Foodborne Viral Gastroenteritis: Challenges and Opportunities

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Norwalk-like viruses (NLVs) are estimated to be the most common causes of foodborne disease in the United States, accounting for two-thirds of all food-related illnesses. The epidemiologic features and disease burden associated with NLVs have, until recently, been poorly understood because of the lack of sensitive detection assays and the underuse of available diagnostic tools. However, the application of molecular techniques to diagnose and investigate outbreaks of infection during recent years has led to a growing appreciation of the importance of these agents. NLVs are a principal cause of outbreaks of acute-onset vomiting and diarrhea in all age groups—most commonly, via contamination of uncooked foods by infected food-handlers, but also via foods contaminated at their sources, such as oysters and raspberries. NLVs may also account for >10% of sporadic cases of gastroenteritis in children and adults. Future research will focus on the development of easy-to-use diagnostic assays based on antigen and antibody detection as well as vaccine development. Implementation of simple prevention measures, including correct food-handling practices, will continue to be a priority.

Gastroenteritis remains one of the most common causes of morbidity and mortality worldwide. The impact is most dramatic in developing countries, where an estimated 2.5–3.2 million children aged <5 years die each year [1], and the World Health Organization estimates that a large number of these deaths are caused by foodborne pathogens. In the United States, although the number of deaths associated with enteric infections is low, gastroenteritis associated with contaminated food remains a common cause of disease and hospitalization, accounting for an estimated 76 million cases and 325,000 hospitalizations annually [2]. The growing appreciation of the dramatic disease burden of foodborne illnesses in the United States has increased interest among federal and state public health agencies and led them to study these diseases, to evaluate prevention strategies, and to improve regulatory oversight of the food industry. Much of the recent attention has been given to prevention of infection with bacterial agents; however, viral agents, principally the Norwalk-like viruses (NLVs), are estimated to account for more than two-thirds of the foodborne illnesses caused by known pathogens [2]. Efforts to understand and prevent viral foodborne gastroenteritis have been particularly challenging because of a number of obstacles. First, no simple, sensitive detection assays are available, and access to laboratories that are capable of establishing a proper diagnosis is limited. Second, the lack of a specific treatment option for viral gastroenteritis has limited enthusiasm among physicians and public health authorities to seek diagnosis, even when diagnostic tools are available. However, recent advances in detection methods have led to a revolution in our understanding of these agents and their importance, and these advances promise to lead to the development of effective prevention methods in the coming years.

THE AGENTS: NLVS AND EVERYTHING ELSE

Although several viruses have been implicated in foodborne outbreaks of diarrhea, NLVs account for the overwhelming majority of cases of foodborne viral illness. NLVs are a group of related viruses named after the prototype strain Norwalk virus, which was first discovered in 1972 by Albert Kapikian

Received 8 February 2002; revised 13 May 2002; electronically published 21 August 2002.

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Clinical Infectious Diseases 2002; 35:748–53

1058-4838/2002/3506-0016

at the National Institutes of Health, who used immune electron microscopy to examine fecal samples obtained from infected human volunteers [3]. The volunteers had been given filtrates of stool samples collected by Centers for Disease Control and Prevention (CDC) epidemiologists during an outbreak of vomiting at an elementary school in Norwalk, Ohio, in 1968 [4]. Initially, no agent could be identified in these specimens, but the filtrates given to the volunteers induced gastrointestinal disease, indicating the likelihood of an infectious cause. Dr. Kapikian's discovery marked the first time that a virus had been definitively demonstrated to cause diarrheal disease in humans. Research conducted during the following decade confirmed that Norwalk virus was a significant cause of epidemics of gastroenteritis in a wide variety of settings, and the distinct clinical and epidemiologic features of these outbreaks served as a diagnostic guide in the absence of routine laboratory testing [5].

NLVs are small, single-stranded RNA viruses that were previously called "small round-structured viruses" because of their appearance under an electron microscope (figure 1). They constitute a genus in the family Caliciviridae and are divided into 3 distinct genogroups (GI, GII, and GIII), which, in turn, are divided into ⩾15 genetic clusters [6]. Multiple strains of NLVs circulate at any given time, although there is evidence that one strain or group of strains may occasionally predominate: for example, in the mid-1990s, a single, common strain demonstrated extensive global spread in a short period of time [7].

Other enteric viruses have been identified to be important causes of gastroenteritis, such as rotaviruses, astroviruses, adenovirus 40 and 41, and Sapporo-like viruses (SLVs), but few of these infectious agents have been associated with foodborne transmission, and then, only rarely. Rotaviruses have occasionally been implicated in epidemics attributed to contaminated food, such as a recent outbreak of infection among college students, which was presumed to have been caused by the contamination of uncooked food that was prepared by an ill food handler [8]. In the 1970s, rotavirus was implicated in a large, community-wide waterborne outbreak of gastroenteritis associated with a contaminated reservoir that provided drinking water to a small town [9]. Similarly, astroviruses have been reported as causes of foodborne [10] and waterborne [11] epidemics, but these are exceptional cases. Of the other 3 genera of the family *Caliciviridae* (SLVs, *Vesivirus,* and *Lagovirus*), only SLVs are common causes of infection in humans, although few reports of transmission through contaminated food exist [12]. In a review of the etiology of outbreaks of nonbacterial gastroenteritis reported to the CDC, NLVs accounted for 96% of the 90 outbreaks investigated during an 18-month period [13]. Similar frequencies have been reported in Europe [14]. For this reason, in a foodborne outbreak, public health laboratories should rule out NLVs before testing for other viruses.

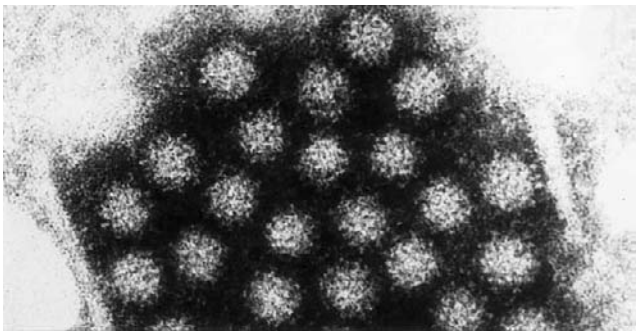

Figure 1. Electron micrograph of Norwalk virus showing calyices around the capsid—hence, the name "calicivirus."

CLINICAL PRESENTATION

NLV-associated viral gastroenteritis generally presents as vomiting; watery, nonbloody diarrhea; abdominal pain; and nausea [15]. Older references to NLV infection as "winter vomiting disease" reflect that vomiting is often the first symptom and is frequently the reason that a person presents for medical attention. Vomiting is more common among children, whereas diarrhea is more likely to predominate among adults. However, both symptoms occur in most patients regardless of age. Fever occurs in one-third to one-half of patients; it is usually low-grade and lasts for <24 h. A variety of other symptoms, including myalgia, headache, and chills, are also commonly reported. Symptoms generally last for 1–3 days. Dehydration remains the most common complication of disease, especially in young children and elderly persons, and no other severe sequelae have been reported. NLV-associated deaths are described in the context of outbreaks of infection, generally among elderly patients residing in nursing homes [16]. Finally, asymptomatic infection is probably quite common [17] and may play an important role in transmission [18, 19].

Symptoms of NLV-associated gastroenteritis are sufficiently nonspecific to make the diagnosis of NLV disease in a sporadic case impossible on clinical grounds alone. However, the epidemiologic setting and the occurrence of other contacts with similar illnesses in the households, schools, or workplaces of affected patients support the diagnosis. In addition, the predominance of vomiting as an initial symptom is helpful for diagnosis. In the early 1980s, Kaplan and colleagues from the CDC [5] found that outbreaks of gastroenteritis that met some simple and easily determined criteria were likely to have been caused by NLVs. These criteria included (1) failure to detect a bacterial or parasitic pathogen in stool specimens, (2) the occurrence of vomiting in >50% of patients, (3) a mean duration

Table 1. Characteristics of caliciviruses that facilitate their spread in epidemics.

Feature	Observation	Consequences
Low infectious dose	Dose, $<10^2$ viral particles	Permits droplet/person-to-person spread, secondary spread, and spread by food handlers
Prolonged asymptomatic shedding	Duration, up to 2 weeks	Increased risk of secondary spread; problems with control of food handlers
Environmental stability	Survives in up to 10 ppm of chlorine, freezing, and heating to 60°C	Hard to eliminate from contaminated water; virus survives in ice and partially cooked oysters
Great strain diversity	Many genetic and antigenic types	Requires composite diagnostic tools; repeated infections with many different antigenic types; easy to underestimate prevalence
No induction of lasting immunity	Disease can occur with reinfection	Childhood infection does not protect against disease in adulthood; difficulty in developing vaccine that confers lifelong protection

NOTE. Data are adapted from [31].

of illness of 12–60 h, and (4) a mean incubation period of 24–48 h. These criteria were particularly useful as a diagnostic tool in the absence of specific laboratory methods for diagnosis, and they remain widely used by local health departments.

PATHOPHYSIOLOGY AND IMMUNITY

NLVs are transmitted primarily by the fecal-oral route, usually either by consumption of contaminated food or water or by direct person-to-person spread. Some evidence exists that NLVs are spread through large droplets or aerosolization of vomitus during outbreaks of infection [20–22]. Finally, environmental contamination has been implicated as a reservoir of infection that sustains outbreaks [23]. Secondary person-to-person transmission in foodborne outbreaks is characteristic of NLVs [18]; this can help health care personnel further distinguish NLV-associated outbreaks of gastroenteritis from those caused by bacteria. Symptoms of NLV disease usually begin 12–48 h after exposure to NLV. At this time, shedding of virus in the stool also generally begins, although presymptomatic shedding may occur [24]. Viral shedding was traditionally thought to cease within 2–3 days after clinical improvement, but the recent use of molecular detection methods indicates that viral antigens can be found in stool samples for a week or longer after recovery [17], and some outbreak reports suggest prolonged infectivity [19]. It is not clear how these findings affect recommendations for infection control in outbreaks, with particular regard to the exclusion of possibly infected food handlers and health care personnel from the workplace.

The mechanisms of immunity to NLV infection are unclear. Volunteer studies originally showed that some subjects developed short-lived immunity, which correlated with an increase in serum antibody levels, but the patients were susceptible to illness again when rechallenged with the same strain >3 years later [25]. Immunity appears to be strain specific, and, given the genetic variability in circulating NLVs, individuals are likely to be repeatedly infected with NLVs during their lifetimes. This observation explains the high attack rates among persons of all ages often reported during outbreaks of gastroenteritis, and it presents steep challenges to the development of a vaccine. Of interest, in challenge studies, individuals with high levels of preexisting serum antibody paradoxically seem predisposed to illness, whereas a proportion of individuals remain asymptomatic, with low antibody titers, despite repeated challenges [17, 26]. These findings have led to hypotheses that susceptibility to infection is genetically determined (e.g., it is determined by presence of a receptor gene, as is the case for cholera).

DISEASE BURDEN AND EPIDEMIOLOGIC FEATURES OF NLVS

Accurate estimates of the true magnitude of the disease burden associated with NLVs are not available, but NLVs are probably the most common cause of epidemics of gastroenteritis and may be the most common cause of sporadic cases of gastroenteritis as well. Outbreaks occur in a variety of different settings, including nursing homes, restaurants, schools, day care centers, and cruise ships [13]. Waterborne outbreaks in community settings have been caused by contaminated wells and recreational water. For instance, contamination of a public water system was estimated to have caused up to 3000 cases of NLV-associated illness in Finland [27].

Most foodborne outbreaks of NLV infection arise though direct contamination of food or water by a food handler immediately before its consumption. Consequently, cold foods, including various salads, sandwiches, and bakery products, are classically implicated in outbreaks of infection [28]. Food can also be contaminated at its source; oysters from contaminated waters are classically associated with widespread outbreaks of gastroenteritis [29]. Other foods, such as raspberries, ice, and

salads, have also been contaminated before widespread distribution and have subsequently caused extensive outbreaks of gastroenteritis (table 1) [30].

Although published epidemiologic criteria allow outbreaks of gastroenteritis to be classified and reported as being possibly due to NLV, the lack of validation with a confirmatory assay means that these reports are only useful for rough estimates of disease burden. The fraction of all foodborne epidemics that are definitively attributable to NLVs depends directly on the quality of surveillance and the availability of sensitive diagnostic tools. During the 1970s and 1980s, when electron microscopy and serologic assays were the primary methods for detection of NLVs, <50% of outbreaks of nonbacterial gastroenteritis were determined to be associated with NLV [5]. With use of more-sensitive molecular techniques, >90% of outbreaks of nonbacterial gastroenteritis have been attributed to NLVs, of which ~40% have been due to foodborne transmission [13]. In 2000, FoodNet, the nationwide sentinel surveillance system in the United States, found that 38 (56%) of 68 outbreaks of gastroenteritis of known etiology were caused by NLVs (FoodNet Surveillance Preliminary Report, 2000; unpublished report). Even so, health care professionals do not commonly test for NLVs, and NLVs remain underappreciated as a cause of outbreaks of gastroenteritis. In 1993–1997, only 9 of the 2751 foodborne outbreaks of gastroenteritis reported to the CDC were classified as having been caused by NLVs [8], and, in 1999, NLVs accounted for 7% of all reported foodborne outbreaks (CDC, unpublished data). So, although the wider availability of more-sensitive diagnostic tools in recent years has helped to increase the number of outbreaks of NLV infection that are diagnosed, relatively few laboratories are able to make a proper diagnosis. Improved access to technology and education are clearly needed.

More recently, NLVs have been found to be a common cause of endemic gastrointestinal disease in developed countries. In a recent Dutch study, NLV was the leading cause of diarrhea in the community, with 11% of patients testing positive for NLV [31], although the fraction of these cases caused by contaminated food was unknown. Furthermore, although it was once thought to cause disease predominantly among adults, NLV clearly is a common infection among all age groups. For instance, a recent study in Japan found that 18% of hospitalizations for gastroenteritis in children aged <14 years were associated with NLV infection, second only to rotavirus infection in frequency and severity [32]. In Finland, NLVs were the second most common cause of acute diarrhea (after rotavirus) among a cohort of children enrolled in a rotavirus trial [33]. Evidence supports the idea that elderly persons are at higher risk for NLV-associated illnesses and probably for more-severe disease [34]. This could be owing to the occurrence of epidemics of infection in assisted-living environments or to reporting bias because of the increased severity among these persons. Finally, although the incidence of NLV-associated disease peaks in the winter months in temperate countries [35], this pattern is less pronounced than those of rotavirus and astrovirus. NLV infection is diagnosed year-round in the United States.

DIAGNOSTIC OPTIONS

The primary reason for the underappreciation of the disease burden of NLV infection has been the difficulty in developing and applying sensitive, easy-to-perform diagnostic assays. The virus cannot be cultivated from clinical samples, and no animal models (other than humans) are available to study NLVs. Until recently, the primary diagnostic methods were electron microscopy, a technique that is relatively insensitive and that requires expensive equipment and expertise, and serologic assays, which were available only in reference laboratories and which use reagents obtained from human volunteers. In the 1990s, electron microscopy was gradually replaced by the more sensitive and specific RT-PCR used to detect viral genome in clinical samples (usually stool samples). Assays have also been developed to detect viral genome in shellfish, which accumulate NLV from contaminated water. The sequencing of the viral genome has aided epidemiologic investigations by linking cases to each other and to a common source and by differentiating outbreaks that were mistakenly thought to be connected. For instance, in 1993, NLVs with identical sequences were found in cases from 23 clusters of gastroenteritis in 6 states and in the implicated oysters [29]. Assays to detect NLVs have to be adapted to each particular food matrix and are, as yet, rarely used. Despite the fact that RT-PCR requires expensive equipment and expertise and is time-consuming to perform, an increasing number of US state health departments and university laboratories are currently using the technique, aided by recent efforts by the CDC to distribute primers and reagents. At present, ~25 state and local public health laboratories have the technical capability to diagnose NLV infection. Nonetheless, testing for NLVs remains restricted to public health laboratories during outbreaks of infection, and tests are not routinely available to the clinician.

The genetic variability of NLVs and the lack of significant cross-reaction between strains have limited the sensitivity of antigen-detection methods for NLVs, which tend to be strain specific. Efforts to characterize currently circulating strains of NLV strains and to detect common epitopes should allow the development of broadly reactive assays.

TREATMENT AND PREVENTION

No specific therapy exists for viral gastroenteritis. Therapy for the symptoms consists of replacement of lost fluids and correction of electrolyte disturbances through oral and intravenous administration of fluids. Although several vaccines against rotavirus are in the late stages of development, these will not be of importance in the prevention of foodborne viral gastroenteritis. The early phase of development of NLV vaccines has begun. Because of the inability to culture NLVs, most of this work has focused on recombinantly expressed viruslike particles that do not replicate. These viruslike particles have proven to be immunogenic in humans when administered orally and, interestingly, also when delivered in transgenic potatoes, which raises the possibility of an edible vaccine. It is presently not known whether protective immunity is induced, and the lack of a culture system means that levels of neutralizing antibodies cannot be measured. Nonetheless, there is hope that viruslike particles of NLVs will induce antibody responses with more cross-reactivity than does infection with the live virus [36].

Prevention of foodborne NLV disease lies in the provision of safe food and water. NLVs are more resilient than are other gastroenteric pathogens; they are able to survive freezing and temperatures of up to 60°C, and they have even been associated with illness after being steamed in shellfish. In addition, NLVs can survive chlorine levels up to 10 ppm, which is well in excess of levels routinely present in public water systems [28]. However, despite this, it is likely that simple measures, such as correct handling of cold foods, frequent handwashing, and paid sick leave, will significantly reduce foodborne transmission of NLV infection.

FUTURE DIRECTIONS

The recent development of improved diagnostic assays and surveillance is helping to define the nature and extent of NLV disease. The next few years are likely to be dominated by research to define the disease burden of NLVs, which we hope will be aided by the successful development of straightforward, sensitive, and specific diagnostic assays. In addition, vaccine development will continue in parallel with epidemiologic studies to define the groups most in need of vaccination. Successful efforts to grow NLVs in cell culture will be a key breakthrough for vaccine development and will revolutionize diagnostic strategies. Cell culture will also allow inactivation studies to examine the stability of NLVs to temperature, disinfectants, and industrial processes. Another important step will be the evolution of assays to reliably detect NLV contamination in different foods. All of these developments will not only make understanding of NLVs even more important for public health authorities, but they will also make NLVs increasingly relevant to clinical physicians in the near future. At this time, however, communication to the public health community of what is already known about NLVs and implementation of simple infection-control measures will continue to be a priority.

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