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Novel Viruses Associated with Gastroenteritis

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Acute infectious gastroenteritis is an extraordinarily common and universal illness that is extremely costly in terms of health care expenditures and lost days of productivity. In the United States, gastroenteritis is probably responsible for the hospitalization of >200,000 people and for the deaths of >500 children each year (1, 2). Worldwide, gastroenteritis probably results in 3 to 5 billion episodes of diarrhea and 5 to 10 million deaths each year (1–3). The etiologic agents of most cases of gastroenteritis are bacteria, parasites, and certain members of two well recognized groups of viruses (rotavirus and adenovirus; Table 1). Investigators have assumed for many years that the agents of the remaining cases of gastroenteritis of unknown etiology are viruses (3). Paradoxically, conventional gastrointestinal enteroviruses have never been associated with gastroenteritis.

In the early 1970s, increasing use and refinement of two investigative techniques (transmission electron microscopy [EM] and cell culture) and a serious local public health problem (an epidemic of winter vomiting disease in Norwalk, Ohio) initiated interest and facilitated research in the viral etiology of gastroenteritis. Subsequently, several novel viruses have been associated with gastroenteritis (Table 1). Many of these novel viruses have several characteristics in common: (i) small size, simple structure, and EM and physical characteristics; (ii) fastidious nature (inability to be cultured routinely); (iii) ability to elicit severe diarrhea that is easily alleviated by fluid and electrolyte replacement; and (iv) previously recognized and well known abilities to cause gastroenteritis in animals.

The following are brief descriptions of two well recognized and several novel viruses associated with gastroenteritis. References 1 through 8 are excellent sources of additional information on these viruses.

TABLE 1. Viruses associated with gastroenteritis

Well recognized viruses	
Rotavirus group A (typical)	
Adenovirus types 1–39	
Novel viruses	
Rotavirus groups B-F (atypical)	
Adenovirus types 40 and 41 (enteric, fastidious)	
Norwalk virus (small, round, structured)	
Norwalk	Taunton
Hawaii	Amulree
Snow Mountain	Sapporo
Montgomery County	Otofuke
Calicivirus	
Astrovirus	
Coronavirus	
Pestivirus	
Parvovirus	
Picobirnavirus	

Rotavirus

Rotavirus is a 70-nm, nonenveloped, 11-segment double-stranded (ds) RNA virus with two icosahedral capsids. It infects humans and most species of animals. Rotaviruses are members of the family *Reoviridae* and are named as such because of their wheellike appearance when observed by EM.

Typical rotaviruses are intimately associated with severe gastroenteritis worldwide in the very young of all susceptible host species. The most commonly affected humans are children age 6 mo to 2 yr old.

Until 1982, rotaviruses were thought to be comprised of only one antigenic group (group A or "typical" rotaviruses) which is defined by the group antigen associated with the inner capsid and which causes diarrhea predom-

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inantly in neonates and young children. In 1982, rotavirus group B was recognized as a human pathogen when it caused epidemics of gastroenteritis in China (9). An interesting observation in the Chinese epidemics was the more common occurrence of illness in adults rather than in young children. Rotavirus group B has been detected sporadically in Australia, Brazil, and the U.S., and there is serological evidence that rotavirus group B has infected people in Southeast Asia (1). However, significant rotavirus group B gastroenteritis has not occurred outside of China. Because rotavirus group B is a common diarrheal pathogen in swine, and has a segmented genome similar to that of influenza viruses (and is even grouped alphabetically like influenza viruses), phenotypic changes due to reassortment of the swine rotavirus group B genome could (or perhaps can in the future) have facilitated the ability of the swine virus to infect humans (1).

Rotavirus group C is primarily a swine virus but has been detected in humans in the U.S. and other areas of the world, particularly England, Japan, Australia, and Brazil (1, 4, 10). In the U.S., serological evidence suggests that exposure to rotavirus groups B and C has been minimal. Rotavirus groups D, E, and F have been found only in animals.

Rotavirus has been cultured in vitro in CV-1, LLC-MK2, AGMK, PCMK, and MA-104 cells. However, culture is not commonly performed in clinical microbiology laboratories because culture of rotavirus often requires special procedures such as previous passage in fetal or gnotobiotic cell lines, pretreatment of specimens with trypsin, or the addition of trypsin to maintenance media. In addition, cytopathic effect is not consistently present or reproducible (4).

Historically, rotavirus has been detected directly in stool specimens by EM. Specimens can be homogenized and diluted, negatively stained with phosphotungstic acid, and examined. Two reasons that this relatively simple and sensitive (albeit expensive) technique is still considered by some workers to be the standard method for detecting rotavirus are the large amount of virus shed in stools and the often relatively bacteria-free (clean) state of rotavirus-elicited stools. The EM technique requires the presence of $>10^6$ virus particles/g feces for optimum sensitivity. In a technique known as immune electron microscopy (IEM), convalescent antirotavirus serum can be mixed with rotavirus purified from specimens and examined by EM. The resulting aggregates of virus are usually easily observed and exclude irrelevant particles with morphology similar to that of rotavirus. In addition, the method can provide information as to the serotype of the virus. Some workers have coated EM grids with *Staphylococcus aureus* protein A, adsorbed rotavirus-specific antiserum to the protein, and used these grids to serotype rotavirus (11, 12).

Enzyme immunoassay (EIA) and latex agglutination (LA) are more commonly used to detect rotavirus in stools than is EM because the former methods are considerably less expensive and labor-intensive, and do not require special equipment. EIA is probably at least as sensitive as EM. In addition, the large amount of antigen in rotavirus-elicited stools is usually ample for detection. Several commercial rotavirus EIA (both monoclonal and polyclonal antibody-based) and LA kits are available. The literature is replete with interproduct comparisons as well as comparisons of products with EM (4, 13, 14). Reported sensitivities and specificities of EIA kits range from 50 to 100% and from 71 to 98%, respectively. Reported sensitivities and specificities of LA kits range from 61 to 96% and from 72 to 100%, respectively. Sensitivities and specificities appear to depend on the studied patient population (both are highest in neonates) and on the standards to which the products are compared (EM or other kits).

Hemagglutination, counterimmunoelectrophoresis, immunofluorescence, complement fixation, and radioimmunoassay also have been used to detect rotavirus antigens; however, these methods are not widely accepted because they are relatively insensitive, may require radiolabeled reagents, or are not practical for other reasons.

Several methods to detect antirotavirus IgG, IgM, and IgA have been described but these are not useful in the diagnosis of rotavirus infection and, therefore, are not commercially available.

Adenovirus

Adenovirus is a 70-nm, nonenveloped single-segment ds DNA virus with an icosahedral capsid which has a single fiber/spike at each vertex. Serological specificity is determined by the protein antigens of the capsid. All mammalian adenoviruses have a common antigen that can be easily detected by complement fixation.

Adenoviruses are typed according to their serological specificities (41 known serotypes/species), protein composition (groups A through F), and nucleic acid restriction endonuclease patterns (groups A through G). In general, serotypes 1–3 and 5–39, 4, 40, and 41 correspond to groups A–D, E, F, and G, respectively.

For many years, adenovirus has been cultured from and has been observed by EM in stools from healthy individuals and from patients with gastroenteritis. However, an association between adenovirus and gastroenteritis has been made only over the last 15

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yr. During this time, "enteric" or "fastidious" (nonculturable except under special conditions) adenovirus types 40 and 41 and culturable type 31 have been found in patients with diarrhea significantly more often than in healthy individuals. Now, adenovirus types 31, 40, and 41 are recognized as occurring worldwide and as significant causes of diarrhea in children ≤2 yr old (1, 4, 15).

Electron microscopy, EIA, nucleic acid probe hybridization, and special cell culture techniques have been used to detect adenovirus types 40 and 41. An EIA kit for adenovirus types 40 and 41 (Adenoclone-type 40/41; Cambridge BioScience, Worcester, Mass.) is available commercially. Adenovirus types 40 and 41 have been cultured in HeLa cells, conjunctival cells, and specially treated CMK cells (4, 16, 17).

Norwalk Virus and Norwalklike Viruses

Norwalk virus (Norwalk type virus) and Norwalklike viruses (also known as small, round, structured viruses) are 27-nm, round, and nonenveloped. The type of nucleic acid in Norwalk viruses is not known. The type virus of the group is the Norwalk virus (agent). Norwalk viruses have a simple but obvious structure that is characterized by an amorphous surface and irregular edges. Norwalk and Norwalklike viruses have similar structures, cause similar clinical gastroenteritis in older children and adults (rotavirus usually affects children <2 yr old), and are present in small numbers in diarrheal stools (rotavirus is present in large numbers). Norwalk viruses are firmly established as agents of gastroenteritis, and have caused many outbreaks of illness.

Norwalk viruses have not been cultured in vitro. Physicochemical characteristics of the viruses have been determined by examining viruses partially purified from diarrheal stools. IEM has been the most commonly used method to examine stools for Norwalk viruses, to examine the structure and antigenic relatedness of the viruses, and to determine diagnostic rises in antibody titers. EIA and RIA tests to detect Norwalk viruses have been developed and described (4, 6, 7) but are used almost exclusively for research and epidemiologic purposes.

Calicivirus

Caliciviruses are 30-nm, nonenveloped, single-stranded (ss) RNA(+) viruses that have surface cup-shaped indentations that give the periphery of the virus a scalloped, 5- or 6-point star appearance. Calicivirus-elicited gastroenteritis is worldwide in distribution, does not occur frequently, usually affects children <5 yr old, and is not often distinguishable from that caused by rotavirus.

Human calicivirus is not culturable in vitro (although animal calicivirus is readily culturable) and is usually detected in stools by EM or IEM. EIAs and RIAs have been developed to detect calicivirus and antibody to calicivirus (4, 18). The morphology, buoyant density, structural proteins, and antigenic characteristics of calicivirus are similar enough to those of Norwalk viruses to suggest that the two may be closely related (3, 4).

Astrovirus

Astrovirus is 30 nm in diameter, contains ss RNA(+), and does not have an envelope. The periphery of the virus is smooth, and the surface structure appears as a 5- or 6-point star, the points of which radiate from the center of the virus. There are at least five serotypes of astrovirus and at least one group antigen.

Astrovirus is associated with food- and waterborne gastroenteritis, usually affects children <7 yr old, and is not as severe as infection caused by rotavirus and Norwalk viruses (3). Astrovirus usually is shed in extraordinarily large amounts.

Astrovirus has been cultured in vitro in HEK, LLC-MK2, and CaCo-2 cells (4, 7). EM and IEM are used to detect the virus in stools and to serotype the virus. Immunofluorescence, IEM, and EIA (both mono- and polyclonal antibody-based) have been used to detect astrovirus antigens in stools and to detect diagnostic rises in antibody titer (19).

Coronavirus

Coronavirus contains ss RNA(+) and is round, 80 to 140 nm in diameter, and enveloped. Coronavirus is morphologically distinct because of its clublike surface projections (peplomers), the distal ends of which surround each virion and give a coronalike appearance to the particle. Coronaviruses cause many diverse diseases in animals worldwide, respiratory diseases (predominantly common colds in children), and possibly, gastroenteritis in humans.

The presence of coronaviruslike particles in stools from people with gastroenteritis has been well documented by EM. However, a positive correlation between the presence of coronavirus and disease has not been demonstrated. Coronavirus is extremely difficult to culture in vitro and is often structurally distinct from classical (respiratory) coronavirus.

The laboratory diagnosis of coronavirus gastroenteritis is made by EM examination of stools and/or by demonstration of a rise in antibody titer after an acute infection. Antibody concentration can be measured against only two strains (229E and OC43) but can be detected by several techniques: neutralization, complement fixation, EIA, and immunofluorescence (4, 20).

Pestivirus

Pestivirus is a ss RNA(+) virus which is 50 nm in diameter, pleomorphic, probably icosahedral, enveloped, and difficult to identify by EM. Pestivirus is widely recognized as a cause of highly economically important diseases in animals, but has never been demonstrated in or to cause disease in humans, either directly or indirectly (21, 22).

Recently, a monoclonal antibody-based EIA was developed and used to demonstrate pestivirus antigens in 23% of the stools of children <2 yr of age with gastroenteritis that was not attributable to recognized enteric pathogens (22). Similar methods have been used to detect pestivirus-associated gastroenteritis and pestivirus exposure in the United States, Bangladesh, and Peru (1, 2).

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Picobirnavirus

Picobirnavirus was previously thought to cause diarrhea in animals; however, human cases of picobirnavirus-associated diarrhea have been reported in Brazil (1).

Parvovirus

Parvovirus are ss DNA viruses that are 20 nm in diameter, nonenveloped, icosahedral, and not culturable in vitro. Parvoviruses are most recognized for causing numerous diseases in animals and erythema infectiosum (fifth disease) in children. However, parvoviruslike particles have been observed (by EM) in the stools of gastroenteritis patients and healthy individuals, and have been associated with shellfish-related gastroenteritis (1).

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Editorial

Infectious Wastes: Myths and Realities

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Public concern over potentially hazardous solid wastes of hospital/medical origin peaked in the summer of 1988, prompted by highly publicized incidents of such wastes washing ashore on a variety of the nation's beaches. Undoubtedly, concern was fueled by the growing fear of AIDS and public inability to distinguish between genuinely infectious wastes and other items that looked bad but were only of aesthetic concern. Nationally, there was a demand for regulatory action, and the American politicians were only too anxious to respond. The federal Medical Waste Tracking Act of 1988 (Luken Bill) was passed and many, often restrictive, state laws followed. The race was on to save the nation from the "evils" of infectious waste.

Hospitals and other medical facilities have been caught in a crossfire which threatens to add considerably to the already skyrocketing costs of medical care. Attempts to apply principles of logic and scientific evidence to arrive at reasonable approaches to managing medical wastes are being thwarted by extreme application of the NIMBY (not in my backyard) principle. We are left with a hodgepodge of inefficient, environmentally unsound, and sometimes outright ridiculous practices in place of sensible solutions. For example, some two-thirds of American hospitals are currently incinerating their wastes onsite. Many of these incinerators are old, inefficient,