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Clinical Microbiology Newsletter

Vol. 6, No. 19 October 1, 1984

Viral Gastroenteritis

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Viral gastroenteritis is estimated to be one of the most common clinical illnesses in the United States, being second in frequency only to the common cold (5). Its clinical manifestations are familiar ones and include nausea, vomiting, and/or diarrhea. Infants and small children with gastroenteritis frequently require hospitalization because the vomiting and diarrhea can lead to severe dehydration and its associated electrolyte imbalance. Unless these patients are treated with intravenous or oral fluids and electrolytes, severe morbidity and even mortality can ensue (4).

A wide variety of viruses have been implicated in gastroenteritis. Bacterial infection accounts for a minority of the gastroenteritis cases in temperate climates, especially during the cooler months (11). Rotaviruses and the Norwalk group of viruses are probably the most common of the gastroenteritis viruses. During their winter epidemics, rotaviruses are responsible for approximately one-half of all viral gastroenteritis occurring in hospitalized children under two years of age (8). The Norwalk and Norwalk-like viruses are also prevalent, but they primarily infect older children, adolescents, and adults (2). Norwalk viruses cause a debilitating but rapidly resolving gastroenteritis that rarely requires hospitalization. The diversity of other viruses implicated in gastroenteritis, primarily affecting the pediatric population, include the minirotaviruses, enteric adenoviruses, small round viruses structurally similar to parvo- or picornaviruses, astroviruses, caliciviruses, and coronoviruses (10–12, 14, 17).

Originally all of these viruses were noncultivable. They were discovered during the past decade by the electron microscopic (EM) examination of negatively stained stool samples obtained from patients with gastroenteritis (1). Since many of these viruses are still noncultivable, their nucleic acid type and their polypeptide number, size, and composition are often unknown and they are therefore unclassified (1). Because they are noncultivable, EM is the preferred method for their detection and identification in most laboratories (15). Although EM is routinely used in Canada, England, and Australia, its potential has not been fully developed in the United States. It entails relatively rapid and simple techniques that are described below (7, 13, 15). While the enzyme-linked immunosorbent assay (ELISA or EIA) and other antigen detection systems have been developed in research laboratories for some of these viruses, ELISA reagents are commercially available only for rotavirus detection (16, 20). (For a discussion of rotaviruses see *Clin. Microbiol. Newsl.* 6:8, 1984.)

Types of Gastroenteritis Viruses

The types of gastroenteritis viruses detectable by EM are described in Table 1. The *rotaviruses* are nonenveloped, icosahedral, double-shelled, wheel-like virions approximately 70 nm in diameter that are in the Reovirus family. The *minirotaviruses* are round, wheel-like viruses which, like the rotaviruses, appear to have a double-shelled capsid (17). However, with a 30–32-nm diameter, the minirotaviruses are about half the diameter of the rotaviruses. The minirotaviruses are sometimes called minireoviruses.

The *enteric adenoviruses* cannot be cultivated in the routine cell cultures and thus differ from other adenoviruses that primarily cause respiratory disease (19). However, these enteric adenoviruses resemble other adenoviruses in being nonenveloped single-shelled, icosahedral viruses with a diameter of approximately 75 nm.

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Table 1
Types of Gastroenteritis Viruses

Name	*Size (nm)*	*Distinguishing Morphology*
Rotavirus	70	Icosahedral, double capsid, nonenveloped
Minirotavirus	30–32	Icosahedral, double capsid, nonenveloped
Adenovirus, enteric	75	Icosahedral, nonenveloped
Small round	22–30	
Norwalk		Round, nonenveloped
Norwalk-like		Round, nonenveloped
Parva/picorna		Icosahedral, nonenveloped
Astrovirus	28–30	5- or 6-pointed star, smooth periphery
Calicivirus	30–40	6-pointed Star-of-David, spiky periphery
Coronavirus	80–130	Round or irregular shape; club-shaped surface projections giving crown-like appearance

There are a number of *small round viruses* that have a rather nondescript morphology. One of the first to be described was the Norwalk agent, isolated from an outbreak of gastroenteritis in Norwalk, Ohio (2). This agent is a small round 27-nm virus with no distinctive morphology. Similar viruses have been isolated from epidemics in the United States, England, and Australia and are called Norwalk-like. Some of these were found to be antigenically related to the Norwalk agent and others are not (1, 6). Other small round viruses in the 22–30-nm range have been described as parvovirus-like or picornavirus-like, since their capsid symmetry and size resemble the small DNA-containing parvoviruses or the small RNA-containing picornaviruses (10, 17). However, their nucleic acid type is not known.

Astroviruses are small round 28–30-nm viruses that have a distinctive morphology (9). A five- or six-pointed star-like configuration is seen on the surface of these viruses, and the center of the star is solid, with no hollow in the center. They have a smooth, circular, well-defined periphery.

The *caliciviruses* are also small round viruses that have a star-like configuration (9). However, they differ from astroviruses in several ways. First, they are slightly larger, ranging from 30–40 nm. Second, only six-pointed, and not five-pointed stars are seen in these viruses. In addition, the stars have a hollow area in their centers, giving them a Star-of-David appearance. Lastly, their edges are not smooth, having feathery or spikey appearance.

Coronaviruses are large (80–130 nm), round or irregular viruses, with club-shaped projections on their surface that give them the appearance of a crown or the corona of the sun (1). Many of the above viruses, including the minirotaviruses, the small round viruses, the astroviruses, and the caliciviruses, cannot be classified into families until their nucleic acid type and other biochemical and biophysical characteristics are more fully determined.

Epidemiology of the Gastroenteritis Viruses

Although rotaviruses account for approximately 50% of all gastroenteritis cases during its peak epidemic months, other viruses account for much of the remaining gastroenteritis, both sporadic and epidemic. Like the rotaviruses, the adeno-, astro-, and caliciviruses cause significant clinical illness in infants and small children, frequently necessitating hospitalization. Older children and adults are less frequently and/or less severely affected by these viruses. Adeno- and calicivirus infection may last from several days to a week or longer, although astrovirus infection is short lived, lasting 12–24 hr. The adeno-, astro-, and caliciviruses are also similar to rotaviruses in that they are responsible for outbreaks of nosocomial infections. Because of the hospitalization of these pediatric patients, both for admitting diagnoses of gastroenteritis and also for nosocomial infections, the laboratory disgnosis by EM should be carried out. Although cases of adeno- and calicivirus gastroenteritis occur throughout the year, they occur more frequently during the winter months. The astro-, minirota-, and parvo/picornaviruses are seen primarily during the winter months (1).

The Norwalk group causes short epidemics through the year that usually last about a week. In contrast to the other viruses, the Norwalk group of viruses primarily infect older children, adolescents, and adults. The clinical manifestations last only 24–48 hr, the patients recover completely, and hospitalization is rarely required (1). Thus routine laboratory diagnoses are rarely carried out on these patients and most of the studies that have been carried out on these viruses were epidemiologic in nature.

Several extensive studies have been done by investigators in the United States, Canada, England, and Australia to determine which are the most commonly occurring enteric viruses. These results are summarized in Table 2. These studies showed that rotaviruses were almost always the most common. Adenoviruses were usually the second or third most common virus, and the small round viruses were about the third or fourth most commonly occurring virus. Astroviruses were one of the least common viruses. However, it is difficult to draw conclusions about the incidence and importance of the minirota- and caliciviruses. The minirotaviruses were the second most common virus in two studies (10, 17) and were nonexistent or not described in six (3, 8, 11,

Table 2
Gastroenteritis Viruses Listed in Descending Order of Incidence

Rotaviruses
Adenoviruses
Small round viruses
Astroviruses
Not known:
Minirotaviruses
Caliciviruses

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12, 14, 18). The caliciviruses were the most common in one study (17), the least common in two studies (11, 14), and nonexistent or not described in five other studies (3, 8, 10, 12, 18).

Laboratory Diagnosis

Because most gastroenteritis viruses are neither cultivable nor reagents, EM is the method of choice for the detection and identification of these viruses in hospital laboratories. EM also has the advantage of ease and rapidity, resulting in same-day results. Enteric adenoviruses can now be propagated in the 293 cell line, but their isolation may take from several days to several weeks and may require one or more blind passes (19).

Like the rotaviruses, many gastroenteritis viruses such as the adenoviruses and astroviruses are frequently present in large enough numbers to be detected directly by EM (1, 13). A major exception are the Norwalk and Norwalk-like viruses that occur in relatively small numbers in stools. However, because these viruses affect older children and adults who rarely require hospitalization, the laboratory diagnosis for these viruses is normally not carried out.

Some investigators have examined stool suspensions directly by EM without clarification (13–15), while others recommend clarifying stool samples to get rid of the large number of bacteria and debris which can interfere with the detection of viral particles (7, 12). Specimens that are not clarified are initially suspended in phosphate-buffered saline or Hanks' balanced salt solution. A drop of suspension is then placed on a Formvar®-coated EM grid, and the excess is removed by touching the edge of the grid with filter paper. A drop of 2.0% phosphotungstic acid (PTA), a negative stain, is added and the excess is blotted off. The grid is then exposed to UV light for 3–5 min to inactivate any viral particles present. Minor variations in this technique exist and each individual laboratory should chose the method that suits it best (13–15).

To clarify stool suspensions, prepare 10–20% suspensions in buffer, centrifuge at 1000 × *g* for 30 min at 4°C, and use the supernatant fluids for preparing grids as described above. Higher *g* forces should not be used, since any viral clumps in the suspension may be removed.

Since at least 10^6 viral particles per ml of sample are needed to be visualized by EM, viral particles in stool suspensions may need to be concentrated before they can be visualized. There are two simple methods for concentrating viral particles in clarified stool samples. One is the pseudoreplica technique, and the other is the agar-diffusion-filtration method (7, 13).

For the pseudoreplica technique, a drop of viral suspension is placed on a small block of 2.0% agar or agarose on a microscope slide and allowed to dry. The aqueous solution and salts are absorbed into the agar, while the viral particles remain on the top of the block. A drop of 0.5% Formvar® solution is added to the top of the block and allowed to dry, so that a Formvar® membrane forms on the top of the block. The agar block is then cut from the microscope slide and immersed in a solution of 2.0% PTA. This causes the virus–Formvar® membrane to float on the surface of the PTA and to be stained at the same time. An EM grid is then placed on the membrane and the grid is removed from the solution and allowed to dry. It is further examined by EM after UV inactivation of viruses is carried out.

For the agar-diffusion-filtration method, a drop of viral suspension is placed on a block of agar. A Formvar®-coated grid is placed upside down over the viral suspension until the aqueous solution diffuses into the agar and the suspension is dry. The concentrated viral particles on the agar surface adhere to the Formvar®-coated grids. These grids are removed, stained with PTA, and examined by EM after UV irradiation (7).

Immune EM (IEM) can be carried out using antisera to clump and concentrate viral particles that may be present in small numbers in a suspension. The method can also be used to show a rise in titer between acute and convalescent sera obtained from gastroenteritis patients, or to study cross-reactivity between related viruses such as with some of the Norwalk-like viruses. IEM is carried out by first mixing a viral suspension with acute and convalescent sera or known immune serum, and allowing the mixtures to set for 30–60 min at 37°C (7, 13). The virus–antiserum mixtures are then placed on a grid, or they are first centrifuged at 15,000 rpm for 30 min to pellet virus–antibody aggregates prior to adding the resuspended pellet to the grids. The grids are then stained with PTA and observed for virus–antibody aggregates. All of these concentration methods are relatively simple, and can be done with a minimum of supplies and equipment. Because all of these methods are relatively rapid, same-day results should be possible.

Conclusions

Viral diagnosis, especially rapid viral diagnosis of viral gastroenteritis, is important for several reasons. First, infants and children hospitalized for viral gastroenteritis should be isolated to prevent nosocomial infections in others. This is usually not done if a specific pathogen is not detected and reported. Second, much less is known about the relative importance and incidence, both epidemic and endemic, of many of the gastroenteritis viruses when compared to the rotaviruses. More long-term, prospective, epidemiologic studies, carried out in various locales, need to be done to determine the relative importance of these other viruses in the epidemiology of gastroenteritis. These studies will be necessary before vaccines or other preventative measures can be developed to control these viruses. Human rotaviruses and enteric adenoviruses can now be propagated in cell cultures. This advancement has paved the way for the production of future rotavirus, and possibly adenovirus vaccines, which will prevent a substantial percentage of viral gastroenteritis in the future.

References

1. **Barnett, B.** 1983. Viral gastroenteritis. Med. Clin. N. Am. **67**:1031–1058.

0196-4399/84/$0.00 + 02.00

2. **Blacklow, N. R., and G. Cukor. 1981.** Viral gastroenteritis. N. Engl. J. Med. **304**:397–406.
3. **Brandt, C. D. et al.** 1981. Comparison of direct electron microscopy, immune electron microscopy, and rotavirus enzyme-linked immunosorbent assay for detection of gastroenteritis viruses in children. J. Clin. Microbiol. **13**:976–981.
4. **Carlson, J. A. et al.** 1978. Fatal rotavirus gastroenteritis: an analysis of 21 cases. Am. J. Dis. Child **132**:477–479.
5. **Dingle, J. W., G. F. Badger, and W. S. Jordan, Jr.** 1964. Illness in the home: a study of 25,000 illnesses in a group of Cleveland families. The Press of Western Reserve University, Cleveland.
6. **Greenberg, H. B. et al.** 1979. Role of Norwalk virus in outbreaks of nonbacterial gastroenteritis. J. Infect. Dis. **139**:564–568.
7. **Hsiung, G. D.** 1982. Diagnostic virology, 3rd ed. Yale University Press, New Haven.
8. **Kapikian, A. Z. et al.** 1976. Human reovirus-like agent as the major pathogen associated with "winter" gastroenteritis in hospitalized infants and young children. N. Engl. J. Med. **294**:965–972.
9. **Madeley, C. R.** 1979. Comparison of the features of astroviruses and caliciviruses seen in samples of feces by electron microscopy. J. Infect. Dis. **139**:519–523.
10. **Middleton, P. J., M. T. Szymarski, and M. Petric.** 1977. Viruses associated with acute gastroenteritis in young children. Am. J. Dis. Child. **131**:733–737.
11. **Murphy, A.** 1981. Aetiology of viral gastroenteritis. Med. J. Aust. **2**:177–182.
12. **Narang, H. K., and A. A. Codd.** 1981. Frequency of preclumped virus in routine fecal specimens from patients with acute nonbacterial gastroenteritis. J. Clin. Microbiol. **13**:982–988.
13. **Palmer, E. L., and M. L. Martin.** 1982. An Atlas Mammalian viruses. CRC Press, Boca Raton.
14. **Riepenhoff-Talty, M. et al.** 1983. Potential specturm of etiologic agents of viral enteritis in hospitalized infants. J. Clin. Microbiol. **17**:352–356.
15. **Riepenhoff-Talty, M. et al.** 1983. Negative staining and immune electron microscopy as techniques for rapid diagnosis of viral agents. *In* E. Buetner (ed.), Defined immunoflurescence and related cytochemical methods. Ann. NY Acad. Sci. 420:391–400.
16. **Rubinstein, A., and M. Miller.** 1982. Comparison of an enzyme immunoassay with electron microscopic procedures for detecting rotavirus. J. Clin. Microbiol. **15**:938–944.
17. **Spratt, H. C. et al.** 1978. Nosocomial infantile gastroenteritis associated with minirotaviruses and caliciviruses. J. Pediatr **93**:922–926.
18. **Suzuki, H. et al.** 1981. Rotavirus infection in children with acute gastroenteritis in Ecuador. Am. J. Trop. Med. Hyg. **30**:293–294.
19. **Takiff, H. E., S. E. Strauss, and C. F. Garon.** 1981. Propagation and in vitro studies of previously non-cultivable enteral adenoviruses in 293 cells. Lancet **ii**:832–834.
20. **Yolken, R. H. et al.** 1977. Enzyme-linked immunosorbent assay (ELISA) for detection of human reovirus-like agent of infantile gastroenteritis. Lancet **ii**:263–266.

Editorial

Bacterial Antigen Detection: Sensitivity Measurements

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A variety of new immunodiagnostic tests for the direct detection of antigens in patient samples are becoming more available. The sensitivity of the tests is of particular importance but there are different ways of expressing it, some of which can be misleading. The sensitivity of a diagnostic test is defined as the proportion of patients with the disease who give positive results in the test (4). This can be calculated from the outcome of clinical trials of the test and the information is usually provided in package inserts or other literature available from manufacturers. The determination of this value can be difficult; it is not always easy to determine with confidence the total number of patients with the disease, because not all cases may be detectable by the conventional techniques used for reference purposes. It is difficult to obtain a significantly large number of clinical samples to reliably calculate sensitivity. Clinical samples may be obtained in larger numbers in some geographical areas (for example, the "meningitis belt" of Africa) but data generated in this way should be interpreted with caution since the absence of or delay in the institution of chemotherapy in these areas may result in larger quantities of bacterial polysaccharides in the body fluids. Different manufacturers of similar kits may have used different reference methods to calculate sensitivity. In any given case different samples were used and unless very large numbers were tested, comparisons could be inaccurate.

It is perhaps because of these difficulties that it has become fashionable to refer to the sensitivity of latex and other test systems in terms of a minimum quantity of antigen which is detectable. Strictly speaking, the figures quoted, usually in ng/ml, refer to *polysaccharide* concentrations determined either colorimetrically or by dry weight and not to *antigen* concentration, which would need to be determined immunologically. More sense could be made of the data if the antigenicity of a polysaccharide correlated directly with its concentration, but this is not the case (2). Other factors such as molecular weight and fine details of primary or secondary structure influence polysaccharide antigenicity (13). The following extreme examples provide illustrations of this.

Neisseria meningitidis group B an-

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