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Viruses that multiply in the gut and cause endemic and epidemic gastroenteritis

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Abstract

Background: Acute infectious diarrhea in young children is a leading cause of morbidity and mortality in developing countries. Even in developed countries, infectious enteritis is second only to respiratory infections as a cause of morbidity in early childhood.

Objective: To nominate the various viral agents that cause enteritis, discuss the pathogenesis, clinical features, epidemiology and diagnostic procedures employed.

Study design: Pertinent literature was reviewed and the findings of investigations carried out on viral enteritis by various colleagues recalled.

Results: The viruses causing gastroenteritis include: Rotaviruses; Adenoviruses—especially Ad 31, Ad 40 and Ad 41; members of the Caliciviridae, e.g. Norwalk virus, Hawaii virus, Snow Mountain virus, Taunton virus, Southampton virus, Toronto virus (formerly mini-reovirus) and others; Astrovirus; Coronavirus; Torovirus; Cytomegalovirus (CMV) and possibly Picobirnavirus. Enteritis-producing viruses replicate in columar epithelial cells in the distal parts of villi of the small intestine. Two mechanisms are addressed to explain why diarrhea occurs. Clinically, the main expression of illness is a watery diarrhea that lasts 24 h to about 7 days. Vomiting is of shorter duration and may not always accompany the diarrhea. Fever is generally $\leqslant 38.5°C$. Virus is shed in the stool for about 3–7 days. Diagnostic procedures employ electron microscopy (EM), immune electron microscopy (IEM), enzyme-linked immunosorbent assay (ELISA), time-resolved fluoroimmunoassay (TR-FIA), latex agglutination, polyacrylamide gel electrophoresis (PAGE) and the polymerase chain reaction (PCR).

Conclusion: In developed countries viral enteritis among young children may be up to three times more common than bacterial gut disease. With the exception of CMV enteric involvement, the stool is characteristically not bloody and white blood cells are not found. Patient management may involve the employment of IV replacement therapy to counter dehydration and electrolyte imbalance. Milder cases may be managed with oral rehydration.

Keywords: Gastroenteritis; Enteritis-producing viruses; Pathogenesis of viral enteritis; Viral epidemiology; Diagnostic procedures

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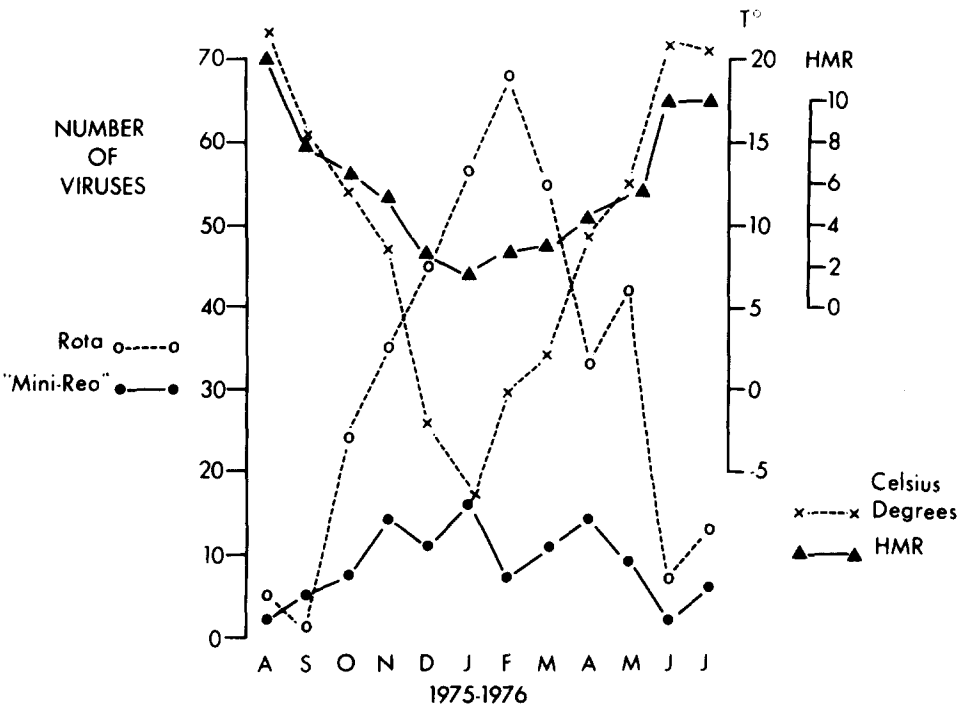

Fig. 1. Influence of season on rotavirus during a 12-month period. HMR indicates the humidity mixing rate. Figure derived from Middleton et al. (1977) Am. J. Dis. Child. 131, 733-737. Copyright 1977, American Medical Association.

1. Introduction

The viruses implicated include: Rotaviruses; Adenoviruses (especially Ad 31, Ad 40 and Ad 41); members of the Caliciviridae, e.g. Norwalk virus, Hawaii virus, Snow Mountain virus, Calici virus, Taunton virus, Southampton virus, Torontovirus (formerly mini-reovirus); Astrovirus, Coronavirus; Torovirus; Picobirnavirus, and Cytomegalovirus (CMV). Photomicrographs of many of these agents may be seen in figures presented in ASM CUMITECH No. 26 (Sherlock et al., 1989).

Acute infectious diarrhea in young children is a leading cause of morbidity and mortality in developing countries. Even in developed countries, infectious enteritis is second only to respiratory infections as a cause of morbidity in early childhood (Middleton, 1982). Viral enteritis may be endemic or epidemic and most often affects immunologically normal hosts early and late in life. Two populations of immunocompromised patients—transplant recipients and human immunodeficiency virus (HIV) subjects—may be infected by the above agents, and experience prolonged virus shedding and chronic diarrhea (Grohmann et al., 1993).

2. Epidemiology

Rotavirus has been the most intensively and systematically studied enteritis-producing virus. Records at the Hospital for Sick Children, Toronto, beginning in January 1972, indicate the striking seasonal incidence of rotavirus disease (Middleton, 1982). This seasonal pattern is illustrated in Fig. 1 and shows a peak of rotativrus gastroenteritis during the cold season when, in addition to low temperatures, the humidity mixing rate (HMR) is lower (HMR = grams of water vapor per kg of dry air) (Middleton et al., 1977). Similar data is available from many children's hospitals in temperate parts of the world. The

seasonal pattern January 1974–July 1982 of rotavirus gastroenteritis at the Children's Hospital National Medical Center, Washington, D.C. is well illustrated by Brandt et al. (1983). Seasonal peaks of rotavirus disease are not nearly so pronounced in more tropical countries (Hieber et al., 1978). In the studies reported by Brandt et al. (1983), Flewett et al. (1974) and Middleton (1982), rotavirus disease was always more numerous in males. The majority of patients studied by these authors was less than 3 years of age.

An analysis of 127 patients with adenovirus gastroenteritis (mainly serotypes 40, 41 and 31) seen at the Hospital for Sick Children, Toronto between January 1985 and August 1986 showed no seasonal variation. There were 69 males and 58 females. Ninety-four percent of the patients were less than 4 years of age (Krajden et al., 1990).

The epidemiology of the various caliciviridae that are associated with epidemic and sporadic enteritis is confounded by the presence of an array of closely and more distantly related causative agents (Lew et al., 1994). Based on serological studies, Kaplan et al. (1982) noted that Norwalk virus could be water-borne, food-borne and also spread by person to person contact. No seasonal trends were apparent. In the longitudinal study by Black et al. (1982) among children from rural Bangladesh, 47% had Norwalk virus antibody at less than 6 months of age. By 2–5 years, 80% of children had detectable antibody. Seroconversion for antibody to Norwalk virus was most frequent during the cool dry period of the year. The review by Kapikian and Chanock (1989) states that, by the fifth decade, 50% of subjects in developed countries showed the presence of antibody to Norwalk virus.

When another member of the Norwalk complex was studied in a hospital setting in Toronto, Canada, quite a different picture emerged (Middleton et al., 1977). Diagnosis of Toronto virus (formerly 'mini-reovirus') was made by electron microscopy (EM). Virus was generally found in young children; in males more often than in females; and more identifications were made during the colder months (Fig. 1).

In a six-year retrospective surveillance of gastroenteritis viruses by EM at 10 centres in the United States and Canada (Lew et al., 1990), astroviruses were the third most commonly encountered etiologic agents (after rotaviruses and adenoviruses). Sixty-four percent of astrovirus detections were in children younger than 12 months. Astroviruses were more likely to be encountered in the colder months of the year. Males were in greater number than females.

3. Pathogenesis of viral enteritis

The pathophysiology and clinical features of viral enteritis, both in humans and animals, has been reviewed by Hamilton and Gall (1982) and also by Bachmann and Hess (1982). Viruses replicate in columnar epithelial cells in the distal parts of villi of the small intestine. Light microscopy (LM) and scanning electron microscopy (SEM) show shortening of the villi. LM also reveals an elongation of crypts and increased numbers of inflammatory cells in the lamina propria. The columnar epithelial cells may assume a cuboidal shape and exhibit vacuolation. These changes are typically patchy with more normal-appearing intervening mucosa. Viral antigen and virions in villus epithelial cells may be demonstrated by immunofluorescence microscopy (IFM) and by thin-section EM.

Two mechanisms are advanced to explain why diarrhea occurs. The more important mechanism is the functional immaturity of villus epithelial cells; the other involves a reduction in total absorptive area resulting from shortening and clumping of villi. Immature epithelial cells are generated in the crypts. These cells are 'secretory' in that there is a net flow of water and electrolytes from the extracellular fluid compartment (ECFC) to the lumen. Mature cells at the villus tips produce a net flow of water and electrolytes from the lumen to the ECFC. Virus infection of the mature epithelial cells leads to a premature shedding of these cells resulting in a migration of immature 'secretory' cells to the distal villi. Hence the expanded luminal contents of both water and electrolytes. Vomiting is thought to be mediated by the stimulation of vagal and sympathetic afferent nerves that are connected to the vomiting centre

in the medulla oblongata. In viral diarrhea there are profuse watery stools containing increased concentrations of electrolytes. Characteristically leucocytes and erythrocytes are not seen. This stands in sharp contrast to feline, canine and mink parvovirus enteritis. These viruses cause an intense inflammatory change in the crypts and a hemorrhagic enteritis results. The pathogenesis of CMV enteritis, which may be bloody, is described later.

4. Clinical features

Nausea, vomiting and diarrhea are common to all viral enteritides. Asymptomatic infection may be witnessed in newborns and in subjects previously infected with the same agent. Severe vomiting and diarrhea, abdominal cramps, fever, malaise and significant diarrhea may be seen in patients if the encounter is the initial one with rotavirus beyond the newborn period. Fever is generally $\leqslant 38.5$°C; vomiting, when present, is of shorter duration ($\leqslant 3$ days) than diarrhea (3–7 days). Virus is shed in the stool for 3–7 days or more. In immunocompromised subjects, chronic diarrhea and prolonged viral shedding has been noted. The incubation period of viral enteritis is usually brief, i.e. 1–2 days, although longer intervals have been recorded for adenoviruses (Sherlock et al., 1989).

5. Characteristic features related to specific viruses

5.1. Rotaviruses

In 1973, Bishop et al. (1973) employed thin-section EM of intestinal biopsy specimens from young children with enteritis. Virus particles later to be called rotavirus were revealed. Soon thereafter, 70-nm wheel-like viruses were visualized by negative contrast-staining EM in stools from children with acute gastroenteritis (Flewett et al., 1974; Middleton et al., 1974). Rotavirus eventually became established as the leading worldwide cause of serious viral enteritis in infants and young children. Rotaviruses are non-enveloped, and have a double-stranded, segmented RNA genome (there are 11 segments). These segments code for the structural and non-structural viral proteins (VP). Thus, VP1, VP2, VP3 form the core of the virion; VP6 is the subgroup antigen and determines if the virus is subgroup A–F. All A subgroup strains have a common antigen. There are two neutralization antigens, namely VP4 and VP7. These antigens are expressed on the outer surface of the virion. There are 11 serotypes determined by VP7 (Sherlock et al., 1989).

The environment around an infected infant or young animal becomes heavily contaminated with virus particles, thus facilitating transfer to adjacent patients by attending staff or visitors. Nosocomial infections may be a substantial problem in pediatric institutions. Waterborne outbreaks can occur. The virus is robust and is not readily inactivated in the environment.

5.2. Adenoviruses

Fortsas et al. (1993) recently reported that enteric adenoviruses (Ad) are important agents of pediatric gastroenteritis. This group succeeded in producing a monoclonal antibody (MAb) which recognises a distinct epitope on protein VI. Their Ad MAb recognises an epitope shared by subgroup A (Ad 12, 18, 31) and subgroup F (Ad 40, 41) adenoviruses, but not representatives of subgroups B, C, D or E. Krajden et al. (1990) reviewed the clinical features of 127 cases of pediatric adenovirus enteritis. Serotyping results obtained by microneutralization tests and restriction endonuclease analysis were available in 105 of the 127 cases. Ad 40 and Ad 41 accounted for 56% of established serotypes. Ad 31 was associated with 17% of the 105 cases. This work establishes the primacy of adenovirus serotypes 31, 40 and 41 as a cause of enteritis. It furthermore reveals that these three serotypes rank second only to rotaviruses in causing viral diarrhea as witnessed in a tertiary care pediatric hospital in a temperate climate.

The average body temperature (rectal) was 38°C (range 36.2–40.8°C). The average duration

of fever was 1.6 days, and the average duration of clinical illness was 8.8 days (range 1–32 days). All fecal samples in the study were tested for bacterial pathogens including *Salmonella*, *Shigella*, *Campylobacter*, *Yersinia*, enteropathogenic *Escherichia coli* and verotoxin-producing *E. coli*. Five of the patients in the study died. All deaths occurred in patients with underlying diseases associated with abnormal host defense and one patient, a bone marrow transplant recipient, had disseminated Ad at postmortem. The serotypes isolated from patients who died were Ad 12, 31, 40 and 41 (2 cases) (Krajden et al., 1990).

5.3. Caliciviridae

The unmasking of this group of related agents proved to be a most virologically challenging task. The agents concerned did not propagate in cell culture or in readily available experimental animals. In addition, the virions were small, i.e. ~30 nm, and in some cases lacked distinctive morphological characteristics when examined by contrast-stain EM. The best-studied member of this family became known as the Norwalk agent (so called because of a large outbreak of enteritis that occurred in Norwalk, Ohio). Kapikian et al. (1972) described their studies using immune electron microscopy (IEM) and human volunteers. They initiated the modern era of laboratory diagnosable viral enteritis.

Norwalk and Norwalk-like viruses produce an illness of short duration, usually of 12–48 h. Infection can be asymptomatic, or vomiting, diarrhea, fever and malaise may be evident. These symptoms are very much more benign than those associated with rotavirus and adenovirus primary infections.

5.4. Astroviruses

These viruses were first described in Glasgow, Scotland, by Madeley and Cosgrove (1975). The virions were 28 nm in diameter and exhibited a star-like surface configuration. Astroviruses cause mild diarrhea and vomiting in children and adults. The virus propagates in monolayers of human embryonic kidney cells without producing cytopathic effects. EM, IEM and IFM of inoculated cell cultures permit the detection of these viruses. Naturally infected patients and volunteers develop astrovirus antibody. Calves and lambs have counterpart astroviruses which cause diarrhea in their respective species (Bishop, 1982).

5.5. Coronaviridae

Caul and Egglestone (1982) have reviewed the evidence implicating human enteric coronavirus as a possible cause of enteritis. On the whole, their evidence is not nearly so compelling as that which has been established for porcine and bovine enteric coronavirus infection (Garwes, 1982).

Toroviruses (also a member of the family Coronaviridae) have recently been shown to cause enteritis in children. Animal counterparts in horses (Berne virus) and calves (Breda virus) are associated with diarrheal illness in these species. The virions are doughnut-shaped (torus-shaped) and measure 35 × 170 nm. In the report by Tellier and Petric (1993), a method of purifying the human Torovirus was described and immunoblot reactions results with human convalescent serum and calf hyperimmune Breda antiserum were detailed. They also intimated that their virus had an RNA genome.

5.6. Picobirnavirus

Picobirnavirus represents a relative newcomer on the scene in terms of an etiologic agent causing human enteritis. The name Birnavirus derives from the suffix 'bi' which signifies both a double-stranded and bisegmented virus (RNA) genome. The two most characterised Birnaviruses are those of infectious pancreatic necrosis virus (IPNV) of fish and infectious bursal disease virus of chickens (Doane and Anderson, 1987). These agents present a hexagonal profile on contrast-stain EM and measure ~60 nm in diameter. In the paper by Grohmann et al. (1993), which addresses the question of enteric viruses and diarrhea in HIV-infected patients, it is concluded that astrovirus and picobirnavirus may be important etiologic agents of diarrhea in such patients. Picobirnaviruses (small, i.e. 35 nm) are found in feces of

rats, swine, guinea pigs and hamsters as well as humans. These viruses are 35 nm in diameter and possess no distinct surface structures by negative contrast-stain EM. Their buoyant density has been calculated at 1.4 g/ml which is greater than that of the 'macro' parent. Polyacrylamide gel electrophoresis (PAGE) reveal two bands of double stranded virion RNA.

Studies of prepared fecal samples from HIV-infected patients and control subjects revealed that 9% versus 2% (with and without diarrhea respectively) shed picobirnavirus.

5.7. Cytomegalovirus (CMV)

Substantial involvement of the gastrointestinal tract may be witnessed in subjects who are immunocompromised, e.g. HIV patients with low CD4 counts, transplant recipients, infants with severe combined immunodeficiency disease and infants with generalized CMV disease following intrauterine infection. The colon is the most common site of involvement, followed by the oesophagus, rectum and small bowel in that order, according to Alford and Britt (1990). Profuse watery diarrhea with or without bleeding and inflammatory changes characterize CMV colitis. Diarrhea in advanced HIV infection has been associated with increased small intestinal permeability as measured by a lactulose/mannitol test (Kapembwa et al., 1990).

6. Laboratory diagnoses

6.1. Specimens

A fecal sample of about 1 g is usually required. Nonenveloped virions and viral antigens are relatively resistant to destruction. Therefore, continuous refrigeration is not required. In a World Health Organization survey in which twelve laboratories worldwide exchanged stool samples containing virus, by means of unrefrigerated air freight services, good agreement was observed among different laboratories using EM detection for the more structurally robust viruses such as rotavirus and adenovirus. On the other hand, the receiving laboratories found it was difficult to detect astrovirus, calicivirus, Toronto virus ('mini-reo') and coronavirus, presumably because these viruses readily lose their structural integrity (C.R. Madeley, personal communication). For long-term preservation of virions, storage at $-70°C$ is recommended. Cycles of freezing and thawing lead to disintegration of virions. Enteric viruses have also been recovered from duodenal aspirates and jejunal biopsies (Sherlock et al., 1989).

In the case of CMV gastrointestinal (GI) involvement, positive cultures may be obtained from urine and tissue obtained by endoscopic biopsy.

Serological determinations employing enzyme-linked immunosorbent assays (ELISA) or IEM are of particular interest in epidemiological and research studies rather than a diagnostic approach to acute disease. Antigens or virions that form the basis of these serological tests may be obtained by purification procedures on infected stools; by cell culture cultivation, e.g. adenovirus, rotavirus and, in the case of Norwalk virus (NV), by self-assembled recombinant NV (rNV) particles expressed in a baculovirus system (Jiang et al., 1990, 1993).

6.2. Electron microscopy

EM is rapid, moderately sensitive, simple to perform and has the distinct advantage of uncovering a diagnosis for a wide range of enteric viral pathogens. Details of EM and IEM are given in CUMITECH No. 26 (Sherlock et al., 1989). Airfuge or immunoconcentration procedures are required to visualize virions of astrovirus and the calicivirus groups. The turn-around time is rapid, i.e. same-day-specimen submission and answer.

6.3. ELISA procedures

These are highly sensitive assays but each assay is limited to a particular virus or virus group, e.g. rotavirus and adenovirus. The basis of most ELISAs involves coating a plastic bead or well with a virus-specific capture antibody. Homologous virus in the fecal specimen binds to the antibody. An enzyme-labelled antibody is then bound. Finally, an enzyme substrate is added and

this results in a colour production which indicates a positive test. ELISA also provides a rapid turn-around time.

6.4. Time-resolved fluoroimmunoassay (TR-FIA)

This methodology was popularized by Halonen et al. (1983). The assay uses a capture format similar to that described above for ELISA. Europium-chelate-labelled anti-indicator species antibody provides the final fluorescence signal that is read in a gated fashion by the fluorometer. Halonen's groups employed TR-FIA on fecal specimens to diagnose adenovirus and rotavirus enteritis.

6.5. Latex agglutination

These assays are both simple and rapid. Some commercial kits are available for rotavirus and adenovirus antigens. On the whole, these assays can be quite specific but their sensitivity falls below that of EM or ELISA.

6.6. Polyacrylamide gel electrophoresis (PAGE)

Rotaviruses can be recognized and identified by virtue of the patterns of migration of the 11 segments of RNA upon PAGE electrophoresis. This system permits the identification of various electropherotypes and provides a useful epidemiological investigative tool.

PAGE has also been used in presumptively identifying picobirnavirus in fecal samples and to distinguish various adenoviruses following restriction endonuclease digestion of viral DNA extracted from infected cells.

6.7. Dot blot hybridization

This methology has not gained very wide acceptance for the diagnosis of enteritis-producing viral infections.

6.8. Polymerase chain reaction (PCR)

This approach not only serves to increase the detection of viruses but also allows related agents to be grouped (Lew et al., 1994). A PCR approach to diagnosis would not be appropriate for laboratories that cannot accommodate a clear separation of product-containing areas and reagent preparation unit. Other guidelines to prevent contamination are given by McCreedy and Callaway (1993).

7. Patient management

Antiviral drugs effective against the pathogens that are associated with viral enteritis are not available. Oral immune globulin, however, has been shown to ameliorate or prevent rotavirus infection in infants and neonates (Barnes et al., 1982). Immunity to infection at a mucosal surface, whether it be respiratory or gastrointestinal, is generally short lived. Thus, throughout life we are infected by various respiratory viruses and viruses that are associated with enteric disease. It is in our middle years that enteric viruses have little impact. However, early in life and in our senior years these pathogens exact their toll. The status of rotavirus vaccines has recently been addressed by Kapikian and Chanock (1990). Preventive measures like hand washing, pathogen-free water supply, patient isolation and breast feeding reduces the burden of disease from the various viruses associated with enteritis.

In gastroenteritis, it is important to establish a precise etiology so that inappropriate antibiotic therapy might be avoided, and appropriate infection control procedures implemented, in order to curtail the spread of infection within institutions.

Severe viral diarrhea may be associated with dehydration and electrolyte imbalance. Patients exhibiting these features should be admitted to hospital for remedial IV replacement therapy. McCarthy (1992) provides a means based on signs and symptoms to grade the degree of dehydration into categories of mild, moderate and severe. Those who exhibit minor abnormalities may be managed by oral rehydration (OR) using WHO guidelines. The standard WHO oral glucose-electrolyte formula is made by adding 3.5 g sodium chloride, 1.9 g trisodium citrate (dihydrate), 1.5 g potassium chloride and 10 g anhydrous glucose to

1 l of water. Sodium bicarbonate (2.5 g) may be substitued for the 2.9 g of trisodium citrate (dihydrate) (Kapikian and Chanock, 1990).

Current diagnostic techniques in virology are rapid and sensitive, so that it is now possible to rule in or rule out a viral etiology of an enteric upset and plan an appropriate management regimen.

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