Enteric Viruses

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Introduction

Viruses interact with the gastro-intestinal tract in a number of ways. Some viruses such as hepatitis A virus and the enteroviruses use the intestine as a portal of entry and rarely, if ever, produce diarrhoeal disease. Others cause diarrhoeal disease only when the immune system is compromised, for example, HIV and cytomegalovirus (HHV-5). Human papillomaviruses and Kaposi's sarcoma associated herpesvirus (HHV-8) can affect the gastro-intestinal tract causing local tumours. On stool electron microscopy, bacteriophages can be seen (Fig. 1) which can be mistaken for other viruses. Bacteriophages are viruses that infect bacteria and are involved only indirectly in human disease, for example, acting as vectors for toxin genes (e.g. shiga toxins 1 and 2 in *Escherichia coli* 0157). However, here we will concentrate on the virology and laboratory diagnosis of the enteric viruses that are primary pathogens causing diarrhoeal disease (Table 1). The relative importance of viruses and the various enteric viruses depends upon the patient's age and their state of immunity. Undoubtedly, viruses are the most important causes of diarrhoeal disease in infants and young children whether HIV-infected or not [1].

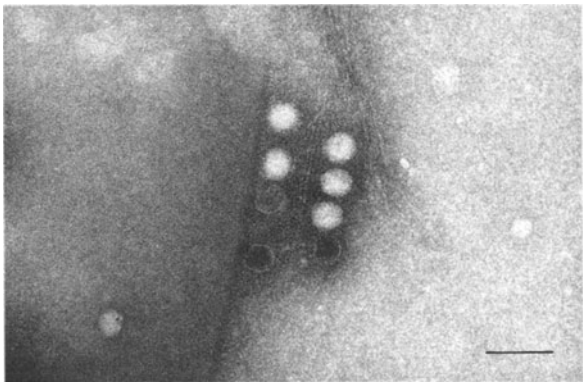


Fig. 1. Bacteriophage particles can easily be misidentified as potential viral enteropathogens. Bar, 100 nm

Table 1. The enteric viral enteropathogens

	Genome	Size	Relative importance in children	Relative importance in adults	Causes outbreaks
Rotavirus	dsRNA, linear segmented	80 nm	++++	+	+
Adenovirus	DNA, linear	80 nm	++	+	-
Astrovirus	ssRNA, linear positive-sense	28–34 nm	++	+	+
Calicivirus					
Norwalk-like	ssRNA, linear, positive-sense	28–32 nm	++	+	+
Sapporo-like	ssRNA, linear positive-sense	28–32 nm	++	++	+
Coronavirus	ssRNA, linear, positive-sense	60–200 nm	+	?	?
Torovirus	ssRNA, linear, positive-sense	100–140 nm	+	?	?
Picobirnavirus	dsRNA, linear, segmented	40–50 nm	+	?	?
Pestivirus	ssRNA, linear, positive-sense	45–55 nm	+	?	?

D. Dionisio (ed.), *Textbook-Atlas of Intestinal Infections in AIDS*

Rotavirus

Human rotavirus was first described as a human pathogen in 1973, when Ruth Bishop and colleagues observed virus particles, which they named duovirus, on thin-section electron microscopy of duodenal biopsy specimens from an infant with acute watery diarrhoea [2]. Subsequently, it was found that virus could be easily detected by negative stain electron microscopy of faeces. The virus was re-named rotavirus because of its characteristic wheel-shaped (rota is Latin for a wheel) morphology (Fig. 2).

Virology

Rotavirus is a member of the family *Reoviridae* and has a double-stranded segmented RNA genome. There are 11 genomic segments and each encodes one or more polypeptides (Table 2). It is 75–80 nm in diameter with a characteristic double-shelled capsid that gives it a wheel-shaped appearance. The core of the virus contains VP (virus proteins) 1, 2 and 3 and the 11 genomic segments. The inner capsid is composed entirely of VP6 and the outer capsid is composed of VP4 and VP7 (Fig. 3). The remaining proteins are involved in replication and are found only

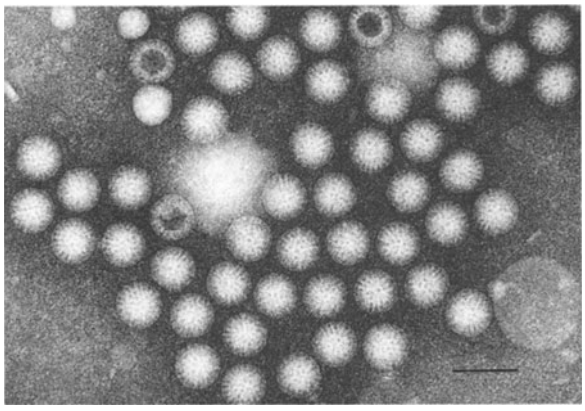


Fig. 2. A negative stain electron micrograph of faeces showing a cluster of rotavirus particles. Bar, 100 nm

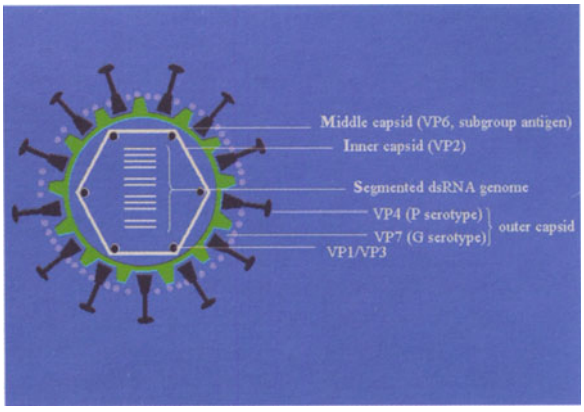


Fig. 3. A cartoon showing the structural proteins and genome of rotavirus

Table 2. Rotavirus genome and gene products

Genome segment	Molecular size (bp)	Gene product	Molecular weight (kDa)	Location in virion	Function
1	3,302	VP1	125	Core	RNA polymerase
2	2,690	VP2	94	Core	RNA binding
3	2,591	VP3	88	Core	Guanylytransferase
4	2,362	VP4 (VP5*+VP8*)	88[a]	Outer capsid	Cell attachment and penetration; Haemagglutinin; neutralizing antigen [P-(protease sensitive) serotype]
5	1,581	NSP1	53	Non-structural	RNA binding (zinc finger)
6	1,356	VP6	41	Inner capsid	Group and subgroup antigen
7	1,104	NSP3	34	Non-structural	RNA binding
8	1,059	NSP2	35	Non-structural	RNA binding
9	1,062	VP7	38	Outer capsid	Neutralizing antigen [G-(glycoprotein)-serotype]
10	751	NSP4	28	Non-structural	Virus assembly; enterotoxin
11	667	NSP5	26	Non-structural	RNA binding

[a]Virus protein (VP)4 must be cleaved into VP5* and VP8* by proteolysis for full infectivity.

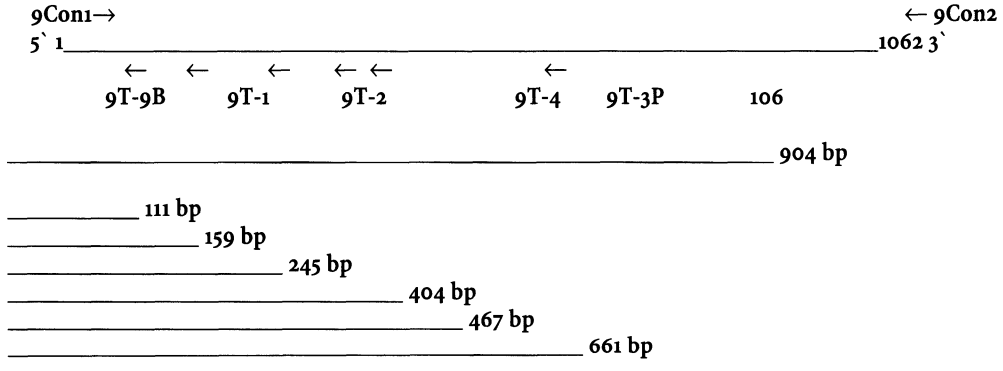
in infected cells and are thus termed NSP (non-structural proteins) of which there are 5 (NSP1-5). VP4 (VP5*, VP8*) and VP7 are involved in attachment to and penetration into host enterocytes. These outer capsid proteins carry neutralising epitopes, and antibody to these epitopes confers immunity to infection. There is still debate over whether the inner capsid protein VP6 carries neutralising epitopes [3].

Epidemiological Markers and Diversity

Rotavirus can be subdivided into one of seven groups (A–G) by epitopes on VP6. However, humans are infectable only by groups A, B and C. Of these, group A rotaviruses are the most important, and all people will have experienced recurrent group A rotavirus infections throughout their lifetime. Approximately 30% of adults have serological evidence of group C rotavirus infection [4]. However, in a survey of childhood diarrhoea in Malawi, only 3.3% of children were found to have group C rotavirus infection and almost two-thirds of these were co-infected with group A rotaviruses [5]. Group B rotaviruses have been responsible for large outbreaks of diarrhoeal disease in adults and children in China and India [6]. Rotaviruses can also infect a large number of animal species, for example, group A rotaviruses can cause infection in primates, cattle, pigs, sheep, horses, dogs, cats and turkeys. It was thought that the viruses were species-specific but there is increasing evidence of cross-species transmission. Group A rotaviruses can be further subdivided into subgroups, again, on the basis of epitopes on VP6. They can have subgroup specificities I, II, I + II or neither. Group A rotaviruses can also be serotyped and there are two major antigenic types. These are G (or glycoprotein) types expressed on the outer capsid protein VP7 of which there are at least 14 types. The P (or protease sensitive) serotypes are encoded on VP4 (VP5*, VP8*); there are at least 20 P types. Antibody to a particular P or G type does not necessarily confer immunity to the others. G and P types were originally delineated by using monoclonal antibodies that neutralised rotavirus growth. However, this required establishing the rotavirus in artificial culture which is not a trivial task and then determining neutralising antibody activity. To overcome this, RT-PCR-based techniques have been developed for P and G typing (Fig. 4). Thus far, all the G serotypes and G genotypes have coincided. Unfortunately, this has not been established for all the P serotypes and P genotypes, thus by convention P serotypes are indicated by rounded brackets, e.g. P(3a) and P genotypes by squared brackets, e.g. P [8]. Variability in the molecular mass of the 11 genomic sequences (as deter-

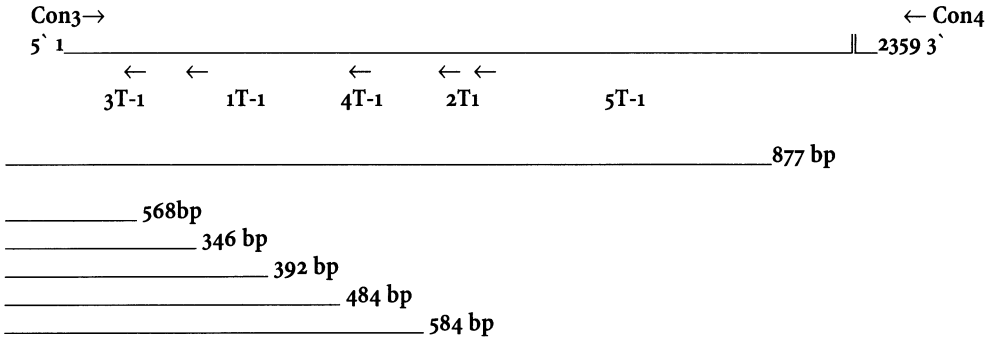
Fig. 4a, b Primers for detection and G- and P- genotyping rotavirus by RT-PCR.
a Primers used for VP7 (G) typing

Primer	Strain	Serotype	NT	Sense	Sequence (5` to 3`)	Primer type
9Con1	Wa	G1	37–56	+	TAG CTC CTT TTA ATG TAT GG	Consensus
9Con2	Wa	G1	922–941	-	GTA TAA AAT ACT TGC CAC CA	Consensus
9T-1	Wa	G1	176–195	-	TCT TGT CAA AGC AAA TAA TG	Type Specific
9T-2	S2	G2	262–281	-	GTT AGA AAT GAT TCT CCA CT	Type Specific
9T-3P	107E	G3	484–503	-	GTC CAG TTG CAG TGT TAG C	Type Specific
9T-4	ST3	G4	423–440	-	GGG TCG ATG GAA AAT TCT	Type Specific
106	HMG89	G8	681–697	-	TCT TCA AAA GTC GTA GTG	Type Specific
9T-9B	116E	G9	131–147	-	TAT AAA GTC CAT TGC AC	Type Specific



b Primers used for VP4 (P) typing

Primer	Strain	Serotype ([GT])	NT	Sense	Sequence (5` to 3`)	Primer type
Con3	Ku	1A[8]	11–32	+	TGG CTT CGC CAT TTT ATA GAC A	Consensus
Con2	Ku	1A[8]	868–887	-	ATT TCG GAC CAT TTA TAA CC	Consensus
1T-1	Ku	1A[8]	339–356	-	TCT ACT TGG ATA ACG TGC	Type Specific
2T-1	RV5	1B[4]	474–494	-	CTA TTG TTA GAG GTT AGA GTC	Type Specific
3T-1	1076	2A[6]	259–278	-	TGT TGA TTA GTT GGA TTC AA	Type Specific
4T-1	K8	3[9]	385–402	-	TGA GAC ATG CAA TTG GAC	Type Specific
5T-1	69M	4[10]	575–594	-	ATC ATA GTT AGT AGT CGG	Type Specific



mined by gel electrophoresis) can also be used to type rotaviruses. In particular the rate of migration of the lowest molecular weight segments (in particular segment 11) splits rotavirus into long, short and super-short electropherotypes. In addition, comparison of the patterns of migration of all the genomic segments when co-electrophoresed can also give evidence of relatedness or otherwise (Fig. 5). Despite this plethora of grouping and typing methods, group A rotaviruses can generally be subdivided into two major genogroups named Wa and DS-1 after their prototype isolates. Members of each genogroup possess particular sets of electropherotype, subgroup and P and G type. The Wa genogroups are predominantly long electropherotype, sub group II with VP4 P[6] or P[8] and VP7 G 1, 3 or 4 serotypes. DS-1 genogroup rotaviruses are generally of short electropherotype, subgroup I and P[4] and G2 serotype specificity. However, this neat segregation is proving to be less than absolute [7]. Because rotaviruses have a segmented genome, novel genogroups can, and have, emerged by re-assortment (exchange of genomic segments in whole or partially when two rotaviruses co-infect the same cell). Novel human-feline, human-canine and human-bovine rotaviruses have been described. In addition, antigenic drift also occurs when point mutations accumulate. Thus there is evidence of a greatly increasing diversity of rotaviruses. Previously, examination of global collections of rotaviruses demonstrated that serotypes G1 to G4 accounted for the vast majority of human infections (80%–90% of strains) [8]. Recently, novel strains including G5 in South America, G8 in Malawi, South Africa and Nigeria and G9 have emerged [9–12]. Indeed, G9 is a globally important serotype being detected in almost every country it has been sought [12, 13].

Rotavirus is the commonest cause of acute dehydrating watery diarrhoea in infants worldwide. It is

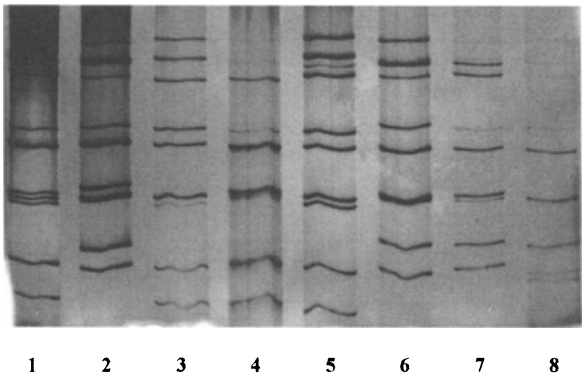


Fig. 5. Polyacrylamide gel electrophorograms of rotavirus dsRNA from faeces

Lanes 1–2: Standard strains showing long electropherotype profile (strain Wa, lane 1), and short electropherotype profile (strain DS-1, lane 2)
Lanes 3–7: Malawi field strains showing long electropherotype profiles (lanes 3–5), and short electropherotype profiles (lanes 6–7)
Lane 8: Malawi field strain showing mixed short electropherotype profile

responsible for 20%–60% of cases of infantile diarrhoea in both hospital and community-based surveys [1]. It is estimated to be responsible for between 500,000 and 800,000 deaths each year in children under 5 years and most (but not all) of these deaths are in developing countries [14]. In temperate countries, there are peaks of infection in the winter months. In sub-Saharan Africa, infection is prevalent throughout the year but with a peak in the dry season [15]. Although most infections present in infants, infections do occur in adults, for example, with group B rotavirus or unusual group A serotypes. It is also a cause of travellers' diarrhoea.

Pathogenesis and Immunity

Rotavirus is transmitted predominantly by the faecooral route although there are some indications of spread by the air-borne route. An infected child can excrete up to 10^{11} rotavirus particles per millilitre of faeces and the infective dose is as low as 10^{2} particles. Rotavirus infects the whole of the small intestine but cannot infect colonocytes. It infects mature villous body enterocytes but cannot infect immature crypt enterocytes. At least four mechanisms have been suggested by which rotavirus causes diarrhoea. Firstly, the disaccharidase enzymes lactase, sucrase and maltase are essential for absorption of sugars. If disaccharides are not cleaved to monosaccharides by these enzymes then they cannot be absorbed, remain in the gut lumen and induce an osmotic diarrhoea. The disaccharidase enzymes are integral proteins in the microvillar membranes. They have a short half-life and are continually replaced by new enzymes synthesised in the enterocyte. Rotavirus appears to interfere with the trafficking of newly synthesised disaccharidases from the Golgi to the microvilli thus leading to a relative disaccharidase deficiency [16, 17]. Secondly, on maturation, rotavirus kills infected enterocytes by oncosis [18]. This leads to a higher rate of death of enterocytes which cannot be replaced quickly enough, and villous blunting occurs leading to a loss of absorptive surface area. Thirdly, there are suggestions that rotavirus affects the intestinal neuroendocrine axis [19]. Finally, recent experiments have delineated the first viral toxin to be described [20]. NSP-4 is involved in viral capsid assembly and entry into endosomes. However, it also has an effect similar to that of cholera toxin in causing a secretory diarrhoea. In immunocompetent children, rotavirus diarrhoea persists for an average of 6–7 days. It appears that cell-mediated immunity is important in resolution of the acute infection [21] and it is noteworthy that children with T-cell deficits exhibit long-term excretion of rotavirus. In general, one infection with rotavirus confers protection against symptomatic re-infection but not silent re-infection [22]. Whether this is true for HIV-infected infants is not known. The role of individual components in immunity is also not entirely clear but the best surrogates of immunity appear to be IgA antirotavirus coproantibodies and serum IgG anti-rotavirus antibody [23].

Laboratory Diagnosis

A patient with acute watery diarrhoea due to rotavirus excretes large numbers of virus particles (ca. 10^{11}/ml). These are easily seen and because of their characteristic shape and size recognised on direct negative stain electron microscopy of stool (Fig. 2). Electron microscopy is the only "catch-all" technique for the diagnosis of infection by rotavirus or other enteric viruses, but is limited by sensitivity (it requires in the order of 10^{5} particles/ml) and the skill of the electron microscopist. The pathogen-specific diagnostic tests for rotavirus include methods for genome and antigen detection. Virus culture is not a feasible routine diagnostic test.

Genome Detection

The most sensitive and specific diagnostic tool is reverse transcriptase polymerase chain reaction (RT-PCR). However, it is not available as a diagnostic kit, therefore RNA extraction, reverse transcription and primer design vary from laboratory to laboratory. However, its use will detect very low numbers of rotavirus particles when not detectable by electron microscopy or antigen detection kits, and if P and G genotype-specific primers are used, will provide valuable epidemiological information. Polyacrylamide gel electrophoresis of RNA extracted from diarrhoeic stool provides an inexpensive and specific diagnostic tool. The 11 dsRNA segments of the genome are unique to rotavirus and comparison of electrophoretic patterns also gives epidemiological information. The whole procedure takes as little as 5 h and although less sensitive than RT-PCR, is equivalent to some antigen detection tests and electron microscopy [24].

Antigen Detection

The two main formats available are antigen capture ELISA and latex particle agglutination (LPA). In general, the former is more sensitive and specific than LPA. LPA has the advantage that it is cheaper and can be used as a rapid "one-off" test, whereas the ELISA

format is better adapted to batch testing. It is possible, depending on the specificity of the antibodies used, for the tests to miss group B and C rotavirus infections.

Treatment and Prevention

There are no specific anti-rotaviral drugs and in most cases (even in HIV-infected children), the infection is self-limiting. The most important therapeutic intervention is to assess the degree of dehydration and institute oral or intravenous rehydration therapy as appropriate. Adjuncts to therapy include the administration of immunoglobulin orally [25,26] and the use of probiotics such as *Lactobacillus casei* spp. *rhamnosus* (strain GG), or *Bifidobacterium bifidum* [27]. Sources of immunoglobulin have included bovine colostrum, pooled human gamma-globulin fractions or hyperimmunised chicken egg yolk immunoglobulin (IgY). Each has worked well in decreasing the duration and frequency of diarrhoea, and oral immunoglobulin was used successfully to treat severe rotavirus diarrhoea in two bone marrow transplant recipients. Probiotic administration has a similar efficacy but has the added advantage of enhancing the specific immune response to rotavirus. Neither intervention has been tried in HIV-infected individuals.

Active immunisation against rotavirus infection has been an important goal for many years, which was almost realised in 1998 [14]. Both bovine and simian rotaviruses have been used following "Jennerian" principles (i.e. analogous to using cowpox to prevent smallpox), to protect against rotavirus infection. Both worked well (ca. 80% protection) in trials in Europe and the USA, but when used in developing countries in Africa and South America where they would have had greatest impact, they were far less efficacious. Thus reassortant vaccines were developed which had 10 dsRNA genomic segments of either simian (rhesus) or bovine rotavirus with the remaining segment encoding VP7 being replaced by that from a human rotavirus. Quadrivalent reassortant rhesus rotavirus vaccine incorporating VP7 genomic segments expressing G1, G2, G3 and G4 proved to be the most effective in trials in the USA, Europe and most importantly in South America. In 1998, following a cost effectiveness analysis which showed benefit, the quadrivalent vaccine was introduced into routine use in infants in the USA. From September 1998 until July 1999, an estimated 1.5 million doses were given to 800,000 infants (ca. 25% of the birth cohort). During this time, there were 15 cases of intussusception occurring in infants who had received the vaccine reported to the Vaccine Adverse Events Reporting System (VAERS) in the USA. A case-controlled study estimated an incidence of one case of intussusception for every 4,670 to 9,474 vaccinees [28], and the vaccine was withdrawn from use. Subsequently, a number of authors have disagreed with the association and have suggested that its use would still be very beneficial in developing countries where there is the highest rotavirus disease and mortality burden.

Nevertheless, despite this setback there are a number of new vaccines in production. These include a multivalent human bovine reassortant vaccine, a monovalent (G1) human rotavirus vaccine and a monovalent lamb rotavirus vaccine which has already been licensed in China [29]. However, as yet, there have been no trials giving any oral rotavirus vaccine to HIV-infected children. Since wild rotavirus infection is no more severe in HIV-infected than HIV-uninfected infants and induces a brisk serological response in both, such vaccines should be safe and effective [30]. They should, however, not be given to those with overt AIDS.

Adenovirus 40/41

Adenoviruses have been detected in stool samples from patients with and without diarrhoeal disease. However, only adenovirus types 40 and 41 which are difficult to establish in culture have been firmly associated with diarrhoeal disease [1].

Virology

The family *Adenoviridae* comprises two major genera, Mastadenoviruses and Aviadenoviruses. The former are mammalian pathogens. All have non-enveloped virion particles with icosahedral symmetry from 60–90 nm in diameter (Fig. 6). They are eas-

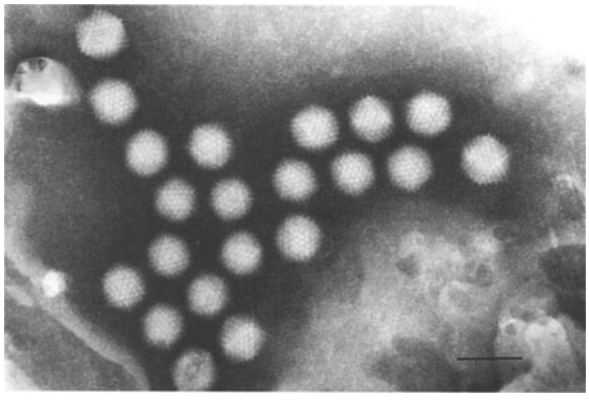


Fig. 6. A negative stain electron micrograph of faeces showing a cluster of typical adenovirus particles. Bar, 100 nm

ily recognisable on electron microscopy. Adenovirus has a linear double-stranded DNA genome from 30 to 38 kbp (kilobase pairs) long. There are at least 51 different human adenoviruses but only those in subgenus F (adenoviruses 40/41) cause diarrhoeal disease. Other adenoviruses, in particular some in subgenera B2 (e.g. types 11 and 50) and D (e.g. types 9, 20, 22, 23, 43–49), are excreted by HIV-infected patients but rarely by HIV non-infected individuals [31]. Although in most instances infection is asymptomatic, in some AIDS patients these viruses can cause hepatitis and colitis (as well as pnemonitis, nephritis and encephalitis) [32]. Except for subgenus F, adenoviruses are easily grown in a variety of cell culture lines and produce cytopathic effects.

Epidemiology, Pathogenesis and Immunity

In most surveys, adenovirus 40/41 is the second commonest cause of viral diarrhoea in hospitalised children, being responsible for 0.9%–11% of cases. However, serosurveys indicate that by the age of 3 years between 30% and 100% of children have neutralising anti-adenovirus 40/41 antibodies. It is also a minor cause of travellers' diarrhoea. However, there are preliminary data to suggest that previous infection does not provide protection against subsequent symptomatic re-infection [33]. Infection is spread by the faeco-oral route but food-borne or water-borne spread has not been described. There is no particular seasonality to infection with adenovirus 40/41. How it causes diarrhoea is not known, nor is much known on immunity to infection. However, in HIV-infected patients who are infected with subgenus D adenoviruses, there is evidence of impaired production of neutralising antibody against homologous virus [32].

Diagnosis, Management and Prevention

Adenovirus can be visualised easily on direct negative stain microscopy of faeces (Fig. 6). However, since a number of different adenoviruses can be excreted in stool, this does not prove that adenovirus is the aetiological agent. Immunoelectron microscopy using anti-adenovirus 40/41 antisera will help, and clumped virus particles should be seen. There are commercially available ELISA-based kits for the diagnosis of adenovirus 40/41 diarrhoea which appear highly sensitive and specific. There have been some indications that the current commercial kits are less efficient at detecting South African strains [34]. PCR-based techniques are available but not in kit form.

There is no specific therapy and management involves assessment of dehydration with appropriate rehydration. There are no vaccines for prevention of adenovirus 40/41 infection although a vaccine for other adenoviruses has been used to prevent infection in military recruits in the USA.

Astrovirus

Astroviruses were first described by Madeley and Cosgrove in 1975 when stools of infants with gastroenteritis were examined by electron microscopy (Fig. 7). They are named for their distinctive appearance on electron microscopy, namely, a smooth outer electron-dense shell with an inner 5- or 6-pointed negatively staining star-shaped core (astron is Greek for a star). Subsequently, human transmission experiments confirmed their role as viral enteropathogens and strains of astrovirus were established in artificial culture.

Virology

Astroviruses are small (28–34 nm), round unenveloped RNA viruses within the family *Astroviridae*. Their genome is positive-sense, unsegmented, linear single-stranded RNA of approximately 6.8 kb. It comprises three open reading frames (ORF). ORF 1a and ORF 1b encode non-structural proteins including a serine protease, RNA-dependent RNA polymerase and a nuclear localisation signal. ORF2 encodes a putative 90-kDa polyprotein that is cleaved by the viral serine protease into three to five structural proteins that make up the capsid. Astroviruses can be grown in the presence of trypsin on primary human fetal cells or more conveniently CaCo2 cells, but can require repeated blind passage. There are at least eight serotypes of human astrovirus. Astroviruses are

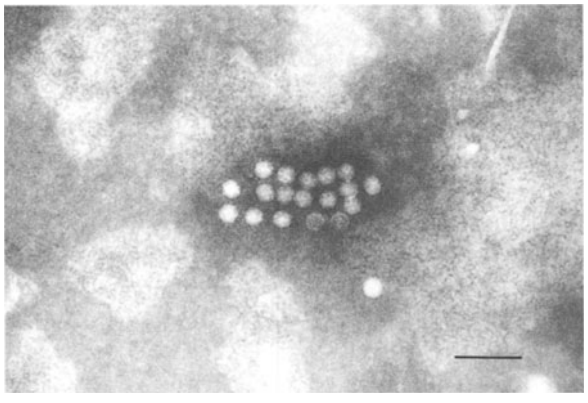


Fig. 7. A negative stain electron micrograph of faeces from a child with astrovirus diarrhoea. Bar, 50 nm

also a cause of diarrhoeal disease in a number of other animal species including birds, cats, dogs, sheep and cattle and there is some evidence of cross-species transmission. Astroviruses survived for 5–6 days when dried at 20°C in faecal material onto porous or non-porous material. Survival was significantly longer at 4°C [35]. This was equivalent to survival of adenovirus but less than that of rotavirus.

Epidemiology and Pathogenesis

Infection is acquired by the faeco-oral route, but the infective dose is not established. Although astrovirus is primarily a cause of childhood gastro-enteritis, it can cause outbreaks in adults especially with the less common serotypes [1]. It is responsible for 2%–11% of childhood cases of gastro-enteritis in hospitalised patients, but in up to 20% of those in community-based studies, thus reflecting its lesser severity compared to rotavirus [36–38]. In temperate countries, the peak of infection is in winter usually a month or so before the rotavirus peak. Although serotype 1 is most often implicated in any season, many serotypes co-circulate [39]. Food-borne outbreaks have also been described most often due to the rarer serotypes [40]. Over 90% of children in developed countries have anti-astrovirus antibodies by 9 years of age [41]. Astrovirus has a world-wide distribution being found in every country where it has been sought. In some studies astrovirus has been identified as a significant cause of chronic diarrhoea [42], for example, in a longitudinal study of HIV-infected adults in the USA [43]. However, no such association was found in smaller studies of HIV-infected children and adults [44, 45]. Prolonged excretion of astroviruses has been detected in immunodeficient and immunosuppressed patients [46]. Apart from this, little is known about immunity to infection, or how astrovirus produces diarrhoeal disease.

Diagnosis

Although electron microscopy was the tool first used for diagnosis, it is less sensitive than either the commercially available ELISAs or RT-PCR. RT-PCR based on conserved regions of the protease gene in ORF 1a is the most sensitive diagnostic tool but this is an "in-house" assay.

Treatment and Prevention

There are no specific antiviral drugs and the management is that of dehydration. There is no vaccine for prevention of infection.

Caliciviruses

In 1968, there was an outbreak of gastro-enteritis in teachers and pupils in a school in Norwalk, Ohio, USA. It was predominantly a vomiting disease and was termed winter vomiting disease. It was shown to be transmissible to volunteers by ultrafiltrates of stool and to be ether- and relatively heat-stable, but could not be established in cell or organ culture. In 1972, 27-nm viral particles were seen by immune electron microscopy using convalescent sera from one of the volunteers for the transmission experiment. The agent was called Norwalk virus and similar outbreaks of infection with similar small round-structured viruses (SRSV) were described elsewhere. In 1976, Madeley and Cosgrove detected calicivirus particles in diarrhoeic stools of children and subsequently similar caliciviruses were associated with outbreaks of infection. Eventually, it became clear that both the SRSVs and caliciviruses were genomically similar and both were assigned to the family *Caliciviridae* [47] in which there are two other genera (*Vesivirus,* feline calicivirus and *Lagovirus,* rabbit haemorrhagic disease virus).

Virology

The human caliciviruses comprise two genera Norwalk-like (NLV, previously SRSV) and Sapporo-like (SLV, previously classical caliciviruses) viruses, and viruses related to these genera can also infect pigs and cattle. Caliciviruses are small (23–32 nm) round unenveloped RNA viruses. Their genomes are single-stranded, linear, unsegmented positive-sense RNA. The genome is polyadenylated and approximately 7.6 kb in length. The genome of NLVs is divided into three ORFs. The first ORF encodes a polyprotein with motifs similar to helicase, cysteine proteinase and RNA-dependent RNA polymerase proteins of picornaviruses. ORF2 encodes the capsid protein and ORF3 encodes a small basic protein of unknown function. The SLV genome differs in that ORF1 encodes both the non-structural and capsid proteins, ORF2 is similar to ORF3 of NLV and ORF3 encodes another small basic protein of unknown function. On electron microscopy, NLVs have an indistinct feathery edge and amorphous substructure (Fig. 8). SLVs have a much more distinct structure. The major structural protein folds into 90 dimers and assembles in an icosehedral form but with 32 cup-shaped depressions (calyx is Greek for a cup), which impart the characteristic "Star of David" appearance on electron microscopy (Fig. 9). Based on RNA sequences of the RNA polymerase or capsid genes, the NLVs can be subdivided into two genogroups but the SLVs are more homogeneous.

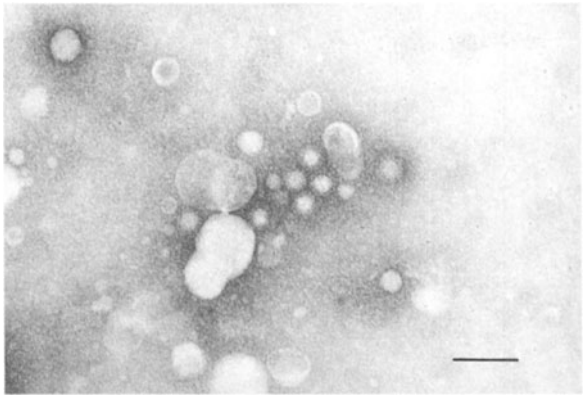


Fig. 8. A negative stain electron micrograph of Norwalk virus (previously termed small round structured virus). Bar, 50 nm

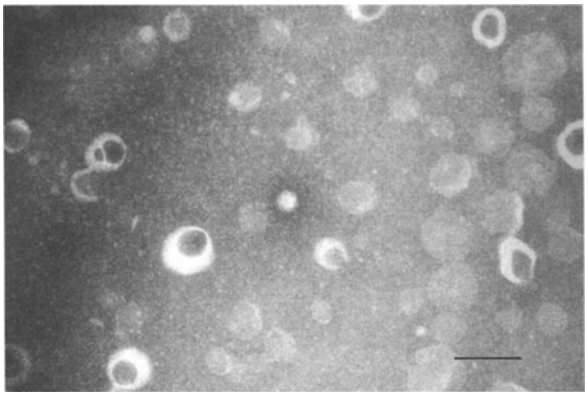


Fig. 9. Negative stain electron micrograph of Sapporo virus showing the "Star of David" shape typical of classical calicivirus. Bar, 50 nm

Calicivirus is currently species infected/virus genus/virus name/strain designation/year of isolation/country of isolation. Thus Norwalk virus is Hu/NLV/Norwalk virus/8FIIA/1968/US and Sapporo virus is Hu/SLV/Sapporo virus/1982/JA [48, 49]. However, common names are easier to cope with (Table 3). With increasing detection, more new human caliciviruses are being described.

Table 3. The human calicivirus

Norwalk-like viruses	
Genogroup I	Norwalk virus
	Southampton virus
	Desert Shield virus
	Cruise Ship virus
Genogroup II	Snow Mountain Agent
	Hawaii virus
	Mexico virus
	Toronto virus
	Lordsdale virus
	Grimsby virus
	Gwynedd virus
	White River virus
	Camberwell virus
	Melksham virus
Sapporo-like viruses	Sapporo virus
	Manchester virus
	Parkville virus
	London virus
	Plymouth virus
	Houston virus

Epidemiology, Pathogenesis and Immunity

The caliciviruses have a worldwide distribution. Some have been detected only in one geographical region but others have been found over the same period in eight countries on five continents [50]. Infections occur sporadically, as community-wide epidemics or as food-borne epidemics [51]. No particular seasonal distribution is evident for SLVs, but in temperate countries NLVs are more prominent in winter. The seroprevalence of calicivirus infection varies by age, virus group and geographical region. For example, in Kenya approximately 60% of adults have antibody to genogroup I NLV (Norwalk virus), 80% to SLV and 100% to genogroup II NLV (Mexico virus), whereas in Japan and parts of Southeast Asia, 82%–88% of adults were seropositive to Mexico virus [52, 53]. In terms of the relative importance of calicivirus in causing paediatric gastro-enteritis, a study in Finland found that of 832 children aged 2 months to 2 years, 20% of cases of diarrhoeal disease were due to NLVs and 9% to SLVs. In comparison, astroviruses were found in 10% of children, enteric adenoviruses in 6% and rotavirus in 31% [54]. Transmission can occur directly by the faeco-oral route or even via aerosols produced by projectile vomiting. Person-to-person transmission has occurred prior to symptomatic disease and contact while playing American football [55, 56]. Food-borne and water-borne outbreaks are not uncommon. Foodstuff implicated as vehicles includes shellfish such as oysters which concentrate human excreta in water, raspberries sprayed with human excreta and even food contaminated by presymptomatic food-handlers [51, 55]. The infective dose is low (<100 particles) and there can be prolonged asymptomatic shedding of virus. In addition, the caliciviruses are quite stable (to 100 ppm chlorine, freezing and heating to 60°C). The incubation period is 1–2 days, and

Fig. 10. Examples of primers used for the detection of human calicivirus from faeces

Primer	Sense	Sequence	Amplicon Size
Norwalk-like viruses (from [70])			
JV12	Outer +	5'ATA CCA CTA TGA TGC AGA TTA 3'	333 bp
SM31	Outer –	5'CGA TTT CAT CAT CAC CAT A 3'	
N1	Inner +	5'GAA TTC CAT CGC CCA CTG GCT 3'	114 bp
E3	Inner –	5'ATC TCA TCA TCA CCA TA- 3'	
(a nested RT-PCR)			
Sapporo-like viruses (from [71])			
Sapp 36	+	5'- GTT GCT GTT GGC ATT AAC A-3'	470 bp
Sapp 35	–	5'- GCA GTG GGT TTG AGA CCA AAG-3'	
Both NLV and SLV (from [72])			
P289	–	5'- TGA CAA TGT AAT CAT CAC CAT A-3'	*
P290	+	5'- GAT TAC TCC AAG TGG GAC TCC AC-3'	
*NLV 319 bp SLV 339 bp			

in general the NLVs produce a predominantly vomiting illness with only 30% also having diarrhoea, whereas the SLVs produce predominantly diarrhoea with some vomiting. The illness is usually short-lived (2–3 days) but in volunteer studies, excretion of NLVs persisted for up to 2 weeks [57].

Following infection there is a homotypic antibody response to the infecting virus but this does not necessarily persist. Repeat symptomatic infections can occur in volunteers after re-challenge and childhood exposure does not protect adults from disease [58]. In addition, there is great diversity in the calicivirus strains that infect humans [59]. There is very little information on calicivirus infection in immunocompromised hosts or those infected with HIV.

Diagnosis

Although these viruses were first discovered by electron microscopy this is a relatively insensitive diagnostic tool, except in the first few days of gastroenteritis [48]. Some antigen detection tests are available using ELISA or radio-immuno-assay formats but they are not able to detect all caliciviruses. RT-PCR is the most sensitive diagnostic tool but no single primer set will detect all viruses (Fig. 10). None of the tests are commercially available.

Treatment and Prevention

There are no vaccines available for prevention of infection.

Other Viruses

For the remaining viruses, either their causative role in diarrhoeal disease is not entirely proven or they are responsible for a minority of cases.

Coronavirus

Coronaviruses are enveloped pleomorphic viruses, 60–200 nm in diameter, which have characteristic club-shaped glycoprotein spikes protruding from their surface (Fig. 11). The genome is single-stranded positive-sense RNA of 27–32 kb in size. Although coronaviruses have been isolated from diarrhoeic stool [60], they are found on electron microscopy as

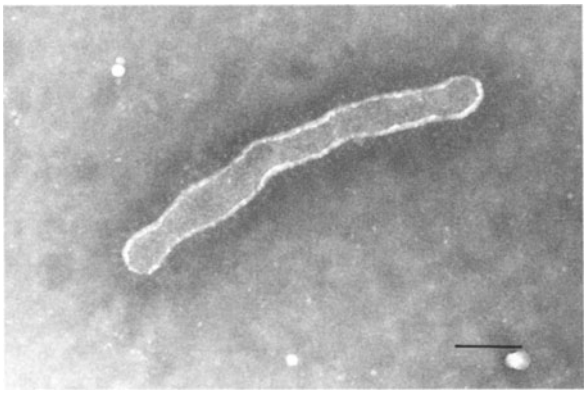


Fig. 11. Negative stain electron micrograph of a coronavirus from stool. Bar, 100 nm

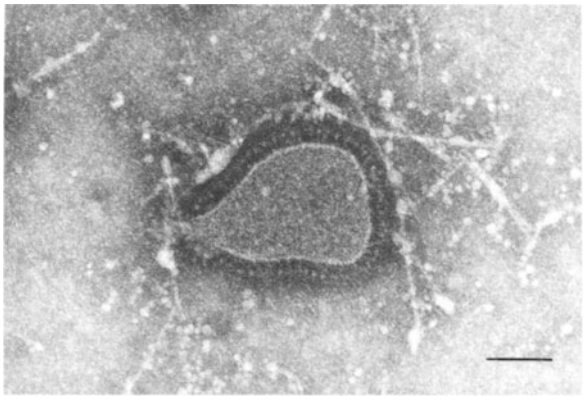


Fig. 12. Negative stain electron micrograph of a torovirus from the stool of a child with gastro-enteritis. Bar, 100 nm

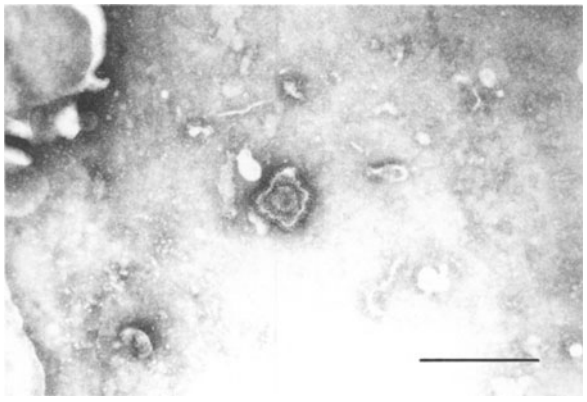


Fig. 13. A pestivirus in the stool of a case of childhood gastro-enteritis. Bar, 100 nm

frequently from controls as from patients with gastro-enteritis. The diagnosis is based entirely on negative stain electron microscopy. Virus culture is lengthy and there are no antigen detection or genomic-based diagnostic methods.

Torovirus

Toroviruses are members of the family *Coronaviridae* and are enveloped with a fringe of glycoprotein spikes (Fig. 12). They are 100–140 nm in diameter and have a tightly coiled tubular nucleocapsid that assumes the shape of a toroid (doughnut shape). This gives them the name torovirus [61]. Their genome is single-stranded positive-sense linear RNA of over 20 kb in size. They were first recognised as causes of diarrhoea in cattle (Breda virus) and horses (Berne virus). Toroviruses are found in 3%–35% of samples from children with gastro-enteritis. Torovirus infection was detected more frequently in immunocompromised and nosocomially infected children in one survey, while in another it was found as frequently in those over 3 years as in those under 3 [62, 63].

Diagnosis is by negative stain electron microscopy of faecal suspensions, where characteristic spherical or kidney-shaped fringed virus particles are seen (Fig. 12). Immunoelectron microscopy has also been used with animal antisera. A human torovirus has been maintained in artificial culture, but this is not a realistic diagnostic method.

Pestivirus

Pestiviruses are a genus within the family *Flaviviridae* and are related to hepatitis C virus. They are enveloped viruses some 40–60 nm in diameter with an external glycoprotein fringe (Fig. 13). Their genome is single-stranded positive-sense RNA approximately 11–12 kb in length. Bovine diarrhoea virus (BDV) is a pestivirus and a survey of 128 children with diarrhoea detected BDV antigens in 30 (23%) of these [64]. A slightly greater proportion of HIV-seropositive patients with chronic diarrhoea (17.8%) were BDV seropositive compared to HIV-infected patients without diarrhoea (15.2%) in a Zambian study [65].

Diagnosis is by antigen detection using monoclonal anti-BDV antibodies, although virus can rarely be seen on direct negative stain electron microscopy of stool (Fig. 13).

Picobirnavirus

Picobirnaviruses (and Picotrirnaviruses) are a novel group of viruses tentatively included within the family *Birnaviridae* [66]. They are small (30–40 nm diameter) unenveloped viruses with icosahedral symmetry. Their genome is double-stranded linear bi- or tri-segmented RNA with segment lengths of 2.6 and 1.9 kbp for picobirnaviruses and 2.9, 2.4 and 0.9 kbp for picotrirnaviruses.

Picobirnaviruses have been described in association with childhood gastro-enteritis and outbreaks of gastro-enteritis in elderly care facilities but their pathogenic role is not entirely clear. They have been strongly associated with diarrhoea in HIV-infected patients in Argentina, but similar studies in HIV-infected adults or children in Venezuela did not confirm this association [43–45, 67, 68].

Diagnosis is by polyacrylamide gel electrophoresis of RNA extracted from faeces. Characteristic bands (2 or 3) of the correct molecular mass range demonstrate the presence of virus. Recently, an RT-PCR has been

developed which should be more sensitive [69]. There are no commercially available kits for detection.

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