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18 Rotavirus and Other Viral Diarrhoea

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KEY POINTS

- Rotavirus, norovirus, sapovirus, enteric adenovirus and astrovirus are established aetiological agents of acute gastroenteritis across all age groups in all settings.
- Rotavirus is the single most important pathogen associated with severe dehydrating gastroenteritis in infants and young children worldwide.
- Norovirus causes sporadic and epidemic disease in adults and children.
- All gastroenteritis viruses infect the epithelial cells of the intestinal mucosa, causing acute, non-inflammatory watery diarrhoea mostly of less than 1 week duration.
- Aetiological diagnosis requires the detection of either specific antigens of each virus with immunologic assays, or the presence of the viral genome with molecular assays or visualization of virus by electron microscopy.
- Treatment is primarily supportive and consists of rehydration and restoration of electrolyte balance.
- Two live, oral, attenuated vaccines, Rotarix and RotaTeq, have been developed to prevent global morbidity and mortality due to rotavirus infection.
- Both rotavirus vaccines are entering childhood immunization schedules following successful clinical trials in representative populations worldwide, with early evidence of substantial impact.

Introduction

The gastrointestinal tract is the commonest portal of entry for a variety of pathogens, including viruses, but not all of these viruses are causally associated with diarrhoeal disease. Among the viruses that infect enterocytes, or at least use them as a portal of entry, there are two major groups. The first group comprises those viruses that cause systemic infections after entering into the body through the gastrointestinal tract, and diarrhoea, if ever present, is not a major feature of infection. This group includes many enteroviruses, including poliovirus and coxsackieviruses, hepatitis A and E viruses and some adenoviruses. The second group comprises viruses that infect the upper small intestine and cause non-inflammatory diarrhoea. There are currently five established gastroenteritis viruses affecting humans, i.e. *Rotavirus*, *Norovirus*, *Sapovirus*, Human *Astrovirus*, and *Human Adenovirus F* (formerly called group F adenovirus).

Rotavirus

Human rotavirus was discovered in 1973 on thin-section electron microscopy of duodenal biopsies from a child with acute gastroenteritis.[1] Virus particles were subsequently identified in large numbers in faeces by direct negative-stain electron microscopy[2] and significant antibody titre rises were demonstrated between acute and convalescent sera from diarrhoeal children by using immune electron microscopy.[3] The virus was named rotavirus because of its characteristic wheel-shaped (*rota* = Latin for wheel) morphology on electron microscopy (Figure 18.1).

EPIDEMIOLOGY

Virtually all children are infected with rotavirus at least once by the age of 3–5 years, whether they live in developing or developed countries. However, the consequences of infection are markedly different according to geographic location, with over 95% of deaths due to rotavirus diarrhoea occurring in the developing countries of the Indian subcontinent, sub-Saharan Africa and Latin America (Figure 18.2).[4–6] Rotavirus diarrhoea occurs at an earlier age in children in developing countries than in children in developed countries (Figure 18.3). The median age of children hospitalized with rotavirus diarrhoea in many African and Asian counties is 6–9 months, and up to 80% are less than 1 year old. In contrast, the median age in developed countries is 13–16 months and the highest proportion of cases occurs in the 2nd year of life.[7,8] In temperate countries, rotavirus infections peak in the winter and early spring, with fewer cases detected at other times. In tropical countries, rotavirus infections characteristically occur throughout the year, although more cases are typically observed in the cooler and drier months. Although the mode(s) of transmission of rotavirus are not completely understood, person-to-person spread of rotavirus by the faecal–oral route is likely to play a central role.

In both developing and developed countries, rotavirus is the major cause of severe gastroenteritis requiring hospitalization. It was estimated that, from 1986 to 1999, a median of 22% (range 17–28%) of acute diarrhoea cases in children less than 5 years of age were due to rotavirus[4] but this proportion nearly doubled from 2000 to 2004 to 39% (range 29–45%),[5] related in part to the widespread application of more sensitive detection methods, and to a decrease in the proportion of gastroenteritis caused by bacterial pathogens. The annual global mortality due to rotavirus diarrhoea among children less than 5 years of age has been estimated as 453 000 in the pre-rotavirus vaccine era, with rotavirus accounting for 37% of all diarrhoea deaths and 5% of all-cause deaths in children under 5 years of age.[9] The Democratic Republic of Congo, Ethiopia, India, Nigeria, and

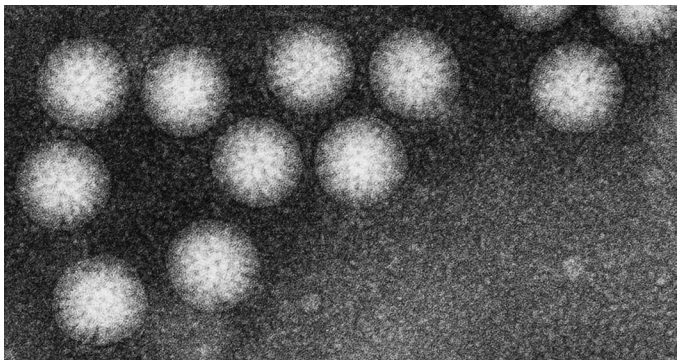

Figure 18.1 Negative-stain electron micrograph of rotavirus (×200 000).

Pakistan accounted for more than half of all rotavirus deaths, with India alone accounting for 22% of deaths.[9]

VIROLOGY

Rotavirus is a genus within the family *Reoviridae*, and within the genus there are seven groups (A–G), each of which represents a separate species, e.g. *Rotavirus A*, *Rotavirus B*, etc.[10] Only group A, B and C rotaviruses are established human pathogens. Group A rotavirus has the greatest medical importance and, unless mentioned otherwise, the word rotavirus usually infers Group A rotavirus. Group B rotavirus infection is rare and affects both adults and children, causing both outbreaks and sporadic infections, primarily in China, India and Bangladesh.[11,12] Group C rotaviruses tend to affect older children than do group A rotaviruses, and up to one-third of adult humans have serological evidence of infection with Group C rotavirus.[13,14]

By conventional negative-stain electron microscopy, rotavirus has a characteristic double capsid appearance measuring approximately 75 nm in diameter (Figure 18.1), but cryo-electron microscopic studies demonstrated that the rotavirus virion comprises a triple-layered capsid with 60 spikes protruding from its surface, making its overall diameter nearly 100 nm. The outermost layer (outer capsid) consists of two proteins, VP4 and VP7, each of which independently serves as a neutralization antigen (Figure 18.4). The serotype defined by the VP4 protein is called the P type, for protease-sensitive protein (because VP4 is proteolytically cleaved into VP8* and VP5*), and the serotype defined by the VP7 protein is called the G type, for glycoprotein. The middle capsid consists of the most abundant viral protein, VP6, which is the major protein against which non-neutralizing antibodies are raised during infection. The core or the innermost layer consists of VP2, a scaffolding protein, and inside this layer are VP1 (viral RNA-dependent RNA polymerase) and VP3 (guanyltransferase), which is present in association with the 11 segments of double-stranded genomic RNA. In addition to these five structural proteins, there are six non-structural proteins (NSPs), each of which is encoded by a single genome segment, except for NSP5 and NSP6 (encoded by RNA segment 11), which carry out various functions during replication and morphogenesis. NSP4 is a chaperone protein enabling the subviral particle to acquire the outer capsid proteins VP4 and VP7 during the later phases of viral morphogenesis. NSP4 also acts as a viral enterotoxin, causing diarrhoea in newborn mice.[15–17]

Rotavirus genomic RNA can be extracted directly from clinical specimens and separated by polyacrylamide gel electrophoresis (PAGE). With this, two major RNA migration patterns are recognized in which genome segments 10 and 11 of long RNA pattern viruses migrate faster than do those of short RNA pattern viruses (Figure 18.5).[18] The precise migration pattern is

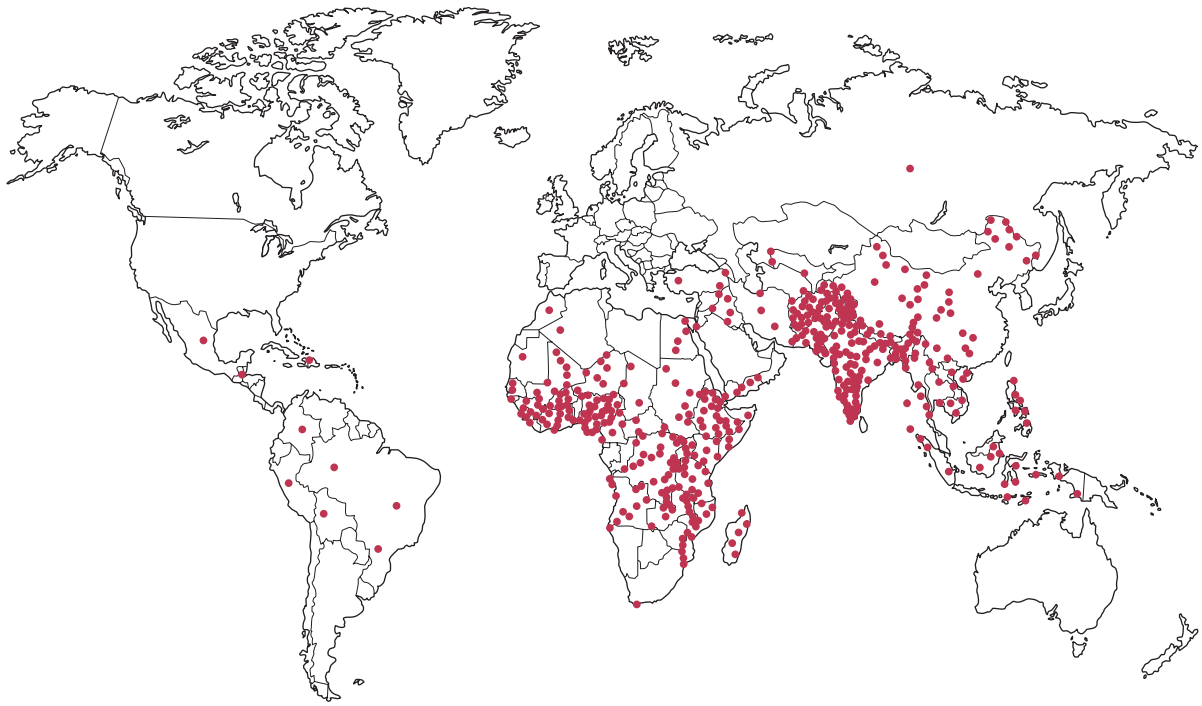

Figure 18.2 Map of global distribution of rotavirus mortality in children less than 5 years of age. Each dot represents 1000 deaths. *(Reprinted from Parashar UD, Gibson CJ, Bresse JS, et al. Rotavirus and severe childhood diarrhea. Emerg Infect Dis 2006;12:304–6.)*

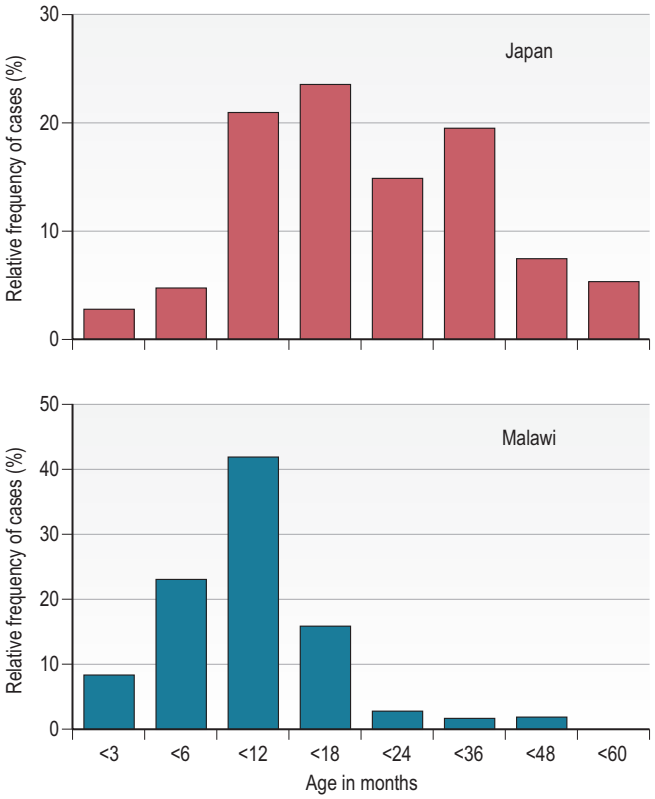

Figure 18.3 Two contrasting patterns of age distribution of rotavirus diarrhoea occurring in Malawi (as an example of a developing country) and in Japan (as an example of a developed country). *(Data from Nakagomi T, Nakagomi O, Takahashi Y, et al. Incidence and burden of rotavirus gastroenteritis in Japan as estimated from a prospective sentinel hospital study. J Infect Dis 2005;192 (Suppl 1):106–10. and Cunliffe NA, Ngwira BM, Dove W, et al. Epidemiology of rotavirus infections in children in Blantyre, Malawi,1997–2007. J Infect Dis 2010;202:S168–74.)*

characteristic for each rotavirus strain and is called an 'electropherotype', which was extensively applied in molecular epidemiological studies, until the use of genotyping and sequencing methods became more widespread.[19]

The serotype is the most important antigenic determinant of rotavirus and is defined by serological assays. However, serological typing methods have been largely replaced by molecular typing (genotyping). In addition to VP7 (G) and VP4 (P) genotyping, a nucleotide sequence-based, complete genome classification system has recently been introduced for rotavirus classification;[20] the genome of individual rotavirus strains is given the complete descriptor of Gx-P[x]-Ix-Rx-Cx-Mx-Ax-Nx-Tx-Ex-Hx. Of these 11 genotypes, the G and P genotypes have been extensively investigated because of their importance in protective immunity. There are thus far 26 G genotypes and 35 P genotypes reported among human and animal rotaviruses, but the G and P type combinations (Figure 18.6) detected in human rotaviruses are mostly limited to G1P[8], G2P[4], G3P[8], G4P[8] and G9P[8].[21,22] However, G12 strains have now emerged across the world,[23] and G8 strains with either P[6] or P[4] account for a significant proportion of human rotavirus strains in Africa.[24,25] Such genetic diversity is generated by frequent reassortment of the genome segments and interspecies transmission of rotaviruses between humans and animals.[26–28]

PATHOGENESIS

Rotavirus exclusively infects the mature differentiated villous enterocytes of the small intestine. Rotavirus attaches to its cellular receptors (sialoglycoprotein and integrins) via the VP4 protein. The minimum infective dose is as low as 10^2–10^3 virus particles in adult volunteers.[29] Progeny virus is produced after 10–12 h, and released in large numbers into the intestinal lumen ready to infect other cells. The pathogenesis of rotavirus diarrhoea includes both malabsorptive and secretory components.[30] Malabsorption may be consequent upon damage to mature absorptive enterocytes resulting in malabsorption of nutrients, electrolytes and water; virus-induced downregulation of the expression of absorptive enzymes; and functional changes in tight junctions between enterocytes leading to paracellular leakage. Biopsies show atrophy of the villi and mononuclear cell infiltrates in the lamina propria. Secretory mechanisms include those mediated by activation of the enteric nervous system and the effect of NSP4, the latter being via activation of cellular Cl^- channels, leading to increased Cl^- and consequently water secretion. Rotavirus infection was previously thought to be limited to the intestine, but rotavirus causes viraemia for at least a short period in the acute phase of infection in immunocompetent infants as well as in experimentally infected animals.[31] The clinical significance of this systemic spread of rotavirus remains unclear.

IMMUNITY

In general, one or more episodes of rotavirus infection confer protection against subsequent moderate or severe rotavirus diarrhoea but not against asymptomatic reinfection or mild diarrhoea. Furthermore, infection with one serotype generally provides serotype-specific (homotypic) protection, and repeated infections lead to partial cross-serotype (heterotypic) protection. These beliefs are supported by the findings of a cohort study in Mexico, where children who had experienced one, two or three episodes of rotavirus diarrhoea had adjusted relative risks of experiencing a further attack of rotavirus diarrhoea of 0.23, 0.17 and 0.08, respectively, and of asymptomatic rotavirus infection of 0.62, 0.40 and 0.34, respectively.[32] However in a cohort study in Vellore, India, while protection against moderate or severe disease increased with successive infections it was only 79% after three infections and no evidence of homotypic protection was demonstrated, indicating that immune protection following natural infection may vary by location.[33] In the Indian study, multiple infections were common, with only 30% of all identified infections being primary. Immunity following natural rotavirus infection is believed to be mediated by both humoral and cell-mediated immune responses.[34] Rotavirus-specific immunoglobulin (Ig) A antibodies on the enteric mucosal surface are thought to be the primary mediator of protective immunity. Cellular immunity is considered to be important in the resolution of rotavirus infection and appears to be cross-protective between the different G serotypes.[35]

Protection of neonates against rotavirus infection appears to be mediated by transplacentally acquired maternal antibody[36,37] and by antibodies and other factors in breast milk.[38] However, a study in Bangladesh showed that hospitalized children with rotavirus diarrhoea were more likely to be breast-fed than were children with diarrhoea due to other infectious agents.[39] Rotavirus infection in neonates often results in asymptomatic infection and

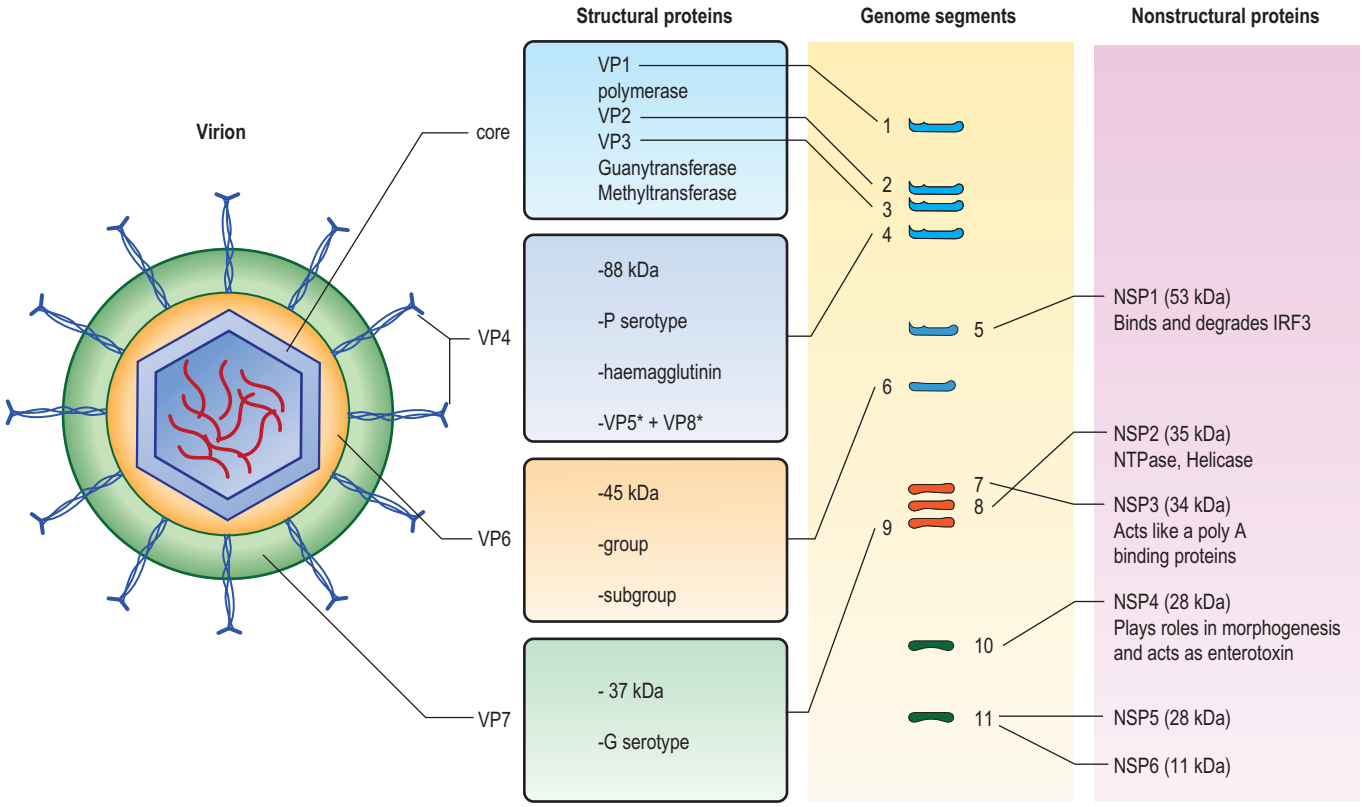

Figure 18.4 Schematic diagram showing the relationships between the structure of the rotavirus virion and the genomic double-stranded RNA segments. IRF3, interferon regulatory factor 3; NTPase, nucleotide triphosphatase.

rotavirus can therefore circulate silently in neonatal units. Neonatal strains are often unusual and can infect even in the presence of high titres of transplacental antibody from the mother. Asymptomatic neonatal infections may induce protection against subsequent severe rotavirus gastroenteritis.[40] Finally, it is increasingly recognized that otherwise healthy adults can experience rotavirus diarrhoea and elderly people can develop severe rotavirus gastroenteritis as their immunity wanes.[41,42]

CLINICAL FEATURES

The outcome of rotavirus infection varies from asymptomatic, through mild short-lived watery diarrhoea, to an overwhelming gastroenteritis with dehydration leading to death. The onset of symptoms is abrupt after a short incubation period of 1–2 days. Fever, vomiting and watery diarrhoea are seen in the majority of infected children and last for 2–6 days. Rotavirus diarrhoea tends to be more severe than that due to other common enteropathogens.[43] Extraintestinal manifestations during rotavirus gastroenteritis, including encephalopathy, have captured attention since rotavirus viraemia was reported.[44] It is not possible to distinguish rotavirus gastroenteritis from other viral causes of diarrhoea solely on clinical grounds.[45] The stools are usually watery or loose, and are seldom blood-stained. In severe cases, the cause of death is dehydration, which can be hypo- or hypernatraemic and is often associated with metabolic acidosis. Underlying conditions such as malnutrition may be a risk factor for a more severe disease outcome. Rotavirus does not appear to produce more severe disease in HIV-infected infants.[46,47]

DIAGNOSIS

Large numbers of rotavirus particles (up to 10^{11}/g of faeces) are excreted during the acute phase of infection. Children with severe diarrhoea excrete more virus than do children with less severe diarrhoea.[48] Rotavirus can be detected in stool specimens by a number of techniques, including electron microscopy, PAGE, antigen detection assays, RT-PCR and virus isolation. Electron microscopy remains a valuable diagnostic tool since it is a catch-all technique that will also detect other potential viral enteropathogens. PAGE is a convenient diagnostic tool for the detection of rotavirus RNA extracted directly from stool specimens (Figure 18.5). The assay also allows detection of non-group A rotaviruses, which fail to react in most antigen detection assays. PAGE is a relatively simple technique with high specificity for rotavirus (100%) and reasonable sensitivity (80–90%), and can be performed in tropical countries relatively cheaply.[49] It has the added advantage of providing epidemiological information because the electrophoretic migration pattern of the 11 segments of the double-stranded RNA genome is specific to each rotavirus strain.[18,50]

Antigen detection tests are currently the most widely used assays for rotavirus infection in diagnostic laboratories and include enzyme-linked immunosorbent assays (ELISAs), and immunochromatographic assays.[51] The sensitivity and specificity of the majority of these tests are generally high (90–95%) but they are designed to detect only group A rotaviruses. Detection of viral genome by RT-PCR is predominantly a research tool which provides information on the genotypes of the circulating strains[52–54] and the duration of viral shedding in stool

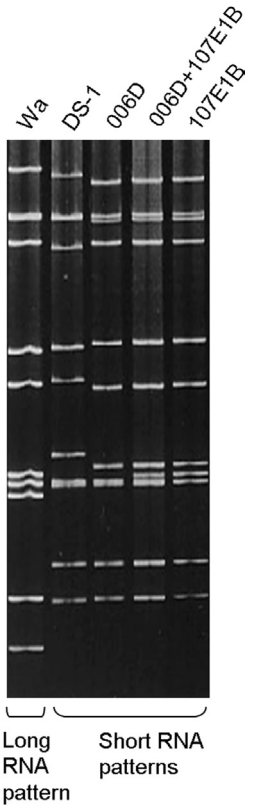

Figure 18.5 Separation of rotavirus genomic RNA into 11 bands by polyacrylamide gel electrophoresis. Two RNA patterns, long and short, are represented by prototype strains Wa and DS-1, respectively. Strains 006 and 107E1B have similar but distinct RNA electropherotypes. The differences in migration of segments 7, 8 and 9 are clearly demonstrated by co-electrophoresis in which RNAs from both 006 and 107E1B were loaded on the same lane. *(Adapted from Nakagomi T, Gentsch JR, Das BK, et al. Molecular characterization of serotype G2 and G3 human rotavirus strains that have an apparently identical electropherotype of the short RNA pattern. Arch Virol 2002;147: 2187–95.)*

which can be prolonged.[55,56] Group A and group C rotaviruses can be isolated in cell culture but viral culture is limited to research purposes.

MANAGEMENT

The mainstay of management consists of assessment of dehydration and replacement of lost fluid by oral rehydration with fluids of specified electrolyte and glucose composition, together with administration of zinc in children over age 6 months and where the prevalence of malnutrition is high.[57] Intravenous rehydration therapy is indicated for patients with severe dehydration, shock or reduced levels of consciousness. Human or bovine colostrum and hyperimmune human serum immunoglobulin have been used to manage chronic rotavirus infection in immunocompromised children. Administration of probiotics such as *Lactobacillus casei* GG also appears beneficial. The antiprotozoal drug nitazoxanide was shown to decrease the median duration of rotavirus gastroenteritis by 44 hours in a randomized double-blind placebo-controlled trial in Egyptian children.[58] As an adjunct to oral rehydration, the antisecretory agent racecadotril reduces diarrhoea duration and stool volume in children with acute gastroenteritis.[59]

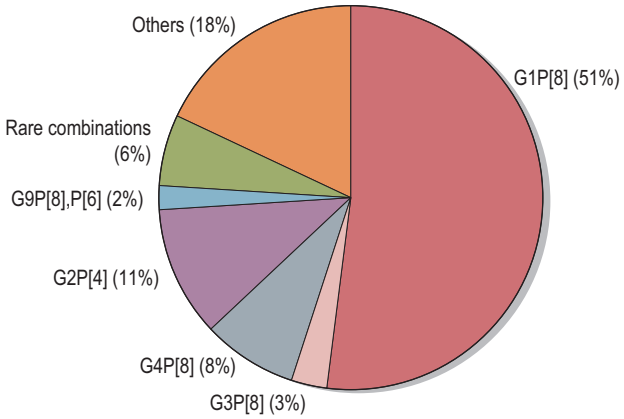

Figure 18.6 Relative frequencies of rotavirus genotypes detected globally among human rotaviruses over the period 1994–2003. *(Adapted from Gentsch JR, Laird AR, Bielfelt B, et al. Serotype diversity and reassortment between human and animal rotavirus strains: implications for rotavirus vaccine programs. J Infect Dis 2005;192(Suppl 1):146–59.)*

PREVENTION AND CONTROL

Since virtually all children will have experienced rotavirus infection by the age of 3–5 years in both developing and developed countries, it is clear that hygiene and sanitation practices are not sufficient to prevent the spread of rotavirus infection within the community. Thus, prevention of severe rotavirus gastroenteritis by vaccines remains the only practical preventive measure.[60] The first licensed rotavirus vaccine, a rhesus monkey rotavirus-based tetravalent human reassortant vaccine (RotaShield®), was withdrawn after this live, oral vaccine was associated with the development of intestinal intussusception in approximately 1:10 000 vaccine recipients in the USA, with intussusception cases occurring disproportionately in those infants who received their first dose of vaccine at over three months of age.[61,62] These unfortunate events stimulated the further development and testing of two additional live, oral rotavirus vaccines, Rotarix® (GlaxoSmithKline Biologicals) and RotaTeq® (Merck & Co.). Rotarix® is a monovalent, human rotavirus vaccine of serotype G1P1A[8] administered as a two-dose schedule, whereas RotaTeq® is a pentavalent, bovine–human reassortant vaccine comprising types G1, G2, G3, G4 and P[8] and is given in a 3-dose schedule.[63] Both vaccines were found to be safe in large, phase III clinical trials, each involving more than 60 000 infants and were 85–95% efficacious in preventing severe gastroenteritis due to rotavirus.[64,65] In countries that have introduced rotavirus vaccine into their childhood immunization programmes, hospitalizations due to rotavirus gastroenteritis have dramatically fallen.[66,67] Evidence of an indirect effect of rotavirus vaccines has been presented from the USA and other settings, with reduced incidence of disease in children too old to have been vaccinated and in adults.[66,67]

Both vaccines have recently been evaluated in clinical trials in Africa and Asia, where efficacy against severe rotavirus gastroenteritis was lower than previously experienced in other settings (50–75%), with poorer countries generally having lower efficacy (e.g. 50% in Malawi).[68–70] The reason for reduced efficacy in low-income countries is not yet known, but other live, oral vaccines against polio and cholera are known to be less efficacious in developing countries.[71,72] Despite more modest efficacy in low-income countries, the high burden of rotavirus disease led the WHO to issue a recommendation that rotavirus

vaccines should be incorporated in all childhood immunization programmes worldwide, with a strong recommendation for countries where diarrhoeal disease accounts for more than 10% of childhood mortality.[73] Because of the age-related occurrence of intussusception, the WHO recommended that the first dose of vaccine should be administered by 15 weeks of age and that the full course should be completed by 32 weeks of age.[73]

An early indication of the major public health benefit that rotavirus vaccination can bring has been demonstrated in Mexico, where diarrhoea mortality has fallen since the introduction of Rotarix into its childhood immunization programme.[74] A small increase in the risk of intussusception noted following the first vaccine dose in Mexico and after the second vaccine dose in Brazil has been noted, but the risk is outweighed by the number of diarrhoea deaths that will be prevented.[75] A re-evaluation of the risk/benefit ratio of rotavirus vaccination, in particular the impact of vaccination on severe disease in high mortality settings, has led to a WHO recommendation that the age restrictions on immunization be removed but that surveillance for intussusception should be implemented to monitor vaccine safety.[76] Additional assessments should include examination of the impact of rotavirus vaccination on rotavirus epidemiology including the distribution of rotavirus strains. Early studies suggested an increase in prevalence of G2P[4] strains following Rotarix introduction in Brazil, but it remains unclear whether this results from immune pressure secondary to vaccine use or natural fluctuation in strain types.[77] Continuous global strain surveillance is required to address this question.[78]

Enteric Adenovirus

Adenovirus is an unenveloped DNA virus with an icosahedral capsid measuring 70–75 nm in diameter (Figure 18.7). Its genome comprises double-stranded linear DNA of 33–45 kbp. Taxonomically, human adenoviruses that primarily cause diarrhoea are classified as species *Human adenovirus F* (formerly called group F) within the genus *Mastadenovirus*, family *Adenoviridae*. Within *Human adenovirus F*, more often referred to as 'enteric adenovirus', two serotypes 40 and 41 are distinguished based upon virus neutralization assays. These enteric adenoviruses account for approximately 5% of cases of infantile diarrhoea, occurring most often in children under 2 years of age without a clear seasonality.[79] However, it was reported that enteric adenoviruses were detected in as many as 20% of hospitalized children in northern Taiwan.[80] Enteric adenoviruses are spread from person-to-person by the faecal–oral route. Adenoviruses were found contaminating water in poor sanitary settings,[81] but these adenoviruses are unlikely to be enteric adenoviruses. No food-borne nor water-borne spread of enteric adenoviruses has been documented.

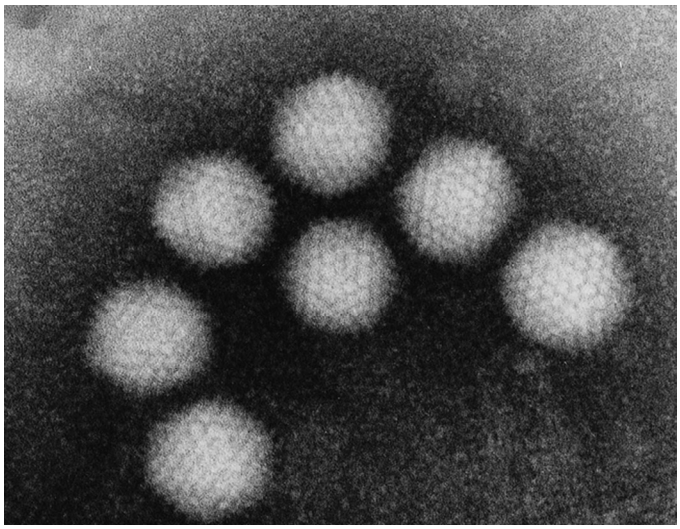

Figure 18.7 Negative-stain electron micrograph of enteric adenovirus (×200 000).

The clinical features of enteric adenovirus gastroenteritis do not differ from those of rotavirus but the duration of diarrhoea tends to be longer in adenovirus infection than in rotavirus infection.[82,83] Other than gastroenteritis, adenovirus is implicated as a cause of idiopathic intussusception in infants.[84,85] These adenoviruses are of serotypes 1, 2, 3 and 5, and rarely of 40 or 41 (enteric adenoviruses).

The diagnosis of adenovirus infection is by visualization of characteristic virions in stool specimens under the electron microscope; demonstration of adenovirus antigens in stool by ELISA or immunochromatography or by detection of the genome by PCR which has much higher sensitivity.[80]

Treatment of adenovirus diarrhoea is by managing dehydration. There is neither a specific therapeutic intervention nor an available vaccine.

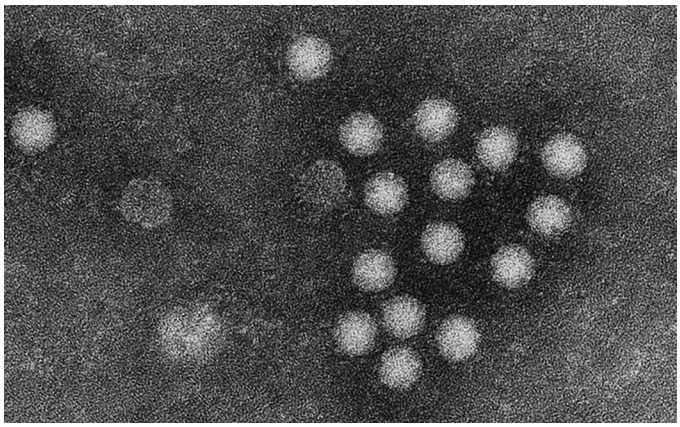

Figure 18.8 Negative-stain electron micrograph of astrovirus (×200 000).

Astrovirus

Human astrovirus, a species in genus *Mamastrovirus*, family Astroviridae, has an unenveloped virion measuring 28–30 nm in diameter with a characteristic star shape 'stamped' on its surface, a five- or six-pointed star with an electron-dense centre (*astron* = Greek for a 'star') (Figure 18.8). Its genome is positive-sense single-stranded RNA approximately 7 kb in length, which encodes an RNA polymerase (ORF1a), a serine protease (ORF1b) and three capsid proteins (ORF2). While astrovirus was first described in humans in 1975, astrovirus can infect a variety of animal species.

There are eight serotypes of *Human astrovirus* with serotype 1 the most frequently detected.[86] Other serotypes can cause outbreaks of food-borne infections and there appears to be more diversity in developing countries.[87] More recently, however, a greater number of astroviruses in human stool have been found far beyond the eight serotypes of *Human astrovirus*;

while their association with disease has yet to be established, there are five additional novel astroviruses, MLB1, MLB2, VA1, VA2 and VA3, described on the basis of phylogenetic analysis.[88] Astrovirus infections predominate in young children aged between 4 months and 4 years, and account for between 2% and 10% of cases of diarrhoea in children. The disease tends to be milder and more frequently encountered in community-based studies.[89] One such study in Mexico estimated the incidence of astrovirus gastroenteritis to be 0.1 episodes/child per year.[90] Seroepidemiological studies have demonstrated that more than 90% of children in the USA will have experienced astrovirus infections by the age of 6–9 years.[91] Astrovirus has been detected in all countries where sufficiently sensitive detection methods have been used. In temperate countries it shows a similar seasonal distribution to rotavirus but peaks earlier.

Astrovirus is transmitted faeco–orally either directly or by ingestion of food. It infects the upper small intestine but the mechanism of diarrhoea is not known. The features of the illness are similar to those of rotavirus but may be milder and its duration is 4–5 days on average. However, in Bangladesh, astrovirus was found to be associated with prolonged diarrhoea.[87]

Diagnosis used to be solely by electron microscopy, but this is now being replaced by more sensitive and easy-to-perform ELISA or by detection of the genome by RT-PCR. Treatment is by managing dehydration. There is no vaccine available and little is known of immunity to infection, other than that children with immunodeficiency syndromes excrete the virus for long periods.[92]

Norovirus

VIROLOGY

Genus *Norovirus*, of which *Norwalk virus*[93] is the type species, belongs to the family *Caliciviridae*. Norovirus has an unenveloped virion with icosahedral symmetry, measuring 27–35 nm in diameter with a feathery-ragged outline (Figure 18.9). Its genome is positive-sense, single-stranded RNA, approximately 7 kb in length, with a stretch of poly A sequence at its 3′ terminus.[94] The genome contains three ORFs, of which ORF2 encodes VP1, a 59 kDa protein, on which the antigenicity of a virus strain is expressed. Since neither animal model nor cell culture systems are available to test the infectivity of norovirus other than human volunteers, serotypes of Norovirus have not been established. The genome of norovirus exhibits a great diversity and there are more than 30 genotypes that are distributed into five genogroups (GI–GV)[95] of which human noroviruses cluster into GI, GII and GIV.[96]

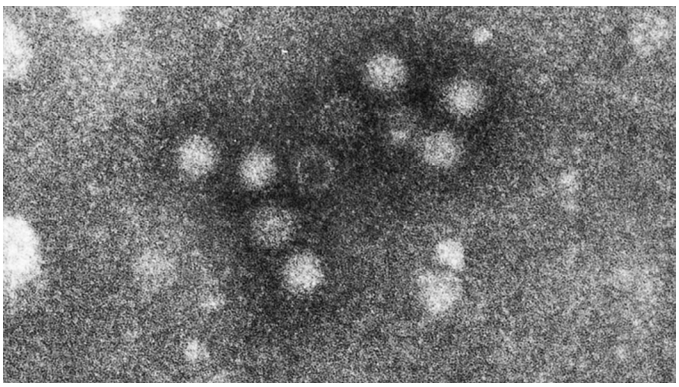

Figure 18.9 Negative-stain electron micrograph of norovirus with a feathery-ragged outline (×200 000).

EPIDEMIOLOGY

Norovirus spreads faeco–orally from person to person and from contaminated food and water; the respiratory route has also been suggested from epidemiological observations that aerosolized saliva or vomitus can be the source of infection.[97] In temperate countries, norovirus gastroenteritis tends to show winter seasonality. Norovirus was initially thought solely to cause epidemic gastroenteritis limited to older children and adults, but it is the cause of gastroenteritis in four epidemiological settings. These include: (1) Epidemic food-borne gastroenteritis that affects a large number of otherwise healthy adults over a short period of time; the illness usually resolves spontaneously without sequelae. (2) Sporadic gastroenteritis in the community.[98] (3) Healthcare-associated infections occurring in semi-closed settings such as hospital wards and residential homes resulting in often prolonged illness with increased severity. (4) Infantile diarrhoea in both developing and developed countries.[99] In a study in Finnish children, norovirus was responsible for 20% of gastroenteritis cases.[100] Similarly, in Iraq,[101] Libya,[102] and Brazil,[103] norovirus was detected in 30%, 18%, 15%, of diarrhoeal children less than 5 years of age, respectively. Norovirus is now the most common virus detected in children with acute gastroenteritis seeking medical attention in the US, where rotavirus vaccine has been part of the childhood immunization programme since 2006.[104]

CLINICAL FEATURES

Norovirus causes an illness with an abrupt onset of vomiting, diarrhoea and abdominal pain following an incubation period of 1–2 days. The illness is generally mild and fever rarely exceeds 38°C. Recovery follows within 1–3 days, but excretion of norovirus into stool lasts longer, sometimes up to 4 weeks. Approximately half of those infected with norovirus remain asymptomatic.

DIAGNOSIS

Similar to other viral gastroenteritis cases, diagnosis is based on the detection of virus in stool specimens. Historically, immune-electron microscopy was used to detect norovirus particles in stool specimens obtained in the acute phase of the illness. However, the diagnosis of norovirus gastroenteritis is now most commonly undertaken by sensitive, real-time PCR assays with genogroup-specific Taqman probes that provide both virus detection and quantification.[105] When the amount of virus is abundant, this will be followed by qualitative RT-PCR to amplify the end of ORF1 and the first part of ORF2 and subsequent sequencing to determine the genotype of a strain. In practice, a web-based genotyping tool is conveniently used (see: http://www.rivm.nl/mpf/norovirus/typingtool). Although much less sensitive than these molecular-based assays, commercial antigen detection kits have been developed; immune-chromatography, in particular, allows diagnosis at the point of care. While the

specificity of such immunoassays is close to 100% for those norovirus genotypes to which antibodies used in the kit are raised, diagnostic accuracy in practice may be lower because of the antigenic diversity likely to be encountered.

IMMUNITY

Short-term homologous immunity (against the infecting strain) appears to follow an episode of norovirus gastroenteritis. However, great antigenic diversity permits repeated illness with different norovirus strains. It was noticed in early challenge studies with volunteers that there are individuals with natural resistance against some strains of norovirus. Recent progress in understanding the relationships between norovirus and histo-blood group antigens has partly solved this mystery.[106] Norovirus appears to use ABO and Lewis blood group antigens expressed on the mucosal surface of the enterocytes as viral receptors; thus, non-secretors, in whom such antigens are not expressed on the intestinal mucosa, are resistant to norovirus infection.[107] Recent studies propose an evolutionary model in which the most prevalent GII.4 noroviruses persist by altering histo-blood group antigen binding targets as well as changing epitopes surrounding the binding pocket over time.[108]

TREATMENT AND PREVENTION

There is no specific therapy. Vaccines using virus-like particles are under clinical development.

Sapovirus

Genus *Sapovirus* (the type species *Sapporovirus* was found in Sapporo, Japan[109]), within the family *Caliciviridae*, has an unenveloped virion with icosahedral symmetry, measuring 30–35 nm in diameter, with characteristic cup-like depressions (*calyx* = Greek for a 'cup', hence, *calici*[110]), on its surface (Figure 18.10). Negative-stain electron microscopy reveals characteristic particle morphology with cup-like depressions, often described as the 'Star of David' (Figure 18.10). Its genome is positive-sense, single-stranded RNA of approximately 7 kb in length with a stretch of poly A sequence at its 3′ terminus. Unlike norovirus, sapovirus encodes the capsid protein contiguous with the large non-structural polyprotein (ORF1). The junction that corresponds to ORF1 and ORF2 of norovirus consists of a one- or four-nucleotide overlap between the stop codon of ORF1 and the first AUG codon of ORF2. This creates a −1 frameshift. The 3′ end of ORF1 encodes a single polypeptide of 62 kDa.

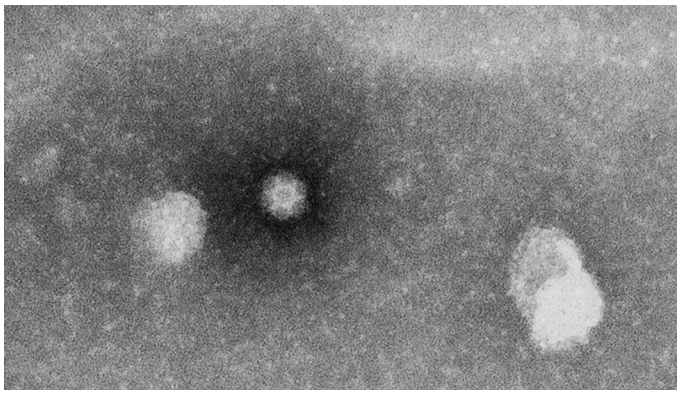

Figure 18.10 Negative-stain electron micrograph of a sapovirus with the classical 'Star of David' morphology (×200 000).

Illness due to sapovirus tends to predominantly occur in young children, and virtually all children appear to have experienced infection by sapovirus by the age of 5 years. Sapovirus gastroenteritis may occur year-round, although it seems to occur more frequently in winter. Sapovirus accounts for approximately 5% of cases of infantile diarrhoea[111,112] and is distributed globally. Sapovirus rarely causes outbreaks of foodborne gastroenteritis.

Sapovirus spreads faeco-orally and infects, and causes predominantly diarrhoea in infants and young children. Protective immunity appears to follow infection, since adults rarely get sapovirus gastroenteritis.

While a typical calicivirus-like morphology under the electron microscope strongly suggests the presence of sapovirus, the definitive diagnosis needs to be made based on either antigen detection or identification of the sapovirus genome by RT-PCR.[106]

Treatment is by management of dehydration. There is neither specific antiviral chemotherapy nor a vaccine available.

Other Viruses

A number of other viruses, including coronavirus[113] torovirus,[114] picobirnavirus[115,116] pestivirus,[117] Aichi virus[118] and bocavirus[119] have been detected in stool specimens of patients with acute gastroenteritis. Their clinical and epidemiologic significance as aetiological agents of diarrhoea is being investigated.

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