals.[19]

An understanding of the mechanisms of replication has been limited by the inability to grow these strains in culture. Following the cloning of Norwalk virus in 1990, it became possible to produce virus-like particles (VLPs) by expression of the capsid protein in insect cells. The expressed capsid proteins self-assemble into VLPs with morphologic and antigenic properties similar to native viruses. This resulted in an increased understanding of structure, antigenicity, and, more recently, host susceptibility factors to infection, with the VLPs being used as antigens for detection of antibodies, to generate specific antisera, and for binding assays to identify receptors.[20–24]

EPIDEMIOLOGY AND THE DISEASE

Clinical disease due to the noroviruses has an average incubation period of 24–48 hours and is characterized by acute onset of nausea, vomiting, abdominal cramps, myalgias, and nonbloody diarrhea. Illness usually resolves in 2–3 days, but can be longer – up to 4–6 days in hospital outbreaks and in young children. Vomiting is relatively less prevalent in infants than in older children and is a characteristic feature of gastroenteritis outbreaks in adults.[25,26] Fever is reported in 30–40% of patients and resolves within 24 hours.[25,27,28] Deaths have been reported in the elderly during outbreaks in nursing homes and noroviruses have recently been reported in necrotizing enterocolitis in neonates.[29,30]

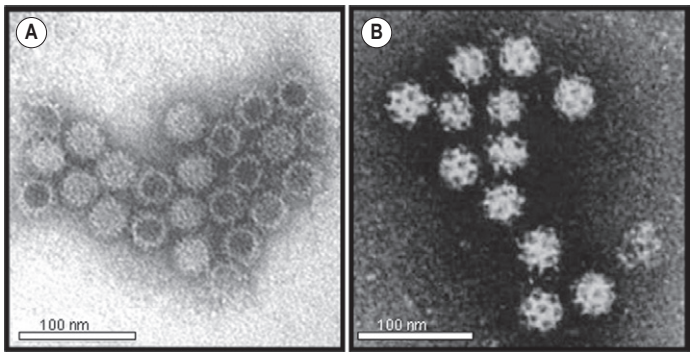

Figure 62.1 Electron micrographs of human caliciviruses. (**A**) Norwalk virus particles from the stool of an ill volunteer. (Reproduced from Guix S, Asanaka M, Katayama K, et al. Norwalk virus RNA is infectious in mammalian cells. J Virol 2007;81:12238–12248.) (**B**) A sapovirus from the stool of a child with gastroenteritis. (Reproduced from Nakata S, Estes MK, Chiba S. Detection of human calicivirus antigen and antibody by enzyme-linked immunosorbent assays. J Clin Microbiol 1988;26:2001–2005.)

Noroviruses are highly infectious and are transmitted primarily through the fecal-oral route, either by consumption of contaminated food or water, or by direct person-to-person spread. Aerosolization of vomitus has also been shown to be a mode of transmission.[31] The ability of the virus to survive in the environment suggests that environmental contamination and fomites are an important source of infection.[32,33] The four distinct patterns of viral gastroenteritis – endemic childhood diarrhea, outbreaks in closed communities, other food- or water-borne outbreaks among wider communities, and viral gastroenteritis in immunocompromised patients – are all seen in caliciviral infections.

In developed countries, diarrhea is mainly seen in children less than 5 years of age, but the peak incidence of diarrhea in developing countries occurs in children less than 2 years of age. In most studies from developed countries, noroviruses are the second most common cause of gastroenteritis in children, following rotaviruses, and, increasingly, studies from tropical countries are reporting patterns similar to those seen in developed countries.[6] There are few community-based studies, but they indicate that in developed countries almost a quarter of all episodes in young children may be due to noroviruses.[34] A summary of 21 studies published in the last 10 years from tropical countries, which identified noroviruses mainly in children hospitalized with dehydrating gastroenteritis, shows a mean prevalence of 11.7% (*Table 62.1*).[35–55]

Studies over the last 30 years, mainly in developed countries, have shown that noroviruses cause large outbreaks of acute gastroenteritis in many settings. Attack rates in outbreaks can be over 50%, and there is often substantial secondary spread. Outbreaks are common in winter, but can occur year-round in closed and semiclosed settings such as nursing homes, hospitals, hotels, and cruise ships.[56] Cruise ship outbreaks can recur even after thorough cleaning and changes of passengers, indicating the ability of the virus to survive in the environment. Nosocomial outbreaks are a particular concern in Europe, where they have led to the closure of wards and significant economic impact, but there are limited data on noroviruses in health care settings in developing countries.[57] Transmission of viruses in such settings can occur from person to person or through fomites, emphasizing the need for hygiene and good infection control practices to limit spread of infections.

Most other outbreaks are believed to be linked to contaminated food and water. A report from the Centers for Disease Control and Prevention (CDC) showed that 39% of 264 confirmed norovirus outbreaks were associated with restaurants or catered events.[58] Foods implicated in outbreaks of norovirus gastroenteritis are usually those contaminated either directly with fecal matter at the source, such as shellfish harvested from sewage-contaminated waters or fresh produce irrigated with sewage or fecally contaminated water, or by infectious food handlers. Shellfish concentrate viruses through filtration and, although depuration reduces fecal coliform contamination, it does not remove noroviruses. In outbreaks identified to be associated with fresh produce, noroviruses were responsible for about 25% of the outbreaks. Most foodborne outbreaks where food handlers are a source of transmission result from foods that require handling, but not cooking, such as salads and sandwiches. Asymptomatic infections and prolonged shedding for several weeks after infection are risk factors for virus transmission if individuals do not maintain high levels of hygiene.[59,60]

Waterborne outbreaks of norovirus gastroenteritis in developed countries have been associated with different forms of recreational water[61] and contamination of drinking water.[62] Although few studies have reported outbreaks in developing countries, it is likely that, given lower standards of hygiene and poor quality of drinking water, such transmission does occur. Although a rapid and accurate diagnostic assay is not widely available for diagnosing norovirus disease, Kaplan et al proposed the presence of four epidemiologic features for confirmation of norovirus as a cause of outbreaks: (1) vomiting in 50% or more of affected persons; (2) incubation period of 24–48 hours; (3) symptoms for 12–60 hours; and (4) absence of bacterial pathogens on culture, which have recently been validated as being sensitive and specific.[26,63]

The few studies that have looked at norovirus infections among immunocompromised individuals have reported both symptomatic and asymptomatic infections in transplant receipients and some increase in viral shedding in persons infected with human immunodeficiency virus.[64–66] Infection in highly immunocompromised transplant recipients can lead to symptomatic diarrhea lasting months to years.[67,68]

Strain characterization studies have suggested that there are differences in the strains detected in outbreaks and in sporadic cases, and some differences in geographic distribution. Analyses of norovirus detected in outbreaks and sporadic cases suggest that infections with GII strains are severalfold more common than those of GI strains. GII.4 norovirus strains have become the predominant cause of epidemic disease in the past decade.[69] Norovirus evolution may be driven by immune selection pressure, with the exposed viral capsid protein that binds carbohydrates in the human gut evolving because of the antigenic drift in the receptor-binding regions of the P2 subdomain.[70]

Seroprevalence studies using recombinant antigens or VLPs of genogroups I and II have been used to assess exposure to infection and have shown that exposure is common, even in isolated populations.[71] However, given the lack of longitudinal data on the persistence of antibodies and the variability in host susceptibility to different viruses, it is difficult to draw meaningful conclusions based on serosurveys in populations that are not well characterized.

PATHOGENESIS AND IMMUNITY

Other than a murine norovirus, noroviruses have not been grown in culture, making studies of pathogenetic mechanisms difficult. In studies carried out on volunteers, infection by Norwalk virus requires a low infectious dose and produces blunting and broadening of the intestinal villi, vacuolation of the villus epithelial cells, and mononuclear cell infiltration into the lamina propria of the proximal small intestine.[72,73] The small intestinal mucosa remains intact and lesions have not been found in biopsies of the colonic and gastric mucosa, suggesting that viral replication may be limited to the upper small intestine.[74] The cause of diarrhea is unknown, although several mechanisms have been proposed, including reduced absorptive capacity of the disrupted epithelium, proliferation of the secretory crypt cells, and reduced expression of certain digestive enzymes, resulting in an osmotic diarrhea. The nausea and vomiting have been attributed to delayed gastric emptying and altered gut motility, although there is no direct evidence to support these proposed mechanisms.

Early volunteer studies showed that infected volunteers developed a short-lived (6–14 weeks) immunity after a Norwalk virus challenge.[75] Symptomatic volunteers could be reinfected with the same virus when challenged 2–3 years later.[75] A second group of volunteers could not be

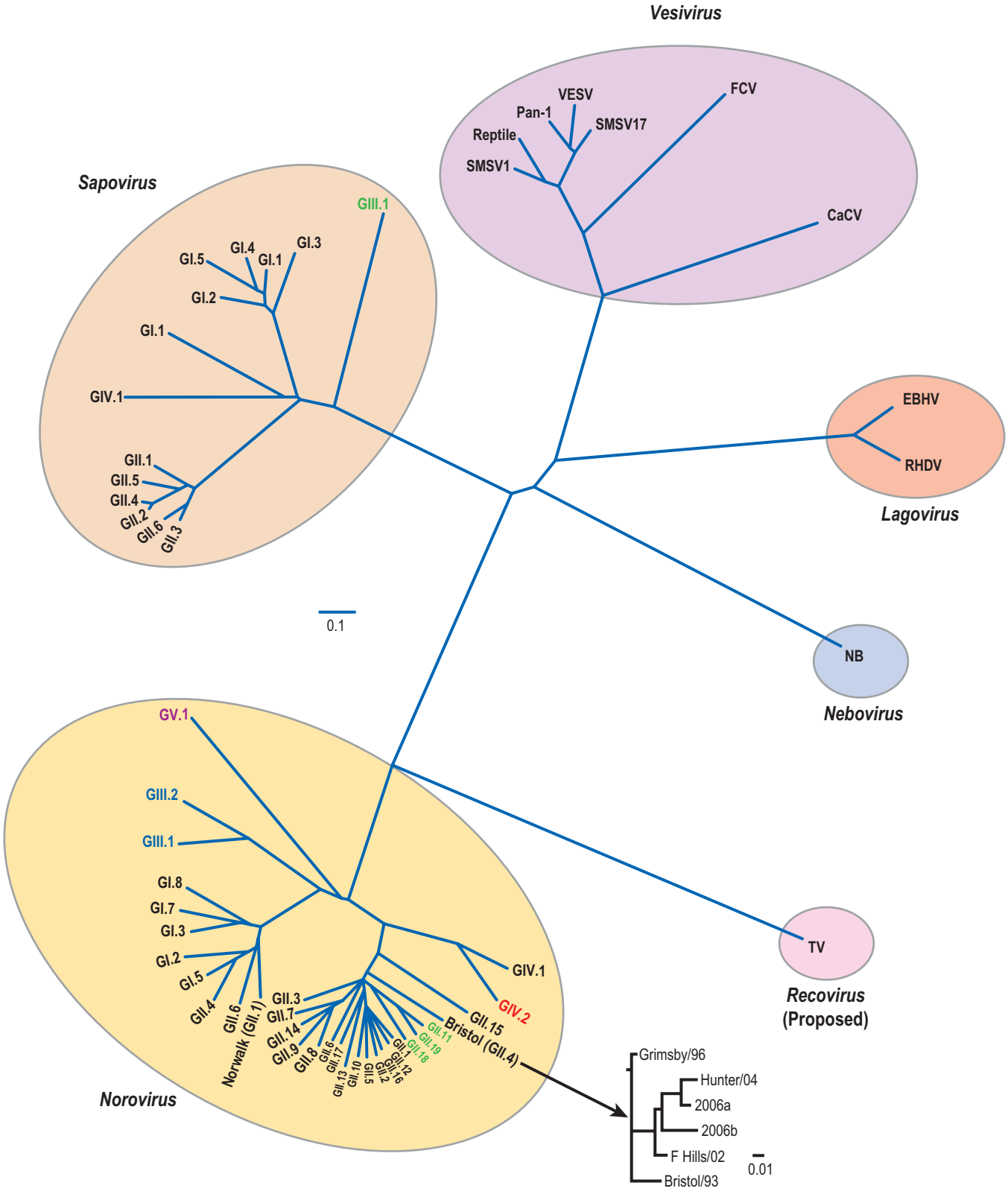

Figure 62.2 Phylogenetic analysis of caliciviruses. A multiple alignment of 57 calicivirus VP1 amino acid capsid sequences was performed using Clustalw (http://www.ebi.ac.uk/Tools/clustalw2/index/html) and the phylogenetic analyses were performed with programs in the PHYLIP v3.6 package. The scale bar represents the unit for expected number of substitutions per site. Within the *Norovirus* and *Sapovirus* genera, human prototype viruses are listed in black, porcine viruses are shown in green, bovine viruses are shown in blue, a murine virus is shown in purple, and a lion virus is shown in red.

Table 62.1 Noroviral Gastroenteritis in Tropical Countries. Studies Published between 2000 and 2009, which Tested >100 Patients Admitted with Dehydrating Gastroenteritis Were Included

Site	*n*	Age in Years	Positive *Norovirus*	Predominant Genogroups	Reference
Dhaka, Bangladesh	917	Children	4.5%	GII.4	35
Delhi, India	226	<5	15.9%	GII	36
Pune, Nagpur, Aurangabad, India	830	<7	6.3–12.6%	GII.4, recombinants	37
Pune, India	236	<5	11.9%	GII.4, GIIb	38
Vellore, India	350	<5	15.1%	GII.1	39
Karachi, Pakistan	517	Children	9.9%	GI, GII	40
Indonesia	218	Children	21%	GII	41
China	1110	<5	10.3%	GII.4	42
Hong Kong, China	995	All ages	16.7%	GII	43
Chiang Mai, Thailand	296	<5	8.1%	GII.4	44
Chiang Mai, Thailand	248	<5	14.1%	GII.4	45
Ho Chi Minh City, Vietnam	502	Children	6.4%	GII	46
Cairo, Egypt	230	<1.5	26%	GII.4	47
Tamale, Ghana	367	<11	7.3%	GII	48
Blantyre, Malawi	398	Children	6.5%	GII.3	49
Leon, Nicaragua	542	<5	12%	GII.4	50
Recife, Brazil	233	Children	15%	ND	51
Rio de Janeiro, Brazil	289	Children	14.5%	GII, GI	52
Rio de Janeiro, Brazil	318	<5	20%	GII.4, GII	53
Goinia, Brasilia, Brazil	1006	Children	8.6%	ND	54
São Paulo, Brazil	234	Children	33.3%	GII.4	55

infected initially or on repeated challenge years later. Recent research has shown that host factors are an important determinant of susceptibility norovirus infections in that Norwalk virus infection depends on the presence of specific human histo-blood group antigen (HBGA) receptors in the gut.[76] Further investigations of the binding of different noroviruses have found that H type 1 and Lewis^b carbohydrate antigens bind strongly to Norwalk virus VLPs.[77] Persons who express a functional α(1,2)-fucosyltransferase-2 (FUT2), which is necessary to make H type 1 and Lewis^b antigens, are called secretors, and secretors can be infected with Norwalk virus. Individuals who are homozygous recessives for the FUT2 gene and do not express H type-1 oligosaccharide (nonsecretors) are resistant to infection with Norwalk virus.[77,78] Norovirus strains belonging to different genotypes may have specific binding properties to different blood group antigens.[79–81] The combination of the strain-specific binding, the variable expression of the HBGA receptors, and acquired immunity may explain the differences in host susceptibility observed in outbreaks and in volunteer studies.[82]

DIAGNOSIS

Electron microscopy was initially used for identification and continues to be used by some laboratories to screen stools for potential viral pathogens, despite the lack of sensitivity.[5] Early antigen detection assays used reagents derived from human volunteers, but had low sensitivity.[83] Recently, commercial stool enzyme immunoassay detection methods mainly based on monoclonal antibodies have been developed and are available in Europe, and they appear to be useful in outbreaks when the outbreak strain is within the range included in the assay.[84] Serologic assays also have been developed using recombinant-expressed norovirus capsid proteins to detect immune responses to infecting norovirus strains, but are used more in epidemiological studies than for diagnosis in individual patients.[85,86]

Currently, RT-PCR assays are the most common approach for establishing a diagnosis of norovirus infection. Virus-specific primers are used to amplify conserved regions of the genome, usually in the polymerase or capsid genes.[5] No single primer pair can detect all norovirus or sapovirus strains because of the high sequence diversity, but, in most geographic regions, more than 90% of currently circulating strains can be detected using separate primer pairs for genogroup I and II noroviruses and sapoviruses. Highly sensitive assays based on real-time RT-PCR have been developed and evaluated for both noroviruses and sapoviruses, and these assays may be useful both for diagnosis and to study patterns of viral shedding.[87–89]

TREATMENT AND PROGNOSIS

Most viral gastroenteritis is self-limiting and no specific therapy is available or required. However, in children, diarrhea, especially with vomiting, can result rapidly in dehydration, which should be corrected immediately. Oral rehydration solutions providing essential electrolyte replacement are given as first-line therapy. Patients with significant dehydration and those unable to tolerate oral fluids require intravenous rehydration. There is no limitation on food intake. In experimentally infected adults, oral administration of bismuth subsalicylate reduced abdominal cramps and gastrointestinal symptoms;[90] but bismuth compounds and antimotility agents, such as diphenoxylate or loperamide, should be avoided in children.[91]

PREVENTION AND CONTROL

Outbreaks

The prevention of outbreaks of viral gastroenteritis relies on the control of contamination of food and water, maintenance of strict hygiene by food handlers, and reduction of secondary transmission through person-to-person spread. Measures to avoid contamination of waters in oyster-harvesting areas are necessary to prevent shellfish-associated outbreaks. Monitoring of food and water for noroviruses is not yet available routinely, although methods have been developed to detect noroviruses directly from food and water.[87,92]

Strict personal hygiene and the proper disinfection of environmental surfaces are critical to prevent food handler-associated transmission. Food

handlers should be excluded from work for 2–3 days after recovery from norovirus illness and perhaps be reassigned to jobs not directly involving contact with food.[93] Similarly, in the care of the sick individuals at home or in hospitals, maintenance of strict hygiene, avoidance of contact with, and appropriate disinfection of, environmental surfaces contaminated with vomitus and feces are required.[83] In situations in which the epidemic is extended by periodic renewal of the susceptible population, such as hospitals, camps, and cruise ships, the facility or institution may have to be closed to interrupt transmission.[94]

Sporadic Disease

Standard hygienic precautions, including frequent hand-washing, careful disposal of waste and thorough cleaning, should help prevent calicivirus infection in all settings. In the absence of a cell culture system for human caliciviruses, it has not been possible to study disinfectants directly, but use of chlorine solutions at a concentration of 1000 parts per million on hard surfaces is recommended in the United Kingdom.[95]

Vaccine Development

With increasing recognition of the widespread incidence and clinical importance of noroviruses, the need for specific prevention strategies, such as vaccines, is becoming apparent. Recombinant VLPs expressed either in baculovirus or in transgenic plants have been shown to be safe and immunogenic when given orally to volunteers.[96,97] These vaccines could be used in settings where it is important to have at least short-term immunity to noroviral gastroenteritis, such as the military, travelers, and the elderly in hospitals or nursing homes. However, the relative roles of mucosal, systemic, and cell-mediated immunity are not understood. Recent reports indicate that noroviruses may not be limited to the intestine, in which case it may be necessary to have both systemic and mucosal responses in order to induce protection.[98,99] An incomplete understanding of the immune correlates of protection, lack of persistent long-term and cross-protective immunity, and the existence of multiple genetic and antigenic types of virus all present challenges to the development of vaccines directed at inducing protection against disease caused by these important agents of viral gastroenteritis.

Access the complete reference list online at
http://www.expertconsult.com