Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Chapter 151

Arturo S Gastañaduy
Rodolfo E Bégué

Acute gastroenteritis viruses

Acute gastroenteritis is a major cause of morbidity and mortality worldwide, most commonly affecting children both in developing and developed countries. Its importance was clearly defined in the 1980s and 1990s (Table 151.1).[1–4] Recent studies indicate that it continues to be a significant burden for human health.[5,6] While fewer than 5% of stool samples in patients with diarrhea show a bacterial or parasite pathogen,[7] recent technology allows for identifying an increasing number of viral etiologies.

APPROACH TO MANAGEMENT

No specific antiviral exists for any of the gastroenteritis viruses. Primary objectives of management are prevention and treatment of dehydration and malnutrition.[8] Most patients will improve with the administration of oral rehydration solutions (ORS) and continuous feeding with their regular diet. Rarely, intravenous fluids may be needed for severe dehydration, intractable vomiting or very high stool output. Although lactose malabsorption develops frequently during acute gastroenteritis, most children can be fed their usual lactose-containing formulas in small frequent feeds. Human milk has high lactose content, yet it reduces stool output and provides excellent nutrients and anti-infectious factors; therefore, breast-feeding should be continued. Moderate to severe malnourished children with diarrhea present a special problem. Malnutrition causes total body sodium excess, potassium and micronutrient deficiencies and increased energy requirements. ReSoMal is recommended for these patients. It is a specially formulated ORS that provides less sodium and more potassium and glucose. Magnesium, zinc and copper are added to ReSoMal.[9]

APPROACH TO PREVENTION

Viruses that cause gastroenteritis are transmitted mainly by the fecal–oral route, through direct person-to-person contact or contaminated food, water and fomites. Aerosol transmission from respiratory secretions or vomit occurs rarely. Hygienic measures decrease person-to-person spread. However, because these measures are difficult to enforce, particularly in young children, and because some viruses are resistant to commonly used disinfectants, transmission of the disease continues even in developed countries. Outbreaks require isolating ill personnel, thoroughly cooking food, disinfecting the environment and proper handling of food, water and sanitation. Careful hand washing cannot be overemphasized, especially among food handlers and personnel from hospitals, schools and day-care centers. Hospitalized or other institutionalized patients should have universal precautions and contact isolation until 48–72 hours after symptom resolution. The best preventive method will be effective, safe and affordable vaccines. Currently, such a vaccine is commercially available only for rotavirus.[10]

APPROACH TO DIAGNOSIS

An etiologic diagnosis is not necessary for the management of most cases of acute gastroenteritis. For viral gastroenteritis, an etiologic diagnosis may be desired in specific clinical circumstances or for epidemiologic and research purposes. Detection of viral antigens by immunoassays (IAs) is the method of choice. IAs are simple and rapid, have good sensitivity and specificity, and are practical

Table 151.1 Morbidity and mortality rates of diarrheal diseases in children*

	DEVELOPING COUNTRIES			USA–CANADA
	Snyder & Merson (1982)[1]	**Claeson & Merson (1990)[2]**	**Bern *et al.* (1992)[3]**	**Glass *et al.* (1991)[4]**
No. of studies evaluated	24	276	22	4
Diarrheal episodes/year (millions)	1000	1500	1000	21–37
Episodes/child/year (median)	2.2–3.0	3.3	2.6	1.3–2.5
No. of diarrheal deaths/year	4.6 million	4.0 million	3.3 million	325–425

*Estimates from longitudinal, prospective, community-based studies in developing and developed countries.

for testing large numbers of samples. IAs are commercially available for rotaviruses, adenoviruses and astroviruses. Reverse transcriptase polymerase chain reaction (RT-PCR) techniques have been developed for many gastroenteritis viruses. Because they are more sensitive than IAs, they allow viral detection not only in clinical specimens (stools, vomitus) but also in contaminated food, water and fomites. These techniques are most useful for caliciviruses. Electron microscopy (EM) and immune electron microscopy (IEM) identify viruses of distinct morphology and excreted in large numbers (>10^6 virions/ml of stools), such as rotavirus or astrovirus. However, these techniques are not suitable for a large number of samples or routine laboratory diagnosis. Viral culture is essential for virus characterization, the study of their pathogenesis and the production of diagnostic reagents but is slow and cumbersome. Not all gastroenteritis viruses can be cultured.

VIRAL AGENTS OF GASTROENTERITIS

The morphologic, epidemiologic and clinical characteristics of the main gastroenteritis viruses are presented in Tables 151.2–151.4. Most cases of acute gastroenteritis worldwide are caused by caliciviruses in all age groups and rotaviruses in children; all the other viruses are responsible for approximately 10% of cases.

Caliciviruses (Norovirus, Sapovirus)

In 1972, Kapikian *et al.*, investigating a gastroenteritis outbreak in Norwalk, Ohio, visualized 27 nm particles in a stool filtrate and noted that infected individuals developed a specific antibody response against the particles. This was the first confirmation of a virus as an etiologic agent of gastroenteritis.[11] The virus was named Norwalk virus (NV) and was believed to belong to the Picornaviridae family. Similar viruses were named by the location where they were first identified (Hawaii, Sapporo, etc.) and, as a group, were known as Norwalk-like viruses or small round structured viruses (SRSVs). Soon, it became evident that the SRSVs were different from the picornaviruses in structure, mode of replication and other characteristics. Cloning of the NV genome[12] led to the classification of these viruses within the Caliciviridae family.

NATURE

Caliciviruses are small (27–40 nm), nonenveloped viruses with a single-stranded, positive-sense RNA genome.[13] They have an icosahedral capsid with cup-like depressions on the viral surface (*calici* is derived from the Latin word *calyx*, which means cup) (Fig. 151.1).

Four genera have been identified in the Caliciviridae family: Lagovirus, Vesivirus, Norovirus (NoV) and Sapovirus (SaV). Lagovirus and Vesivirus only infect animals. Norovirus and Sapovirus have human and animal strains and are further classified into genogroups and genetic clusters (Table 151.5).

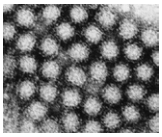
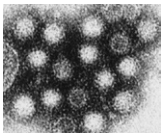
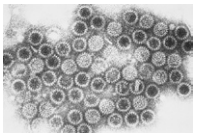
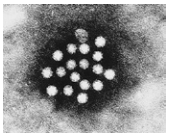
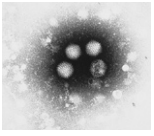
The calicivirus genomes are about 7.3–8.5 kb in length and organized in two or three open reading frames (ORFs). In the genera with three ORFs, Norovirus and Vesivirus, the first ORF (ORF1) encodes for a large protein that by proteolytic cleavage produces the nonstructural proteins. ORF2 encodes for the single major structural protein (viral protein 1, VP1) and ORF3 encodes for a minor structural protein (viral protein 2, VP2). Sapovirus and Lagovirus have only two ORFs. In these viruses, VP1 and VP2 are encoded in ORF1 and ORF2, respectively.[14]

The molecular weight of VP1 is approximately 60 kDa. Ninety VP1 dimers form the capsid with 90 arch-like protruding capsomers arranged in such a way as to leave 32 calices on the viral surface. VP1 has two major domains: the shell (S) domain and the protruding (P) domain. The S domain forms the inner part of the capsid. It has 225 amino acids (aa), including aa 10–49 corresponding to the *N*-terminal region of the protein (N subdomain), which faces the interior of the capsid. The P domain includes amino acids 226–520 and is subdivided into subdomains P1 and P2. The P1 subdomain (aa 226–278 and 406–520) forms the sides of the capsomers, whereas the P2 subdomain (aa 279–405) forms the most protruding part of the capsomer arches (see Fig. 151.1). VP1 self-assembles into virus-like particles (VLPs) without RNA or VP2 participation. The VLPs are morphologically and antigenically similar to natural virions.[15]

Each virion has only one or two copies of VP2 (12–29 kDa). The function of VP2 is unknown; however, in feline caliciviruses, intact VP2 is needed to produce infection. A third structural protein, VPg, is also present as one or two copies per virion. It is linked to the genomic or subgenomic RNA and may function as a nonstructural protein during replication.[14]

The caliciviruses have seven nonstructural proteins (NSP1–NSP7). The function of some of them has been inferred based on similar sequences in picornavirus proteins. NSP3 is a nucleoside triphosphatase,

Table 151.2 Structure and morphological characteristics of gastroenteritis viruses

Characteristics	Norovirus	Sapovirus	Rotavirus	Astrovirus	Enteric adenovirus
Family	*Caliciviridae*	*Caliciviridae*	*Reoviridae*	*Astroviridae*	*Adenoviridae*
Virion size (nm)	27–35	27–40	70–75	41	70–80
Envelop	Non-enveloped	Non-enveloped	Non-enveloped	Non-enveloped	Non-enveloped
Capsid	Icosahedral	Icosahedral	Triple shelled	Icosahedral	Icosahedral
Genome type	Positive sense ssRNA	Positive sense ssRNA	Segmented dsRNA	Positive sense ssRNA	dsDNA
Morphology on electron microscopy	Round surface, cup-shaped indentations	Round surface, cup-shaped indentations	Wheel-like capsid with radiating spokes	Round, 28–30 nm, 5–6-pointed star shape	Fiber-like projections from vertices
Electron micrograph					

ds, double-stranded; ss, single-stranded.
Norovirus and sapovirus electron micrographs courtesy of C Humphrey (CDC); rotavirus, astrovirus and enteric adenovirus electron micrographs courtesy of S Spangenberger.

Table 151.3 Epidemiological characteristics of gastroenteritis viruses

Characteristic	Norovirus	Sapovirus	Rotavirus	Astrovirus	Enteric adenovirus
Age group	All ages	Children	6–24 months	<7 years, elderly	<4 years
Seasonality	No	No	Winter	Winter	Summer
Disease pattern	Outbreaks, endemic	Endemic, outbreaks	Endemic, annual epidemics	Endemic, nosocomial outbreaks	Endemic
Transmission	Person-to-person, water, food, shellfish	Person-to-person, water, cold foods, shellfish	Person-to-person, food, water	Person-to- person, food, water	Person-to-person
Fecal excretion (days)	7–13	–	10	–	Persistent, months
Outpatient prevalence (%)	Endemic: 10–25 Outbreaks: 90	1–10	5–10	7–8	4–8
Inpatient prevalence (%)	Rare	3–5	35–40	3–5	5–20

Table 151.4 Usual clinical characteristics of gastroenteritis virus infections

Signs and symptoms	Norovirus	Sapovirus	Rotavirus	Astrovirus	Enteric adenovirus
Prodrome (days)	1–2	1–3	1–3	3–4	8–10
Diarrhea: watery	66–95% 4–8/day Adults >children	88–95% Mild	96–100% 10–20/day	72–100% 2–4/day	97% 1/3 >14 days
Vomitus	57–95% Children >adults	44–65%	80–90% Early	20–50% 1/day	79% Early
Fever	24–48% Low grade	18–34%	60–65% Moderate	20% Low grade	Occasionally Low grade
Abdominal pain	11–91% Cramps	–	Colicky	50%	–
Dehydration	~1%	Infrequent	Frequent in young children	Infrequent	Infrequent
Other symptoms	Myalgia 26%, headache 22%	Respiratory 22%, myalgia, headache	Respiratory 22–52%	Malaise, respiratory	Respiratory occasionally
Duration of illness (days)	0.5–2.5	4	3–8	2–3	5–12

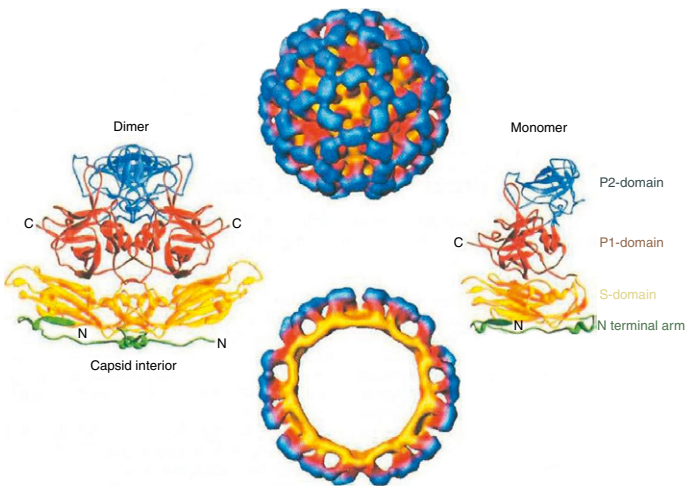


Fig. 151.1 Norwalk virus-like particle. Adapted from Hutson *et al*. Trends Microbiol 2004;12:279–87, with permission.

NSP6 is a proteinase and NSP7 is an RNA-dependent RNA polymerase. While the function of the other NSPs is unknown, they may play a role in the replication process.[14]

EPIDEMIOLOGY

Human caliciviruses (HuCV) have worldwide distribution. Noroviruses infect persons of all ages, both in developed and developing countries. They are the most common cause of gastroenteritis outbreaks and are also recognized as the most common cause of viral gastroenteritis in community-based studies.[16] Studies show an increasing prevalence of specific antibodies since infancy, reaching 80–90% in young adults.[17] The prevalence of specific antibodies rises faster in developing countries, indicating early infections.[18] Genogroups I and II produce the majority of community infections. Most infections are caused by a single circulating strain. For example, Genogroup II.4 became the most common cause of outbreaks around the world in the 1990s. Co-infections occur, giving the opportunity for the exchange of genetic material between strains and the generation of new virus strains.[19] Outbreaks occur mainly in

Table 151.5 Taxonomy of the Caliciviridae family

Genus	Genogroups	Genetic clusters	Representative species	Representative strain*
Norovirus (Nov)	I II III IV V	1–8 1–19 1–2 1 1	Norwalk virus Hawaii norovirus Bovine norovirus Alphatron virus Murine norovirus	Hu/NoV/GI.1/Norwalk/1968/US Hu/Nov/GII.1/Hawaii/1971/US Bo/NoV/GIII.1/Jena/1980/DE Hu/Nov/GIV.1/Alphatron98-2/1998/NL M/NoV/GV.1/MNV-1/2003/US
Sapovirus (SaV)	I II III IV V	1–3 1–3 1 1 1	Sapporo virus London sapovirus Swine sapovirus Houston sapovirus Argentina sapovirus	Hu/SaV/G1.1/Sapporo/1982/JP Hu/SaV/GII.1/London/1992/UK Sw/SaV/GIII.1/PEC-Cowden/1980/US Hu/SaV/GIV.1/Hou7-1181/1990/US Hu/Sav/GV.1/Argentina 39/AR
Lagovirus (LaV)			Rabbit hemorrhagic disease virus European brown hare syndrome virus	Ra/LaV/RHDV/GH/1988/DE Ha/LaV/EBHSV/GD/1989/FR
Vesivirus (VeV)			Vesicular exanthema of swine virus Feline calicivirus	SW/VeV/VESV/A48/1948/US Fe/VeV/FCV/F9/1958/US

Host species abbreviations: Hu, human; Bo, bovine; M, murine; Sw, swine; Ra, rabbit; Ha, hare; Fe, feline. **Country abbreviations**: US, United States; DE, Germany; NL, Netherlands; JP, Japan; UK, United Kingdom; AR, Argentina; FR, France.
*Host species/genus/species or genogroup/strain name/year of occurrence/country of origin.
Adapted with permission from Green *et al.*[13]

day-care centers, schools, colleges, hospitals, nursing homes, military personnel, restaurants, vacation facilities and cruise ships. Noroviruses also cause travelers' diarrhea. They were the only pathogens isolated from Hurricane Katrina evacuees with gastroenteritis in a large shelter in Houston, Texas.[20] Noroviruses can spread internationally through contaminated food or beverages.[21] A study of 8271 food-borne outbreaks of gastroenteritis reported to the US Centers for Disease Control and Prevention (CDC) shows a median of affected persons of 25 vs 10 for the bacterial outbreaks. Ten percent of the individuals required medical care and 1% were hospitalized.[22]

Caliciviruses are ubiquitous and stable in the environment, providing a persistent source of infection. Noroviruses survive freezing, heating to 140°F (60°C) for 30 minutes and are stable in water chlorinated to 6.25 mg/l; most municipal water systems contain <5 mg/l of chlorine. The virus is also acid-resistant and ether-stable.[23,24]

PATHOGENICITY

Caliciviruses are transmitted mainly by the fecal–oral route, but airborne transmission has also been reported.[25,26] The infectious dose has been estimated at less than 10 virions.[16] The incubation period ranges from 10 to 51 hours with a mean of 24 hours.[23,27] Norovirus infection mainly affects the proximal portions of the small intestine with broadening and blunting of the villi, crypt cell hyperplasia, cytoplasm vacuolization and mononuclear cell infiltration in the lamina propria. There is malabsorption of D-xylose, lactose and fat, and a decrease in brush border enzymes.[28,29] Pathologic changes are transient and resolve within 2 weeks. No histologic lesions are seen in the gastric or rectal mucosa, and the secretion of hydrochloric acid, pepsin and intrinsic factor remain normal.[30] Gastric emptying is markedly delayed, which could explain the frequent occurrence of nausea and vomiting; however, the degree of delay does not correlate with the severity of vomiting.[31] Virus excretion in the stool may begin during the prodrome stage,[32] last 7–13 days,[33] and persist for several weeks after resolution of symptoms, especially in infants,[34] and for months to years in immunocompromised patients.[35]

The cell and tissue tropism of HuCV are largely unknown. Calicivirus replication may be similar to that of other positive-strand RNA viruses. Following attachment to specific receptors, the virus enters the cell and is uncoated, and ORF1 produces the NSPs. Synthesis of negative-strand RNA begins. This serves as a template for positive-strand genomic and subgenomic RNA, which will serve as a template for the synthesis of VP1 and VP2. It is known that VP1 can self-assemble to form the capsid, but the process of packing of the viral RNA within the capsid, maturation and release of the virions is poorly understood.[14] It is assumed that replication occurs in the epithelial cells of the upper gastrointestinal tract; however, no viral particles have been observed by EM of the jejunal mucosa. While there is no animal model for the human caliciviruses, it has been shown that radiolabeled Norwalk VLPs bind and, in some cases, enter various cell lines.[36] The development of a human cell line for norovirus and a murine norovirus model will advance the study of norovirus pathogenesis.[37,38]

Susceptibility to NV infection is peculiar. Some individuals have natural resistance to the infection. They lack virus receptors; repeated challenges with the virus fail to produce illness or antibody response. The virus receptors are histo-blood group antigens (HBGAs). These are membrane carbohydrates present on the surface of red blood cells, enterocytes and other mucosal cells. The carbohydrates have an α1,2-linked fucose in common, e.g. Fucoα2Galβ3GlcNac. The protruding domain of VP1 of NV VLPs attaches to this trisaccharide or similar molecules, allowing binding and subsequent internalization of the VLPs. The receptors are host and viral-strain specific. Receptor production is genetically controlled by alleles at the ABO, FUT2 and FUT3 loci. Individuals with O blood group are more easily infected, while B blood group individuals have decreased risk. Individuals with the FUT2 –/– genotype are highly resistant to NV infections and represent about 20% of the European population.[39,40]

Acquired immunity is mostly short term and type specific. Challenges with the virus produce illness and specific antibody response. Although re-exposure to the same virus strain within 6–14 weeks does not result in illness, a re-challenge 27–42 weeks later results in disease and serum antibody response.[23,41,42] Serum or local jejunal antibodies do not correlate with resistance in volunteer studies.[33,43,44]

On the other hand, a rapid mucosal IgA response, associated with resistance to illness, has been shown in volunteers.[40] Cell-mediated immune responses have been observed in volunteers immunized with Norwalk VLPs.

PREVENTION

To date, no commercially available vaccine exists against HuCV. Vaccine development has been hampered by the following factors:

- partial understanding of HuCV immunogenicity;
- multiple virus types and infrequent cross immunity;
- inability to culture the virus; and
- lack of a small animal model for human caliciviruses.[45]

Nevertheless, rNV VLPs given orally to mice and human volunteers are safe and induce IgG_1 and IgA antibody responses.[46] While waiting for the vaccine, preventive measures should follow the procedures mentioned above. Contaminated water supplies can be treated with chlorine concentrations >10 mg/l.

DIAGNOSTIC MICROBIOLOGY

RT-PCR is the most widely used technique. It is very sensitive and allows virus detection in clinical specimens and the environment. Primer selection is important as genetically diverse strains may not be detected; RT-PCR with multiple primers identified Norwalk-like viruses as the etiologic agent of 96% of gastroenteritis outbreaks.[14,16,47] Immunoassays to detect viral antigens in stools are less sensitive; however, IAs to detect antibodies to the viruses have been very useful in sero-epidemiologic and vaccine development studies.

CLINICAL MANIFESTATIONS

The usual clinical characteristics of norovirus and sapovirus infections are presented in Table 151.4. Most of the time, infections are acute, short lived or asymptomatic; however, severe illness, including disseminated intravascular coagulation, has been described in healthy soldiers under severe environmental conditions.[48]

MANAGEMENT

No specific therapy exists for HuCV infections. Treatment is supportive with oral or intravenous rehydration. Bismuth subsalicylate reduces severity and duration of illness in experimental infections in adults,[49] but the use of symptomatic medications for the management of acute gastroenteritis in children is not recommended by the American Academy of Pediatrics.[50]

Rotaviruses

First described in 1973 by Bishop *et al.*,[51] human rotaviruses (RVs) represent the main agent of acute gastroenteritis in infants and young children.

NATURE

Rotaviruses belong to the family Reoviridae.[52] Intact 75 nm particles have a triple-layered capsid with core, inner and outer layers. Sixty spike-like structures (capsomers) radiating from the inner to the outer layer give the virus its characteristic wheel-like appearance. The core capsid encloses the viral genome, consisting of 11 segments of double-stranded RNA. Each segment encodes for one protein, except segment 11 which encodes for two. Six proteins (VP1–VP4, VP6–VP7) form the virion structure, and six nonstructural proteins (NSP1–NSP6) are expressed only in the infected cell (Fig. 151.2, Table 151.6).[53,54] The core is made of VP1, VP2 (its major contituent) and VP3. VP6 is the sole component of the inner capsid. The outer capsid is composed of VP7 (90%) and VP4, which forms the capsomers.

VP6 defines seven antigenic groups (A–G), with group A rotaviruses causing most human infections. The outer capsid proteins VP4 and VP7 determine the serotypes. Those defined by VP7 are called G (for glycoprotein) serotypes, and those defined by VP4 are called P (for protease-sensitive protein) serotypes. There is full concordance between VP7 serotypes and genotypes; there are at least 15 G serotypes designated G1–G15. The concordance between VP4 serotypes and genotypes is poor. There are 14 VP4 serotypes (P1–P14) and 26 genotypes (1–26). P1 and P5 include subtypes A and B; P2 includes subtypes A, B and C; Genotype 5 has a 5A subtype. Rotaviruses are formally designated using the G and P letters followed by the serotype number; a second number in parentheses after the P indicates the genotype. For example, the human RV strain Wa is designated G1P1A[8].

EPIDEMIOLOGY

Transmission of RVs is mainly person to person by the fecal–oral route. Fecal excretion starts immediately before the onset of symptoms and lasts for 5–7 days. Spread is favored by the large number of virions excreted in feces (1 trillion per ml) and the low infective dose (10 RV particles). Contamination of food and water has been implicated in some outbreaks. Since RVs can survive for 60 days on environmental surfaces at different temperatures (39.2–68°F/4–20°C) and humidities (50–90%), fomites may play a role in such settings as day-care centers and nurseries.[55] Respiratory transmission has been suspected in a few outbreaks and is supported by the way the annual RV epidemic spreads in North America. However, attempts to isolate RV from respiratory secretions have been mostly unsuccessful.

Rotaviruses are the most commonly identified viral enteropathogens of infants and young children. The proportion of diarrhea cases caused by RVs increases from community to clinic to hospital populations, reflecting the tendency of the virus to produce dehydration. In the USA, RVs cause 5–10% of all diarrheal episodes and 30–50% of severe diarrhea in children under 5 years of age, resulting in 3.5 million cases, 55 000 hospitalizations, 20–40 deaths and costs in excess of one billion dollars.[56] Worldwide, the estimates are 111 million episodes, 2 million hospitalizations and 600 000 deaths annually. RVs account for a larger proportion of cases in developed countries than in developing ones (38–89% vs 20–46%, respectively) and in high-income groups than in low-income ones (60% vs 4–30%, respectively).[57]

Incidence rates peak at age 6–24 months. Neonates are affected infrequently. Adults exposed to infected children become infected frequently (11–70%) but rarely develop clinical disease.[58,59] Outbreaks have been described in nursing homes for the elderly, hospital wards and military bases. RVs have been detected in as many as 20% of cases of travelers' diarrhea. Asymptomatic shedding of RVs occurs in 10–15% of individuals.

In temperate climates, RVs appear in characteristic and predictable winter epidemics. In North America, the epidemic starts in late fall in Mexico and the southwestern USA, spreads in a northeast direction and ends in the spring in the northeastern USA and the Maritime provinces of Canada.[60] The reasons for this spread pattern are not clear; climate, virus characteristics or other factors may play a role. In tropical climates, RVs are endemic throughout the year, with some clustering in the cooler, drier months. Globally, group A serotypes G1–G4, in conjunction with P[8] or P[4], constitute most human infections. However, there is much geographic and temporal variability, with G9, G8, G5 and others emerging as prevalent in some parts of the world.[53,61] Multiple serotypes can co-circulate during a specific year.

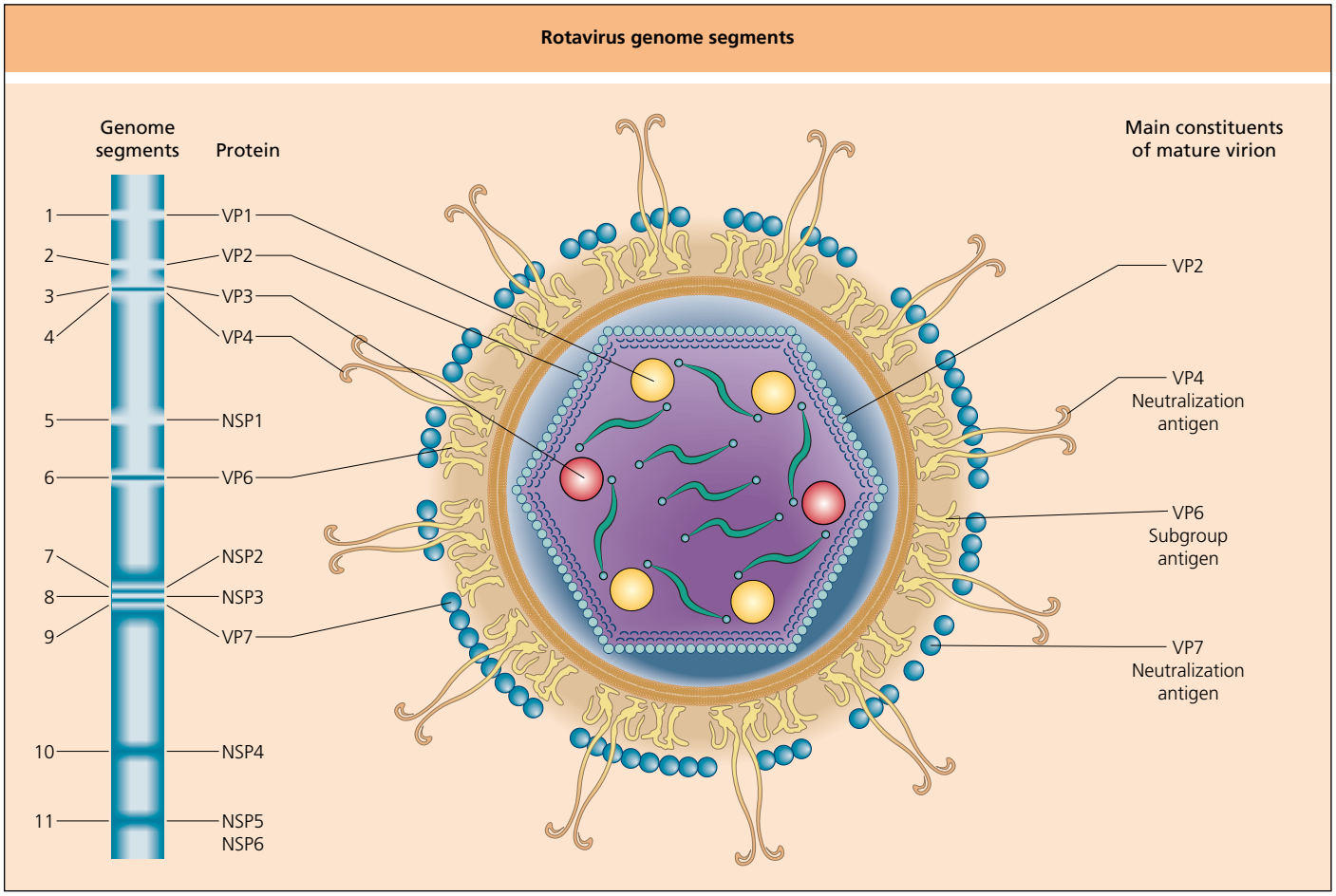


Fig. 151.2 Diagram showing rotavirus genome segments, protein products and their location in the viral particle. Adapted with permission from Gentsch *et al.*[53]

Group B RVs have been associated with epidemics of diarrhea among adults in China, and group C RVs have been identified in Central and South America, Europe, Australia and Asia.

PATHOGENICITY

Rotaviruses preferentially infect the mature enterocytes of the small intestine. Infected cells change from columnar to cuboidal, with enlarged cisternae of the endoplasmic reticulum and fewer and shorter microvilli. The cells are eventually killed and sloughed off and, with denudation of the tip cells, the villi become shortened. Mononuclear leukocyte infiltration is minimal. These changes occur within 24 hours of infection, start proximally and progress caudally.[51,62] Recently, RV RNA has been detected in blood and cerebrospinal fluid, suggesting that RV may represent a systemic infection.[63] Diarrhea results from decreased absorption of salt and water secondary to enterocyte damage and replacement of absorptive intestinal cells by secreting cells from the crypts. Loss of disaccharidases at the damaged brush border results in carbohydrate malabsorption and osmotic diarrhea. NSP4, the first described viral enterotoxin, increases plasma membrane chloride permeability, leading to chloride secretion and secretory diarrhea.[64]

The replication cycle of RV has been reviewed elsewhere.[54] Some key points are noted here. The nature of the receptors on the host cell remains elusive. On the virus side, VP4 mediates attachment, and its cleavage into VP5* and VP8* by proteases-like trypsin is essential for cell penetration. Cell penetration also requires VP7. The virus enters the cell either by direct membrane penetration or by receptor-mediated endocytosis; intracellular levels of Ca^{2+} trigger virus uncoating. RNA synthesis occurs in the core of the virion, mediated by an endogenous RNA-dependent RNA polymerase. The RNA transcripts encode the RV proteins, which are translated by the cellular translational machinery. The initial steps of virus replication occur in cytoplasmic inclusions called viroplasms. The viral subparticles bud through the membrane of the endoplasmic reticulum and become mature particles. NSP4 plays a key role in the assembly process, which is Ca^{2+} dependent. Lastly, mature virus particles are released by cell lysis in nonpolarized cells or by nonclassic vesicular transport in polarized epithelial cells.

Immunity against RV infection and illness are not completely understood.[65] Clinical protection may involve local (mucosal) and systemic (serum) antibodies as well as cellular immunity. VP7- and VP4-induced serum-neutralizing antibodies against the infecting serotype (homotypic) appear within 2 weeks of infection. Heterotypic antibodies against different serotypes also occur, but mostly among adults, and vary with the infecting strain. There is a correlation between the presence of homotypic neutralizing antibodies and protection.[58] The duration of homotypic protection is probably longer than that of heterotypic but is both incomplete and short lived, as shown by the occurrence of re-infections with the same serotype. Antibody levels are high at birth, decline by 3–6 months, rise to a peak at 2–3 years and remain elevated throughout life (this is probably because of repeated, mostly asymptomatic infections).[66] Serum antibodies do not always prevent the infection. Mucosal immunity may be more important than systemic immunity. It develops 4 weeks after the illness and persists for several months, eventually decreasing with advancing age.[67]

Passively acquired mucosal immunity by breast-feeding or orally administered immune globulins has conferred protection to high-risk individuals. Cell-mediated immunity also seems to be important.

Table 151.6 Rotavirus genome segments and their corresponding viral proteins

Genome segment (size, bp)	Protein product (MW, kDa)	Location in virus particles	Function
1 (3302)	VP1 (125)	Core capsid	RNA-dependent RNA polymerase, complex with VP3
2 (2729)	VP2 (94)	Core capsid	Main constituent of core, RNA binding
3 (2591)	VP3 (88)	Core capsid	Complex with VP1, guanylyl and methyl transferase, synthesis of capped mRNA transcripts
4 (2359)	VP4 (86.7)	Outer capsid	Hemagglutinin, cell attachment, neutralization antigen, determines P serotypes, cleaved by trypsin into VP5* (52.9) and VP8* (24.7), virulence
5 (1566)	NSP1 (58.6)	Nonstructural	Basic protein, RNA binding
6 (1356)	VP6 (44.8)	Inner capsid	Main constituent of inner capsid, determines group specificity, protection, required for transcription
7 (1074)	NSP3 (34.6)	Nonstructural	Acidic protein, RNA binding, inhibits host cell translation
8 (1059)	NSP2 (36.7)	Nonstructural	Basic protein, RNA binding, forms viroplasms with NSP5
9 (1062)	VP7 (37.4)	Outer capsid	Glycoprotein, major constituent of outer capsid, determines G serotypes
10 (750)	NSP4 (20.2)	Nonstructural	Role in morphogenesis, interacts with viroplasms, modulates intracellular Ca^{2+} and RNA replication, enterotoxin, virulence
11 (664)	NSP5 (21.7)	Nonstructural	Role in morphogenesis, forms viroplasms with NSP2, interacts with VP2 and NSP6
11 (664)	NSP6 (12)	Nonstructural	Interacts with NSP5, present in viroplasms

bp, base pairs; MW, molecular weight.
Adapted with permission from Estes & Kapikian.[54]

In mice, RV-specific cytotoxic T cells appear in the intestinal mucosa soon after infection. Mice with severe combined immmunodeficiency are able to clear RV infection when reconstituted with CD8 T cells, despite their lack of antibodies against the virus.[68] Recent experimental data suggest a role for non-neutralizing anti-VP6 antibodies in protection, though its significance in human infection is still unclear.[69]

PREVENTION

Breast-feeding reduces the overall incidence of diarrhea. For RVs, however, the effect of breast-feeding may be not so much in reducing incidence as in reducing the severity and duration of illness.[70]

Careful hand washing is important to reduce virus transmission. Fecal contamination of surfaces and objects occurs frequently, and RVs can survive in the environment for weeks.[71] Effective disinfectants are 6% hydrogen peroxide, 2500 ppm chlorine, 80% ethanol, ethanophenolic disinfectants, ultraviolet radiation and heat; drying and phenolic disinfectants are not effective;[72] hypochlorites are inactivated by fecal organic matter. Nondisposable objects are better cleaned by washing at 176°F (80°C) for at least 1 minute. Household laundry should be washed with detergent and bleach, followed by a drying cycle.

The first commercial RV vaccine, RotaShield by Wyeth-Ayerst Laboratories, was licensed in the USA in 1998. It was a rhesus–human reassortant live vaccine containing strains with specificities for G1–G4 serotypes. Three doses given orally at 2, 4 and 6 months of age were 49% effective for all RV diarrhea and 80% effective for severe RV diarrhea, thereby decreasing physician intervention by 73% and basically eliminating all cases of RV dehydration.[73] The vaccine was suspended less than 1 year later due to an association with intussusception, estimated as one additional case/10 000 infants vaccinated.[74]

More recently a new vaccine, RotaTeq, has been licensed in the USA (Table 151.7). This is also a live reassortant vaccine but of bovine–human origin and containing five strains of G1–G4 and P1[8] specificities. Three doses of the vaccine administered at 2, 4 and 6 months of age have demonstrated 74% efficacy to prevent any RV disease and 98% efficacy for severe disease with no detected association to intussusception.[75] A second RV vaccine, Rotarix (Table 151.7), also prevents any (73%) or severe (85%) RV disease without association to intussusception.[76] Rotarix is a live monovalent vaccine derived of a human attenuated strain of G1P[8]; two doses at 2 and 4 months are recommended. It is licensed in Latin America, Europe and recently in the US. Both vaccines decrease the overall burden of diarrhea hospitalization by ~50%, which may significantly improve the worldwide health of children. Both vaccines are recommended for universal immunization of children in the US.

DIAGNOSTIC MICROBIOLOGY

Various antigen detection kits based on enzyme immunoassays (EIAs) and the latex agglutination test are commercially available and represent the tests of choice for most clinical circumstances. They are relatively inexpensive and permit rapid diagnosis with high sensitivity and specificity (70–100%). Newborns and breast-feeding children might have higher false-positive results. Samples should be obtained during the symptomatic period to optimize the performance of the test. If samples are not to be processed immediately, they can be stored at 39.2°F (4 °C) or frozen. In special situations, other tests can be considered, such as EM, gel electrophoresis of viral RNA, hybridization of radiolabeled nucleic acid probes to the viral RNA, amplification by RT-PCR and viral culture. Serologic tests are rarely used. Neutralizing antibodies can be detected by plaque reduction or cytopathic effect inhibition.

Table 151.7 Comparison of two licensed rotavirus vaccines

Name	RotaTeq®	Rotarix™
Producer	Merck & Co., Inc	GlaxoSmithKline
Vaccine type	Live, bovine–human reassortant	Live-attenuated human RV strain (RIX4414)
Serotypes	Pentavalent: G1, G2, G3, G4, P1[8]	Monovalent G1P[8]
Dose	>2 × 10^6 infective doses, each	>1 × 10^6 infective doses
Administration	Oral, three doses at 2, 4 and 6 months of age	Oral, two doses at 2 and 4 months of age
Intussusception risk*	1.6 (0.4–6.4)	0.85 (0.30–2.42)
Efficacy:†		
RV GE, any severity	74 (67–80)	73 (27–91)
RV GE, severe	98 (88–100)	85 (72–92)
All diarrhea hospitalization	59 (52–65)	42 (29–53)
Virus shedding	9%	50–80%

*Odds ratio vaccine vs placebo (95% CI).
†Percent decrease vaccine vs placebo (95% CI).
GE, gastroenteritis; RV, rotavirus.

CLINICAL MANIFESTATIONS

The clinical spectrum of RV infections ranges from asymptomatic to severe disease with dehydration and death. The usual clinical picture is presented in Table 151.4. The disease is self-limiting; chronic infection has not been described in the normal host.[66] Neonatal RV infections are symptomatic in <10–20% of cases, and usually mild. Severe infections may occur among premature infants and in special care units.

MANAGEMENT

Rehydration and appropriate feeding are the main therapies for RV diarrhea. Oral immune globulin and colostrum or human milk containing RV antibodies have been used in the treatment of RV diarrhea with good results.[77] The anti-RV titer is low in human preparations but higher in colostrum from cows immunized against RV. This form of therapy might prove useful for immunocompromised patients and those with chronic or severe disease. Also, formulas supplemented with *Bifidobacterium bifidum* and *Streptococcus thermophilus* reduce the incidence of diarrhea and RV shedding in infected children.[78] Interference with the overgrowth of bacteria and promotion of the intestinal immune response to RV have been suggested as possible mechanisms. Recently, nitazoxanide (a broad-spectrum anti-infective) has been reported to decrease the duration of severe RV diarrhea.[79]

Astroviruses

Astroviruses belong to the family Astroviridae. In 1975, they were described by Madeley & Cosgrove as small (28 nm) particles with a five- or six-pointed star appearance by EM.[80] Later studies showed

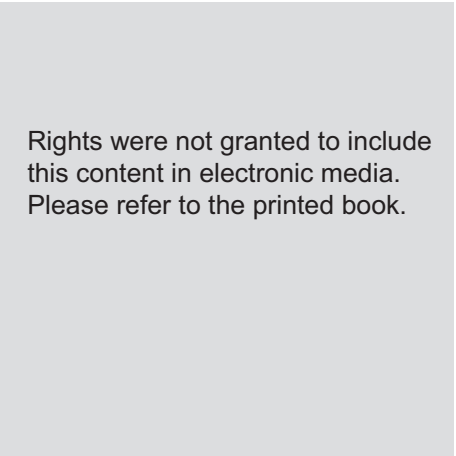


Fig. 151.3 Three-dimensional reconstruction of cryoelectron microscopy image of human astrovirus. Reproduced with permission from Mendez & Arias.[85]

an icosahedral, 41 nm morphology with well-defined spikes (Fig. 151.3). If the viruses are subjected to high pH, they transform to the previously described star.[81] The virus is nonenveloped, with a single-stranded, positive-sense RNA genome that contains three ORFs. ORF1a and ORF1b encode viral protease and polymerase, respectively. ORF2 encodes a protein capsid precursor, which gives rise to VP32, VP29 and VP26 (structural capsid proteins). VP26 and VP29 appear to be responsible for antigenic variation.[82] Eight serotypes have been described (HAstV-1 to HAstV-8).

Human astrovirus are responsible for 2–4% of endemic diarrhea in children worldwide; HAstV-1 is the most prevalent.[83,84] Astroviruses are the 'second or third most common cause of viral diarrhea in young children'.[85] They have been associated with outbreaks in day-care centers, schools and pediatric wards. Infection confers protective antibodies, which become more prevalent with increasing age. More than 80% of adults have antibodies against the virus. Immunocompromised subjects and the elderly are also affected.

Astrovirus pathogenesis is not completely understood. Histopathologic studies in an immunocompromised patient show infection in mature epithelial cells of the small intestine,[86] without inflammatory response. Mild crypt cell hyperplasia, also without inflammation, has been observed in turkeys.

Prevention or control of outbreaks can be accomplished by following the procedures mentioned above. The virus is inactivated by methanol 70–90% and heating at 140°F (60°C) for 10 minutes; however, it is resistant to chloroform and ethanol. As yet there is no vaccine.

Diagnosis can be made by commercial IAs with good sensitivity and specificity when compared with EM and RT-PCR.[84] The clinical characteristics of astrovirus infection are presented in Table 151.4. Symptoms are similar to those of RV infection but less severe. Management is supportive.

Enteric Adenoviruses

The Adenoviridae family has 51 human adenovirus serotypes, which are divided into six species or groups (A–F). Described in 1975,[87] enteric adenoviruses belong to species F and comprise serotypes 40 and 41. Other serotypes have also been associated with gastroenteritis, but their significance is less clear.

Adenoviruses are nonenveloped, with a dsDNA genome surrounded by an icosahedral capsid with fiber-like projections from each of the 12 vertices. Morphologic characteristics are presented in Table 151.2. Each virion contains 240 hexons (major surface protein) and 12 pentons. Each penton consists of a base and a fiber. Genus-specific antigens are located in the hexon. Type-specific antigens are located in the hexon and the fiber, which are in the virion surface and give rise to serum neutralizing antibodies.

The fiber protein of most adenoviruses binds to the coxsackie–adenovirus receptor (CAR) of epithelial cells and the penton base mediates internalization of the virus. Infected cells degenerate in typical ways. The process is related to the E3 virus proteins.[88] No specific receptors to explain species tissue tropism have been identified. The pathogenesis of gastroenteritis produced by serotypes 40 and 41 remains elusive.

Enteric adenoviruses have worldwide distribution, mainly affecting young children, and are responsible for 4% of acute gastroenteritis episodes seen in outpatient clinics and 2–22% seen in hospitalized children.[89] Outbreaks lasting 7–44 days have occurred in hospitals and day-care centers; approximately 40% of children were infected, half of them asymptomatically.[90]

Transmission is person to person by the fecal–oral route. Infected persons developed group- and type-specific antibodies. The latter are needed for long-term immunity and can be measured by neutralization or hemagglutination inhibition (HAI) tests. Stool excretion of adenoviruses lasts 10–14 days, from 2 days before and 5 days after diarrhea. Asymptomatic excretion may last months to years; thus, their isolation in diarrheic stools does not necessarily mean acute infection.

Outbreak control can be accomplished following the procedures mentioned above. Adenoviruses are less resistant than RVs and are rapidly inactivated at 133°F (56°C) and by exposure to ultraviolet light or formalin. No vaccine is under development.

Viral antigen detection by immunoassay is the diagnostic test of choice. IAs are simple, quick and inexpensive; sensitivity and specificity are 98% when compared with EM; however, real-time RT-PCR has proven superior to IAs and EM.[91] Enteric adenoviruses do not grow in routine tissue culture.

The clinical manifestations of enteric adenoviruses are presented in Table 151.4. In general, the disease is milder but more prolonged than that with RV. Treatment is supportive. Cidofovir alone or with ribavirin have been used for immunocompromised patients.

Other Viruses

Cases of human gastroenteritis have been associated with picornaviruses (Aichi virus) coronaviruses, parvoviruses, pestiviruses, toroviruses, picobirnaviruses, Breda viruses and others, but their role as agents of gastroenteritis is under study.

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com