The Epidemiology of Enteric Caliciviruses from Humans: A Reassessment Using New Diagnostics

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In the United States, acute gastroenteritis is one of the most commonly noted illnesses on hospital discharge records and death certificates, yet few of these cases have an etiologic diagnosis. The application of new molecular diagnostic methods has shown caliciviruses (previously referred to as the Norwalk family of viruses or small round structured viruses) to be the most common cause of acute gastroenteritis (AGE) outbreaks in the United States, and they may emerge as a common cause of sporadic cases of AGE among both children and adults. Novel molecular methods have permitted outbreak strains to be traced back to their common source and have led to the first identification of virus in implicated vehicles of infection—water, shellfish, and foods contaminated both at their source and by food handlers. The broad application of these methods to routine diagnosis in hospitals and public health laboratories is advancing our appreciation of the full burden of calicivirus-associated diarrhea, and it is opening new avenues for its prevention and control.

Despite major public health advances to improve the quality of food, water, and sanitation during the past century, acute gastroenteritis (AGE) remains one of the most common illnesses in the United States. The illness ranges in severity from mild vomiting and diarrhea to severe disease with dehydration that can be fatal. While AGE is often thought of as an illness in children, adults are affected as well, and the elderly are at the greatest risk of a fatal outcome. Surveys in the United States suggest that nearly every American will have an average of about 1 episode of gastroenteritis each year [1], and the rate is higher for children <5 years of age and for the elderly. From the 250–350 million episodes that occur each year, ~450,000 adults and 160,000 children are hospitalized [2–4], resulting in >4000 deaths (table 1). In terms of health care delivery, 10%–12% of hospitalizations of children <5 years old [5] and 1.5% of those among adults are associated with gastroenteritis [3, 6]. One in 25 American children will be hospitalized for diarrhea by the age of 5 years, and ~1 in 8 American adults (≥20 yrs) will be discharged from a hospital with a diagnosis of primary or secondary AGE during their adult years [3].

During the last 3 decades, there has been a dramatic increase in the number of newly recognized etiologic agents of gastroenteritis. Before 1970, a pathogen could be identified in fewer than 10% of patients hospitalized with diarrhea in the United States; the remaining 90% of cases without an identified pathogen became a “diagnostic void” consisting of various idioopathic, ill-defined conditions, such as the diarrhea of weaning, malnutrition, or old age. Since 1970, >20 different microorganisms—bacteria, parasites, and viruses—have been identified as etiologic agents, and most cases of AGE are now presumed to have an infectious etiology. Nevertheless, a pathogen is currently identified in only a small proportion of cases.

Furthermore, the reported prevalence of an enteric pathogen more often reflects the ease and availability of diagnostic tests specific for particular organisms than the true prevalence of the agent. Consequently, those pathogens most difficult to detect are considered uncommon and relegated to the diagnostic void. For example, a review of the etiology of nearly 7500 foodborne outbreaks of gastroenteritis reported to the Centers for Disease Control and Prevention (CDC) from 1973 to 1987 identified 25% as bacterial, 1.8% each as viral (Norwalk-like virus [NLV] and hepatitis A virus) and parasitic, and 62% to be of unknown cause [7]. Similarly, an investigation of >30,000 patients hospitalized for diarrhea between 1990 and 1992 at 10 sentinel centers in the United States found that only 5.9% had an identified bacterial pathogen, leaving 94% in the diagnostic void [8].

Enteric caliciviruses from humans—previously referred to as the Norwalk family of viruses or small round structured viruses (SRSVs) and hereinafter referred to as human caliciviruses—now appear to be candidate agents that could fill in a substantial portion of this diagnostic void. Our understanding of their importance evolved rapidly after major advances were made in determining the molecular biology of the viruses and in apply-
Background: The Early Years

The original Norwalk virus (NV) was identified from clinical specimens obtained in 1968 during the investigation of an outbreak of gastroenteritis among schoolchildren in Norwalk, Ohio; the outbreak was investigated by Adler and Zickl [9] of CDC. The prototype strain was discovered by Kapikian [10] in 1972 and was the first virus identified that specifically caused gastroenteritis in humans. This landmark discovery was followed by 2 decades of failed attempts to cultivate the virus, develop an animal model, or prepare simple, sensitive, diagnostic tests that could be widely used to study the extent of disease.

In the absence of an animal model, human volunteers became the sole means to examine questions of natural immunity [11, 12], pathogenesis, and the effects of interventions, such as chlorination of water to inactivate virus [13]. Diagnostic tests were developed that used reagents prepared from clinical specimens from hundreds of human volunteers, and crude assays made from volunteer specimens (i.e., paired sera and fecal specimens) served as reference reagents to compare strains, but these assays were available in few research laboratories [11].

In addition, a morphologically distinct SRSV, “classic human calivirus,” was first described by Madeley and Cosgrove [16] in England, and the prototype strain—the Sapporo agent—was identified by Chiba and colleagues [17] in Japan. This classic human calivirus appeared to have several distinguishing epidemiologic features [17–21]. It initially appeared to preferentially infect small children, and unlike the SRSVs, which were identified primarily in outbreaks and affected people of all ages, this virus was occasionally found in sporadic cases of gastroenteritis.

The confusing early names of these viruses, which were determined on the basis of the locations of their discovery (e.g., Norwalk, Hawaii) or their appearance by EM (e.g., SRSV, classic caliciviruses), have been clarified recently. The names are now based on identification of the genetic sequences of the viruses and determination of their genomic organization. All belong to the family Caliciviridae, and they fall into 2 provisionally named genera—“Norwalk-like viruses” (NLV) and “Sapporo-like viruses” (SLV). Consequently, early papers referring to the NV, the Norwalk family of viruses, or NLVs, should generally be viewed as relating to human caliciviruses of the genus NLV.

Outbreak investigations: the emerging role of NLVs. Once NV was identified as a causative agent in outbreaks, assessment of the disease burden depended on the development of improved diagnostic assays (table 2) [22–25]. Initially, EM and IEM were used to detect the virus. Despite the sensitivity of the assay, $10^6$–$10^7$ particles/g, few microscopists took the time to make a diagnosis. In the absence of a simple sensitive test, NLVs appeared to play an insignificant role in the disease burden of gastroenteritis. A CDC review of 7500 foodborne outbreaks that were investigated between 1973 and 1987 [7] identified NLVs in only 1.5%. An even more recent review of outbreaks traced to drinking water ($n = 22$) or recreational water ($n = 22$) identified NLVs in 5% each [26].

In 1978, a radioimmunoassay was introduced for serologic testing to investigate outbreaks of nonbacterial gastroenteritis.

### Table 1. Annual hospitalizations, deaths, and summary statistics for diarrheal disease in adults; United States, 1979–1995.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Deaths/year</th>
<th>Discharges/year</th>
<th>Deaths/1000 discharges</th>
<th>Deaths/1000 population</th>
<th>Hospitalizations, $\times 10^3$</th>
<th>Gastroenteritis as % of all discharges</th>
<th>Length of stay (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20–49</td>
<td>612</td>
<td>208,185</td>
<td>3.0</td>
<td>5.7</td>
<td>1.9</td>
<td>1.6</td>
<td>4.1</td>
</tr>
<tr>
<td>50–64</td>
<td>392</td>
<td>78,822</td>
<td>5.1</td>
<td>11.8</td>
<td>2.4</td>
<td>1.4</td>
<td>5.4</td>
</tr>
<tr>
<td>65–74</td>
<td>1052</td>
<td>72,937</td>
<td>14.6</td>
<td>60.9</td>
<td>4.2</td>
<td>1.5</td>
<td>6.6</td>
</tr>
<tr>
<td>$\geq$75</td>
<td>2293</td>
<td>92,047</td>
<td>25.3</td>
<td>488.2</td>
<td>19.6</td>
<td>1.6</td>
<td>7.4</td>
</tr>
<tr>
<td>Total</td>
<td>4349</td>
<td>451,991</td>
<td>9.8</td>
<td>25.5</td>
<td>2.6</td>
<td>1.5</td>
<td>5.4</td>
</tr>
</tbody>
</table>

NOTE. Data are from [3].

* Hospital discharges for which outcome is known. Outcome was unknown for ~1.6% of discharged patients.
Table 2. Advances in the detection of human caliciviruses, by year.

<table>
<thead>
<tr>
<th>Year</th>
<th>Investigator, reference</th>
<th>Detection method</th>
<th>Sensitivity</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1972</td>
<td>Kapikian [10]</td>
<td>EM/IEM</td>
<td>~10²⁻⁳</td>
<td>Detection of Norwalk virus and antibody</td>
</tr>
<tr>
<td>1978</td>
<td>Greenberg [14]</td>
<td>RIA</td>
<td>~10²⁻⁴</td>
<td>Antibodies to VLPs formatted into immunoassay</td>
</tr>
<tr>
<td>1992</td>
<td>Jiang [22]</td>
<td>RT-PCR</td>
<td>~10²⁻⁴</td>
<td>Beginning of molecular diagnostics</td>
</tr>
<tr>
<td>1992</td>
<td>Jiang [23]</td>
<td>EIA for serology</td>
<td>~10²⁻⁴</td>
<td>VLPs used as synthetic antigens</td>
</tr>
<tr>
<td>1995</td>
<td>Ando [24]</td>
<td>RT-PCR with multiple primers and probes</td>
<td>~10²⁻⁴</td>
<td>RT-PCR detection extended to characterize strains with probe hybridization</td>
</tr>
<tr>
<td>1995</td>
<td>Jiang [25]</td>
<td>EIA for antigen detection</td>
<td>~10²⁻⁵</td>
<td>Antibodies to VLPs formatted into immunoassay</td>
</tr>
</tbody>
</table>

NOTE. EM = electron microscopy, IEM = immune EM, RIA = radioimmunoassay, RT-PCR = reverse transcription-polymerase chain reaction, EIA = enzyme immunoassay, VLPs = virus-like particles.

In targeted studies, 19%–42% of outbreaks of nonbacterial AGE could be attributed to NLVs [27–29]; however, because there were limited reagents, few outbreaks were tested, and those left untested were mistakenly considered to have “no known agent” and became part of the diagnostic void. From 1995, this situation was corrected when reverse transcription-polymerase chain reaction (RT-PCR), which could detect 10⁻⁴ NLVs, became the laboratory standard. Fankhauser and co-workers [30] identified NLV as the causative agent in 94% of a series of 90 outbreaks of nonbacterial AGE, indicating that NLVs were not only the predominant agent associated with outbreaks of AGE in the United States that went without an etiologic diagnosis but were also the most common agents of foodborne outbreaks. A similar high prevalence was identified in studies in The Netherlands [31], the United Kingdom [32], Japan [33], and Australia [34].

Features of NLVs that relate to issues of public health and prevention. From the many investigations of nonbacterial gastroenteritis outbreaks, we have gained knowledge of public health importance about NLVs (table 3). Most early outbreaks were traced to fecally contaminated food or water, but for some, no clear mode of transmission could be identified. This pattern was explained by the finding that very few viruses (<100) are needed for infection, and therefore transmission by droplets, person-to-person contact, or environmental contamination was possible, and secondary spread to family members and friends was frequent [35]. Because asymptomatic shedding can persist for >1 week, infected food handlers may be an important source of infection [22, 36]. The virus is stable in water chlorinated to 10 ppm [13] and survives freezing [37] and heating to 60°C [38], permitting spread in recreational and drinking water and in contaminated oysters that have been steamed.

NLV strains demonstrate great antigenic and genetic diversity. Therefore, diagnostic tests need to be able to detect the range of many different virus types. Furthermore, these strains may provide little cross-protection, so people can be serially infected with different strains. Volunteer studies showed that immunity to NLVs appeared to be short lived [39], so childhood exposure to one strain may not even protect adults from repeat disease with the same strain. Early volunteer studies had several limitations. The challenge inoculum used now appears to have been relatively large, so immunity measured by a marked decrease in viral shedding could well have been missed. Furthermore, they were conducted extensively with only one of many antigenic variants, so the ability to extrapolate these results to other antigenically distinct NLVs is limited. The only reservoir for NLVs was presumed to be humans, although recent reports of isolates from cows and pigs have challenged this view [40–43].

A few epidemiologic studies, particularly in children, provided some clues to the potential prevalence of infections with these viruses and of their importance as a cause of sporadic disease. Early seroepidemiologic surveys of antibody to NV among children in the United States and Bangladesh suggested that in both settings, most children acquired serum antibodies during their first 5–15 years of life, although the Bangladeshis were infected at an earlier age [44]. Nonetheless, since surveys using EM failed to detect the virus in fecal specimens of children who were hospitalized, the causative role of these viruses in childhood diarrhea remained unclear. Grohmann [19] screened 7400 patients hospitalized in Sydney over an 11-year period and found that some specimens found negative by EM were positive by IEM. Of 800 patients, 16.8%, primarily adults, had SRSVs in their stools as determined by IEM, and 2.3% of children were infected with Sapporo-like viruses. This suggests that both groups of viruses were a cause of sporadic gastroenteritis and that their epidemiologic patterns were distinct [19].

British electron microscopists also found SRSVs in both sporadic and outbreak cases, suggesting that these pathogens were ubiquitous but their prevalence was generally low [45, 46].

The Molecular Era

Virus detection and tracing from fecal specimens. The cloning and sequencing of NV and the Southampton virus opened the way for the introduction of new and more sensitive molecular diagnostics [47, 48]. RT-PCR, probes, and sequence analysis were applied to detect viral RNA in fecal specimens, and baculovirus-expressed capsids were used as antigens in immunoassays to detect antibodies and seroconversion [22, 23]. Early studies with RT-PCR proved disappointing: Primers chosen from the polymerase region of the prototype NV and believed to be conserved between strains could not detect most specimens that were positive by EM and obtained from patients who seroconverted to NV [49]. However, merely changing the
primers significantly increased the sensitivity of the assay and demonstrated that detection rates were much higher for specimens from patients who seroconverted to NV than patients from outbreaks that were attributed to antigenically distinct “Norwalk-like agents.”

To resolve this diagnostic problem, Ando et al. [50] sequenced the polymerase regions of a group of strains that had been missed by RT-PCR [50]. The extent of genetic diversity was unanticipated, and each strain examined had a unique sequence. These sequences fell into 2 distinct groups: genogroup I, with the NV as the prototype strain, and genogroup II, comprised of the Snow Mountain and Hawaii agents. This information was used to develop a new mixture of primers and probes to specifically detect strains belonging to the 2 genogroups by RT-PCR and to characterize them into one of the known antigenic types based on hybridization with specific probes [24].

The usefulness of these new methods for molecular epidemiology became clear in a number of extraordinary outbreaks of gastroenteritis associated with contaminated oysters [51, 52], water [53], and food [54–57] (table 4). In each outbreak, the identification of a single sequence among specimens collected from patients in different locations provided, along with observations from epidemiologic investigations, indisputable evidence that the outbreak resulted from a single contaminated source. Furthermore, by preparing primers to the specific sequence of the outbreak strains, investigators could detect virus in the implicated oysters, food, or water; in each case, these viruses had the same sequence as the outbreak strain, underscoring a causal relationship. At the same time, several serial outbreaks on cruise ships, in a school system, and from oysters that were attributed to a single contaminated source were found to be caused by viruses with different sequences, suggesting multiple etiologic agents and possibly different modes of spread [30]. These outbreaks demonstrated that strain tracing could be of considerable importance in determining whether patients were exposed to the same virus and if the identical virus might be present in the vehicle of infection implicated in the epidemiologic investigation.

The role that caliciviruses might play as a cause of outbreaks of nonbacterial gastroenteritis was further clarified in 1995 when RT-PCR with a cocktail of primers was introduced as a routine diagnostic test. In Minnesota, Hedberg and Osterholm [58] identified an NLV in a majority of outbreaks of gastroenteritis investigated by the state health department, making this the most common agent of outbreaks of gastroenteritis of all causes. At CDC, NLVs were detected by RT-PCR in 86 (96%) of 90 outbreaks of nonbacterial gastroenteritis for which fecal specimens were submitted [30]. This finding was in sharp contrast to our results a decade before, when only 19% of such outbreaks were thought to be caused by NV and 39% of patients tested were seronegative [29]. This earlier observation meant that any other etiologic agents for nonbacterial AGE were likely to be relatively unimportant and indicated that we should place greater emphasis on simplifying the diagnostic methods for the NLVs. Furthermore, while NV was the prototype strain of this family, our review indicated that, similar to the original NV, genogroup I strains were less commonly found in outbreaks than were genogroup II NLVs; this finding was also observed in the United Kingdom, where only genogroup II primers were needed to detect most outbreak strains [32, 59].

**Use of expressed antigens in viral diagnosis.** Jiang et al. [23] showed that the capsid gene expressed in baculovirus produced self-assembled virus-like particles (VLPs). Their findings led to the rapid development of immunoassays for both antibodies and antigen. The availability of assays that used VLPs reproduced in high concentration and purity replaced early assays that had required viral antigen extracted from fecal specimens of human volunteers. Antibody assays were soon applied in various settings from seroepidemiologic surveys (which also examined risk factors for virus exposure [60]) to the examination of serologic responses to specific outbreaks [61]. The serosurveys indicated, as did the radioimmunoassay studies before them, that infection with NLVs occurs early in childhood and that most adults have circulating antibodies to these viruses. Patients in outbreaks tend to have a specific antibody response to the genogroup I or genogroup II viruses of the outbreak, although some cross-reactivity occurs [57]. This pattern of antibody response suggests that to get a proper estimate of seroprevalence, an investigator would have to screen sera with a variety of antigens.

Efforts to use expressed capsid proteins to develop simple immunoassays to detect viral antigen in fecal specimens have had a tortuous path. The first enzyme immunoassay for NV could detect antigen in volunteers with good sensitivity many days after challenge, suggesting that soluble antigen persisted

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**Table 3. Key features of Norwalk-like viruses that relate to issues of public health prevention.**

<table>
<thead>
<tr>
<th>Feature</th>
<th>Observation</th>
<th>Consequences</th>
</tr>
</thead>
<tbody>
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<td>Low infectious dose</td>
<td>&lt;10&lt;sup&gt;12&lt;/sup&gt; virus particles</td>
<td>Permits droplet/person-to-person spread, secondary spread</td>
</tr>
<tr>
<td>Prolonged asymptomatic shedding</td>
<td>Up to 2 weeks</td>
<td>Increases risk of secondary spread, problems with control of food handlers</td>
</tr>
<tr>
<td>Environmental stability</td>
<td>10 ppm chlorine; stable with freezing and at 60°C</td>
<td>Hard to eliminate from contaminated water; virus maintained in ice and partially cooked oysters</td>
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<td>Great strain diversity</td>
<td>Many genetic and antigenic types</td>
<td>Requires composite diagnostics; multiple episodes can occur from many different antigenic types</td>
</tr>
<tr>
<td>Lack of longtime immunity</td>
<td>Repeat symptomatic infection on rechallenge</td>
<td>Childhood exposure does not protect adults from disease</td>
</tr>
<tr>
<td>Reservoir</td>
<td>Humans</td>
<td>Occurs only in human host—recent identification of related animal strains</td>
</tr>
</tbody>
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**Table 4. Characteristics of outbreaks of gastroenteritis investigated by the Minnesota state health department.**

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</tr>
</tbody>
</table>
after visible virus was no longer present [62]. However, when this assay was applied to a collection of fecal specimens from a panel of outbreaks, it was very specific and detected only those specimens from outbreaks in which the strain was genetically similar to the original NV strain. Consequently, an immunoassay would require a collection of antibodies to the many antigenically distinct strains unless a common epitope or cross-reacting protein could be identified.

**Implications of New Diagnostics for Public Health**

Application of new molecular diagnostics has demonstrated some clear lessons for public health and prevention and provided directions for future research efforts. The new diagnostics have only recently begun to be applied to understand the burden of disease associated with the caliciviruses. For outbreaks of AGE in the United States, the United Kingdom [59], Japan [33], Australia [34], and The Netherlands [31], it is now clear that most are attributable to caliciviruses, and laboratories investigating such outbreaks must develop the appropriate diagnostic capability. Some states in the United States, such as Minnesota, have taken an early lead to establish credible diagnostic capacity and have already demonstrated the high proportion of outbreaks attributable to NLVs. Application of molecular diagnostics to screen fecal specimens from children with diarrhea indicates that NLVs are second only to rotavirus as causative agents [63].

The ability to trace outbreaks of disease to contaminated food [64] and water using both [53] sequence analysis and newly established methods provides clear directions for molecular epidemiology and public health. For foodborne disease, these methods could shape policy decisions concerning the tracing, screening, and recall of contaminated food products; the closing of contaminated oyster beds; and the exclusion from work of infected food handlers. The methods should also encourage a vigorous program to use rapid virus-detection methods for foods and food handlers who are suspected of contamination. For waterborne disease, the ability to detect NLVs in water implicated in an outbreak provides an entry point for the development of new assays to detect NLVs in water and examine the efficacy of a wide range of water purification methods. While we still cannot measure the viability of an NLV in water, we can measure the gradient of purification of filtration systems for water and depuration systems for oysters.

Our ability to assess the role of NLVs as a cause of hospitalizations for AGE has improved as well, although with some limitations. While RT-PCR diagnostics can detect an etiologic agent in nearly all outbreaks of nonbacterial gastroenteritis, specimens from some patients in these outbreaks remain negative for the virus, suggesting that inhibitors of RT-PCR in fecal samples or improper matches of primers and viral sequence may lower the sensitivity of detection. As such, screening hospitalized patients by using RT-PCR alone may underestimate the prevalence of these agents. On the other hand, serodiagnosis appears to be much more sensitive, and the collection of paired sera from hospitalized patients may provide better estimates of the true prevalence of disease. These studies are ongoing.

**Risk Groups for NLVs**

Studies of outbreaks of gastroenteritis indicate that NLVs infect patients of all ages, a feature that distinguishes them from the other agents of viral gastroenteritis, such as rotaviruses, astroviruses, and adenoviruses, which primarily affect children. This difference suggests that immunity to NLVs may not be long lasting or that, like rhinoviruses, NLVs may have so many distinct types that one never is immune to them all.

Investigation of outbreaks provides some clues to those groups of people who are at greatest risk of disease (figure 1). In the series of 90 outbreaks reported by Fankhauser et al. [30], those among the elderly in institutions were the most numerous (43%) and were followed by outbreaks in restaurants (26%), schools (11%), and vacation settings (11%). A mode of transmission was sought in 51 outbreaks, and of these, food was implicated in 37%, person-to-person contact in 20%, consumption of oysters in 10%, and water in 6%.

Travelers are at high risk as well, and while new diagnostics have been applied to outbreak investigations of people aboard
cruise ships [65, 66], they have not been adequately sought in routine studies of traveler’s diarrhea, for which the diagnostic void remains large [67]. Diarrhea has always been a problem for soldiers on deployment, and a number of large outbreaks attributable to NLVs aboard naval vessels have put these ships out of action for brief periods [68]. Among ground troops in the Gulf War, gastroenteritis was the most common illness of soldiers, and 70% of these cases were attributable to NLVs [69]. Systematic efforts are needed to disseminate methods for diagnosing NLVs so that the full reporting of their impact in different populations can be quantified. In addition, immune-compromised patients, including those infected with human immunodeficiency virus, have had problems clearing infections with NLVs [70].

Discussion

AGE remains a common health problem of unappreciated importance in the United States. While mild illness affects most Americans every year, more-severe illness is associated with ~600,000 hospitalizations and >4000 deaths per year. In the past, surveys of hospitalizations due to AGE and outbreaks of AGE left a large diagnostic void of cases for which no etiologic agent could be identified. The role of caliciviruses was unrecognized and under-appreciated because diagnostics were not commonly available or used. The recent, and so far limited, application of new molecular assays for the caliciviruses suggests a markedly different conclusion: NLVs appear to be the second most common agent of severe diarrhea in children after rotavirus and the most common cause of outbreaks of AGE, including those that are foodborne. Their role in adults hospitalized for diarrhea and fatalities remains to be established, but outbreaks with fatalities in the elderly are common.

The availability of new assays, together with these preliminary observations, requires that improved diagnostic tools, now resident in research centers, be moved to local public health and hospital laboratories. Moreover, given the potential role that caliciviruses play in food and waterborne disease and the recent success in tracing outbreak strains back to contaminated food and water sources, groups concerned with food and water hygiene should play a more active role in adapting these new diagnostic methods for their own prevention efforts. Current methods have not been simplified and are not well suited to routine use; therefore, research needs to be targeted to development of diagnostic techniques that are simple, sensitive, and able to detect the great diversity of viruses in this group. Given the extensive burden of calicivirus-associated gastroenteritis and its high prevalence in outbreaks and sporadic cases of disease, the commercial market for these diagnostic tests for use in the health sector and in the protection of food and water sources should be substantial.

The ability to trace strains by their genetic sequence has further opened new avenues for public health interventions. The identification of single strains of NLVs that have caused multistate outbreaks of AGE in the United States or global outbreaks linked to contaminated foods has demonstrated the value of molecular epidemiology to link diverse patients to a single common exposure and to trace single food items back to a common source that is suitable for control. More challenging for epidemiologists has been the identification of a com-
mon strain of global distribution for which the mode of spread could not be determined. National and global use of common molecular diagnostics and the exchange of sequence information offer the possibility of identifying common outbreak strains early and determining a common source of exposure in real time so that measures for prevention and control can be implemented quickly.

For 25 years, NLVs have avoided attention by being difficult to detect, impossible to trace, and hard to put into their proper perspective as a major cause of AGE in both disease outbreaks and sporadic cases leading to hospitalization. This era has ended with breakthroughs in understanding the molecular virology of the organism that have led to major diagnostic advances. The challenge ahead is to seize upon these new methods, develop and simplify them further, and ensure their application in the broadest possible context. The results will be to rapidly fill in the current diagnostic gap in our understanding of the unknown agents of AGE and the recognition of the primary role that NLVs play in food and waterborne disease, thus permitting a reallocating of efforts and resources to address this urgent need.

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