The Impact of Foodborne Calicivirus Disease: The Minnesota Experience

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The first outbreaks of Norwalk virus gastroenteritis in Minnesota were confirmed in 1982. Since then, Norwalk-like caliciviruses have been recognized to be the most common cause of foodborne disease outbreaks, accounting for 41% of all confirmed foodborne outbreaks in Minnesota from 1981–1998. Although laboratory confirmation of caliciviruses in stool samples was not attempted in most of these outbreaks, all conformed to epidemiologic criteria for defining outbreaks of Norwalk virus. Since 1996, the availability of polymerase chain reaction testing at the Minnesota Department of Health has allowed for the confirmation of calicivirus infection among patients involved in epidemiologically defined outbreaks of viral gastroenteritis. Results have confirmed the usefulness of characterizing foodborne disease outbreaks by epidemiologic criteria and also confirmed the importance of human caliciviruses as the leading cause of foodborne disease outbreaks in Minnesota.

During the course of 1 week in August 1982, four outbreaks of acute gastroenteritis associated with separate social events were reported to state and local public health officials in Minnesota. Epidemiologic investigation of each outbreak implicated cakes from a single bakery as the source of the outbreak. All implicated cakes used frosting made by 1 bakery worker who was ill with vomiting and diarrhea during his work shift. Immune electron microscopy of stool samples from outbreak-associated cases revealed 27-nm virus particles. Infection with Norwalk virus was confirmed by serology [1]. This demonstration of the potential for 1 food worker to cause a large outbreak of viral gastroenteritis and the contemporaneous publication by Kaplan and colleagues [2] of epidemiologic criteria for evaluating outbreaks of Norwalk virus infections forever changed the way outbreaks of foodborne disease were investigated in Minnesota.

Methods

In Minnesota, outbreaks of foodborne disease are primarily investigated by epidemiologists from the state health department and by environmental health specialists from local public health agencies or the state health department. In a few large cities and counties that have their own epidemiologists, the local agency may take primary responsibility for both epidemiologic and environmental health investigations. In these situations, the local health department epidemiologists follow state health department protocols, notify the state health department of outbreaks being investigated, and seek consultation and public health laboratory support for their outbreak investigations. This results in Minnesota having a highly centralized and standardized foodborne disease surveillance system. It also allows for rapid epidemiologic assessment of outbreaks and the efficient use of public health laboratory resources.

After 1982, the lack of available antigen and antibody detection assays for Norwalk-like viruses led the Minnesota Department of Health to define an epidemiologic profile of viral gastroenteritis and use this profile to confirm a viral etiology for a foodborne outbreak [3, 4]. Outbreaks that presented with median incubation periods of 24–48 h, vomiting among >50% of the cases (or a higher proportion of cases with vomiting than fever), and resolution of symptoms within 12–60 h were considered to have a viral etiology.

During 1996, the Minnesota Department of Health Public Health Laboratory developed the capacity to test stool specimens for the presence of caliciviruses by polymerase chain reaction (PCR) [5–7]. Outbreak investigation protocols were modified to attempt to collect 3–5 stool specimens from each outbreak to confirm the presence of caliciviruses as well as to rule out bacterial pathogens. Stool specimens were collected in non-nutritive medium for bacteriologic culture (C & S vials; Meridian, Cincinnati, OH) and stored at 4°C or stored unpreserved at −76°C. Virus particles were isolated by use of Sephadex G-200 spun columns equilibrated with 0.1 × PCR Buffer II (Perkin-Elmer, Foster City, CA). Viral RNA was purified by guanidinium-urea-phenol extraction (Ultraspec-3 kit; Biotecx, Houston, TX). cDNA was prepared by reverse transcription, using primer JV13 and GeneAmp RNA PCR Core kit reagents (Perkin-Elmer).

A 327-bp fragment of the calicivirus polymerase gene was then amplified using primers JV12 and JV13 in a 50-μL PCR reaction and visualized on 2% agarose gels stained with ethidium bromide [6]. cDNA reverse transcribed from purified viral RNA was amplified by PCR, using primers JV12/13. PCR products were purified, using Microspin columns (Pharmacia, Piscataway, NJ) or Qiaquick PCR purification columns (Qiagen, Valencia, CA), and sequenced using primer JV12 and Thermosequenase cycle sequencing reagents (Amersham, Arlington Heights, IL). Sequence analyses were performed using an automated sequencer (OpenGene; Vis-

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Table 1. Confirmed foodborne outbreaks of illness by agent—Minnesota, 1981–1998.

Agent	No. (%) of outbreaks		
	1981–1989	1990–1998	Total
Norwalk-like virus	35 (33)	85 (45)	120 (41)
Clostridium perfringens	15 (14)	22 (12)	37 (13)
Salmonella species	12 (11)	21 (11)	33 (11)
Staphylococcus aureus	7 (7)	5 (3)	12 (4)
Chemical	9 (8)	3 (2)	12 (4)
Bacillus cereus	7 (7)	3 (2)	10 (3)
Campylobacter jejuni	4 (4)	6 (3)	10 (3)
Escherichia coli O157 : H7	1 (1)	7 (4)	8 (3)
Other *E. coli*[a]	1 (1)	5 (3)	6 (2)
Hepatitis A virus	1 (1)	5 (3)	6 (2)
Shigella sonnei	2 (2)	4 (2)	6 (2)
Other[b]	2 (2)	7 (4)	9 (3)
Unknown	11 (10)	15 (8)	26 (9)
Total	107	188	295

[a] Includes 4 enterotoxigenic *E. coli,* 1 enteropathogenic *E. coli,* and 1 other diarrheagenic *E. coli.*

[b] Includes 3 scombroid toxin, 2 ciguatera toxin, 2 *Giardia lamblia,* 1 *Listeria monocytogenes,* and 1 *Cryptosporidium parvum.*

ible Genetics, Toronto, or ABI 373; ABI, Norwalk, CT), and sequences were aligned for comparison with genotyping oligonucleotides using Genetics Computer Group (Madison, WI) programs. Outbreak caliciviruses were assigned to a particular genotype on the basis of a 20-bp region of the sequenced PCR products [7].

Results

From 1981–1998, 295 confirmed outbreaks of foodborne disease occurred in Minnesota. Of these, 120 (41%) met the epidemiologic criteria for being considered outbreaks of Norwalk-like viral gastroenteritis (table 1). During this same time period, there were 57 outbreaks caused by the major bacterial foodborne pathogens: 33 (11%) due to *Salmonella* species, 10 (3%) due to *Campylobacter jejuni,* 8 (3%) due to *Escherichia coli* O157:H7, and 6 (2%) due to *Shigella sonnei.* Thus, the combined number of foodborne bacterial outbreaks accounted for less than half the number of outbreaks of Norwalk-like viral gastroenteritis.

Laboratory confirmation of the presence of Norwalk-like caliciviruses was obtained for two outbreaks in 1982, one in 1993, five in 1996, three in 1997, and eight in 1998. From 1996 through 1998, 47 foodborne outbreaks of viral gastroenteritis occurred in Minnesota. Stool samples were available for PCR testing from 23 of these outbreaks (49%). Caliciviruses were detected in samples from 16 (70%) of these 25 outbreaks. Overall, caliciviruses were detected in 34 (47%) of 72 stool samples obtained from cases associated with these outbreaks (table 2). In 10 outbreaks, PCR products were sequenced and calicivirus genotypes were assigned [7]: Six were genotype P2B, two were P1A, and two were P2A. The P2A outbreaks occurred in 1996. Outbreaks caused by P1A and P2B occurred in both 1997 and 1998.

A high proportion of the 120 confirmed foodborne outbreaks of viral gastroenteritis were associated with consumption of cold food items that had been handled by ill food workers. In 53 outbreaks (44%), ill persons who handled the implicated food items were identified. In 21 outbreaks (18%), food workers denied illness but reported illnesses among members of their household, suggesting either the possibility of transmission from persons with asymptomatic infections or their failure to adequately wash hands and remove virus particles acquired at home. In total, 74 outbreaks (62%) were likely the result of contamination of foods by contact with bare hands. In six outbreaks (5%), illnesses among food workers occurred at the same time as patron illnesses. No food-worker source of contamination could be identified in 40 outbreaks (33%).

The occurrence of employee illnesses and the potential for ongoing transmission of illness to patrons led to the temporary closure of 11 restaurants (22%) that were involved in outbreaks. More than 1 ill employee was identified in 29 (58%) of the 50 outbreaks of Norwalk-like viral gastroenteritis that occurred in restaurants.

Fresh fruits or vegetables were implicated as a vehicle in 44 outbreaks (37%). In 29 produce-associated outbreaks (66%), illnesses among food workers (22 outbreaks) or their family members (seven outbreaks) preceded the outbreak. In two outbreaks, illnesses among food workers occurred at the same time as patron illnesses. No food-worker source of contamination could be identified for 13 outbreaks (30%) associated with fresh fruits or vegetables.

The 44 outbreaks of Norwalk-like viral gastroenteritis associated with fresh produce items accounted for 59% of the 75 confirmed foodborne outbreaks associated with fresh produce vehicles in Minnesota during this time period (table 3).

Discussion

Results of statewide foodborne disease surveillance in Minnesota demonstrate that Norwalk-like viruses continue to be the leading cause of foodborne disease outbreaks, accounting for 41% of all confirmed foodborne disease outbreaks that occurred from 1981–1998. The proportion of outbreaks attributed to Norwalk-like viruses is much greater than reported in national foodborne disease outbreak surveillance data.

From 1988–1992, only 2 of 2423 confirmed foodborne out-

Table 2. Detection of calicivirus in 23 foodborne outbreaks of illness epidemiologically identified as caused by Norwalk-like virus—Minnesota, 1996–1998.

Category	No. (%) of outbreaks
Confirmed calicivirus	16 (34)
Not confirmed	7 (15)
Not tested	24 (51)
Total	47 (100)

NOTE. Of 72 stool samples tested from 23 outbreaks of foodborne illness, 34 (47%) were positive for calicivirus RNA.

Table 3. Confirmed foodborne outbreaks of illness, by agent, associated with fresh produce—Minnesota, 1981–1998.

Agent	No. (%) of outbreaks		
	1981–1989	1990–1998	Total
Norwalk-like virus	17 (81)	27 (50)	44 (59)
Salmonella species	1 (5)	6 (11)	7 (9)
Escherichia coli[a]	0 (0)	5 (9)	5 (7)
Campylobacter jejuni	0 (0)	3 (6)	3 (4)
Chemical	1 (5)	2 (4)	3 (4)
Hepatitis A virus	1 (5)	2 (4)	3 (4)
Shigella sonnei	0 (0)	2 (4)	2 (3)
Giardia lamblia	0 (0)	1 (2)	1 (1)
Unknown	1 (5)	6 (11)	7 (9)
Total	21	54	75

[a] Includes 2 *E. coli* O157:H7, 2 enterotoxigenic *E. coli,* and 1 other diarrheagenic *E. coli.*

breaks reported in the United States were attributed to Norwalk virus [8]; however, 1422 (59%) were considered to have an unknown etiology because no agent was confirmed by laboratory evaluation of clinical specimens. The Minnesota experience suggests that a high proportion of these outbreaks are likely to be Norwalk-like viral gastroenteritis and that careful collection and review of epidemiologic summary data should be a routine part of national foodborne disease surveillance. Laboratory testing of stool samples collected from epidemiologically defined outbreaks in Minnesota since 1996 confirms the usefulness of characterizing foodborne disease outbreaks by epidemiologic criteria. In addition, distinguishing between the epidemiologic characteristics of Norwalk-like viral gastroenteritis and other outbreaks can lead to improved detection, laboratory testing, and confirmation of outbreaks due to other agents (e.g., enterotoxigenic *E. coli*) that are not routinely identified by clinical laboratories.

Norwalk-like viruses are important causes of illness in restaurants and illness associated with fresh produce items. These are important food safety issues because of increasing consumption of fresh fruits and vegetables and increasing consumption of meals eaten out [9]. In 11 outbreaks, illnesses among multiple food workers led to ongoing transmission to patrons and the need to temporarily close the involved restaurants.

Development of strategies to prevent outbreaks in restaurants will be complex. A food worker was identified as being ill before preparing the implicated food in less than half of the restaurant-associated outbreaks. The potential for asymptomatic infections or passive transfer of virus particles on hands to cause foodborne outbreaks needs further evaluation. Furthermore, in one-third of outbreaks, no food-worker source of contamination could be identified. This suggests the possibility that the implicated food items were contaminated when they arrived at the kitchen. A recent international outbreak of calicivirus infections associated with frozen raspberries demonstrated that contamination during harvesting or processing potentially can produce the same type of widespread outbreak associated with low-level or sporadic contamination of ready-to-eat foods that has been documented with foodborne bacterial infections [9, 10]. The lack of sensitive surveillance systems to detect foodborne viral gastroenteritis and the lack of available laboratory methods to identify and subtype virus strains has until recently limited public health efforts to identify or investigate outbreaks associated with widely distributed commodities or food products. However, the development and use of PCR testing and gene sequencing will revolutionize our understanding of the epidemiology of this important group of foodborne pathogens.

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