MAJOR ARTICLE

Outbreaks of Acute Gastroenteritis on Cruise Ships and on Land: Identification of a Predominant Circulating Strain of Norovirus—United States, 2002

Marc-Alain Widdowson,[1,2] Elaine H. Cramer,[3] Leslie Hadley,[2] Joseph S. Bresee,[2] R. Suzanne Beard,[2] Sandra N. Bulens,[1,2] Myrna Charles,[2,4] Wairimu Chege,[2,4] Elmira Isakbaeva,[2,4] Jennifer G. Wright,[2,4] Eric Mintz,[2] David Forney,[3] Jeffrey Massey,[5] Roger I. Glass,[2] and Stephan S. Monroe[2]

[1]Atlanta Research and Education Foundation and [2]National Center for Infectious Diseases, [3]National Center for Environmental Health, and [4]Epidemiologic Intelligence Service, Epidemiology Program Office, Centers for Disease Control and Prevention, Atlanta, Georgia; [5]Molecular Biology Section, Michigan Department of Community Health, Lansing

In 2002, a sharp increase in outbreaks of norovirus-associated illness, both on cruise ships and on land, encouraged us to examine the molecular epidemiology of detected noroviruses, to identify a common strain or source. Of 14 laboratory-confirmed outbreaks on cruise ships, 12 (86%) were attributed to caliciviruses; among these 12, outbreak characteristics included continuation on successive cruises in 6 (50%), multiple modes of transmission in 7 (58%), and high (>10%) attack rates in 7 (58%). Eleven of the 12 calicivirus outbreaks were attributed to noroviruses, 7 (64%) of which were attributed to a previously unreported lineage, provisionally named "the Farmington Hills strain." From May 2002 to December 2002, 10 (45%) of 22 land-based outbreaks also were attributed to this strain. Nucleotide-sequence analysis provided insights into norovirus transmission, by documenting links among outbreaks, the introduction of strains onto ships, and viral persistence on board (despite cleaning). Control measures for outbreaks should address all routes of transmission. Better outbreak surveillance and collection of data on sequences will help to monitor norovirus strains and to identify common sources.

Noroviruses, formerly referred to as "Norwalk-like viruses," are the most common etiologic agents of acute gastroenteritis in the United States, where they are estimated to cause 23 million cases annually [1]. They belong to the *Norovirus* genus of the *Caliciviridae* family, which also includes the *Sapovirus* genus. Noroviruses can be classified into 5 different genogroups, GI–V; the 3 genogroups containing viruses that have been found in humans—GI, GII, and GIV—can be further divided into >15 genetic clusters [2].

However, the epidemiology of gastroenteritis caused by these viruses remains poorly characterized, because of the long-standing absence of a simple, accessible, and sensitive diagnostic assay—the lack of which has discouraged investigations of outbreaks with suspected viral etiology [3]. In the early 1990s, the norovirus genome was sequenced [4]; this permitted the development of an assay that is based on reverse-transcription polymerase chain-reaction (RT-PCR) techniques for the detection of noroviruses in stool samples [5, 6]. However, relatively few state laboratories routinely use this technology, and, for many outbreaks with suspected viral etiology, no stool samples are obtained. Moreover, in the United States, the Centers for Disease Control and Prevention (CDC) routinely receives reports only on outbreaks of gastroenteritis that are either foodborne or waterborne. The few data that are available on the frequent outbreaks that are spread from person to person or by environmental contamination come from the portion of these outbreaks in which stool samples are sent to the CDC's viral gastroenteritis laboratory, which tests for caliciviruses.

Received 18 October 2003; accepted 5 December 2003; electronically published 8 June 2004.

Reprints or correspondence: Dr. Marc-Alain Widdowson, Viral Gastroenteritis Section, Respiratory and Enteric Viruses Branch, Centers for Disease Control and Prevention, Mailstop G04, 1600 Clifton Rd. NE, Atlanta, GA 30333 (zux5@cdc.gov).

The Journal of Infectious Diseases 2004; 190:27–36

0022-1899/2004/19001-0004$15.00

One of the only sources of surveillance data on outbreaks of gastroenteritis that are routinely reported, regardless of the mode of transmission, is the Vessel Sanitation Program at the CDC, which requires the reporting of all persons with gastroenteritis aboard cruise ships entering US waters from foreign ports. Cruise ships are required by the Vessel Sanitation Program to submit a report when ⩾2% of the passengers and/or crew report gastroenteritis, defined as either ⩾3 episodes of loose stools within 24 h or vomiting with 1 or 2 episodes of loose stools, abdominal cramps, headache, myalgia, or fever. The Vessel Sanitation Program formally defines an outbreak as the reporting of symptoms of gastroenteritis on any 1 cruise by ⩾3% of passengers and/or crew [7]. Investigations of all outbreaks of disease may involve ⩾1 of the following activities: enhanced surveillance; environmental-sanitation inspections; descriptive and analytical epidemiologic studies, to identify a mode of transmission; and the collection of stool samples, to identify the etiologic agent.

In 2002, the surveillance system of the Vessel Sanitation Program detected a sharp increase in the number of outbreaks of gastroenteritis on cruise ships. From 1 January 2002 to 2 December 2002, 21 outbreaks of gastroenteritis were reported to the Vessel Sanitation Program, compared with 7 in 2001 and 4 in 2000 [8]. Rapid identification and investigation of those outbreaks and the accompanying collection of stool samples provided an opportunity to better characterize the transmission, disease burden, and molecular epidemiology of noroviruses, both to assess whether the outbreaks might be linked to a common strain or exposure and to consider ways to improve strategies for intervention and prevention.

Here, we describe the outbreaks of gastroenteritis occurring on cruise ships in 2002 for which specimens were received at the CDC and subsequently tested for norovirus. We also compare the molecular epidemiology of cruise-ship outbreaks with that of land-based outbreaks occurring in the United States during the same period.

MATERIALS AND METHODS

Data sources. We collected epidemiologic and molecular data on all cruise ship–related outbreaks of gastroenteritis with onset in 2002 that had been laboratory confirmed by the Viral Gastroenteritis Section at the CDC to be attributable to either of 2 calicivirus genera: norovirus or sapovirus. For the purposes of the present study, we defined an outbreak of calicivirus as a cluster of cases of gastroenteritis occurring on a cruise ship from which at least 4 stool samples from different persons were sent to the CDC for testing and of which ⩾2 were positive, by RT-PCR, for either norovirus or sapovirus. In general, stool samples are obtained by the Vessel Sanitation Program when the number of cases on a ship is rapidly increasing toward the threshold (⩾3% passengers and/or crew) at which an outbreak is defined. However, it happens on occasion that the Vessel Sanitation Program's definition of an outbreak is not met (because the percentage of ill persons did not reach 3%) but the Viral Gastroenteritis Section's definition is. We reviewed data from 3 sources: the records of the Viral Gastroenteritis Section, the surveillance reports provided by cruise ships, and the outbreak reports of the Vessel Sanitation Program or of other CDC groups involved in relevant investigations. Once an outbreak was confirmed, all subsequent contiguous cruises on the same ship were considered to be part of the outbreak if ⩾1% of the passengers and/or crew reported illness.

All strains of norovirus that were detected in stool samples during an outbreak were sequenced as described below; sequencing permitted the comparison of strains found on successive cruises, strains found on ships from the same cruise line, and strains found in land-based outbreaks. Thus, we also reviewed molecular and epidemiologic data on land-based outbreaks of gastroenteritis occurring in 2002 that the Viral Gastroenteritis Section had confirmed, on the basis of at least 2 positive stool samples from 4 samples tested, to be attributable to either norovirus or sapovirus. Throughout the United States, these outbreaks had been investigated by personnel from local health departments, which did not have facilities with which to test for norovirus. Stool samples were sent to the Viral Gastroenteritis Section with limited epidemiologic information on the outbreak, including mode of transmission, setting, and the number of persons affected.

Laboratory testing. Stool samples submitted to the CDC for analysis were initially tested by RT-PCR, by use of degenerate primers targeted at a 172-bp region of the norovirus polymerase gene (region B), as described elsewhere [5]. To better characterize detected noroviruses, particularly those that had indistinguishable region B sequences, selected stool samples were also tested for norovirus, by use of primers targeted at a 277-bp region of the capsid gene (region C). If the results of the initial stool-sample tests (by use of region B primers) were inconclusive, then, in addition to testing by use of region C primers, stool samples also were tested for sapovirus, by use of primers targeted at a 331-bp region of the sapovirus polymerase gene [9].

The amplified products from ⩾2 positive stool samples from each outbreak were sequenced. All detected sequences were analyzed by use of the GCG suite of programs [10], and a phylogram was created, by which the similarity or divergence of strains from different cruise- and land-based outbreaks could be compared.

RESULTS

In 2002, the CDC received stool samples from 14 outbreaks of gastroenteritis occurring on 12 cruise ships. Of these 14 outbreaks, 12 (86%) were confirmed to be due to human calicivi-

ruses (11 to noroviruses and 1 to sapovirus) (table 1). Of the remaining 2 outbreaks, 1 was attributed to enterotoxigenic *Escherichia coli,* and 1 was of undetermined etiology. Sequence data were available from stools samples from all 12 cruise-ship outbreaks due to noroviruses or sapoviruses. Of the 11 norovirus outbreaks, 8 (73%) were attributed to a GII norovirus, 1 to a GI norovirus, 1 to 2 different GII noroviruses, and 1 to either a GI or GII norovirus. In 5 (45%) of the 11 norovirus outbreaks, multiple strains were detected, which complicated the assigning of an outbreak strain. In 3 (60%) of these 5 outbreaks, an identical sequence type was detected in ⩾2 stools (and that strain was thus designated to be the outbreak strain), and the other sequence types were detected in only 1 stool each. In 1 outbreak (ship outbreak 10), 2 of the 4 detected sequence types were detected in multiple (16 and 5) stool samples, a finding that suggests that there were 2 outbreak strains. Last, in ship outbreak 11, 2 sequence types were detected each in single stool samples.

Of the 12 calicivirus outbreaks, 4 (33%) were not classified as outbreaks by the Vessel Sanitation Program definition, because the percentage of ill persons was not ⩾3% of the total number of passengers and/or crew (table 2). Six (50%) of the 12 calicivirus outbreaks spanned ⩾2 contiguous cruises of the same ship, with entirely new passengers on each cruise, and 1 outbreak (ship outbreak 7) continued for 42 days on 4 contiguous cruises. Four of the investigations included a survey of all passengers and/or crew and a case-control or cohort study of risk factors for illness. The median attack rate (passengers/cruise) in these 4 outbreaks was 18% (range, 13%–30%), compared with 3% (range, <1%–34%) for outbreaks in which only persons reporting to the infirmary were enumerated. In 10 (83%) of the 12 calicivirus outbreaks, substantial direct person-to-person transmission was suspected. In 6 (50%) of the 12 calicivirus outbreaks, environmental contamination was suspected of playing an important role in the transmission of virus to passengers on successive cruises, and, in 2 (17%) and 3 (25%) of these outbreaks, respectively, water- and foodborne transmission were implicated. In 4 (33%) instances, an affected ship was docked immediately after an outbreak-associated cruise for 1 week of thorough cleaning, with no passengers on board.

Among the noroviruses detected in the 10 cruise-ship outbreaks associated with a GII norovirus, 7 shared an identical region B sequence (figure 1). This lineage of noroviruses within GII cluster 4 (GII/4) has been provisionally named "the Farmington Hills strain," after Farmington Hills, Michigan, where, in 2002, the first cases of gastroenteritis caused by this strain of norovirus were identified. Of the 3 remaining GII norovirus–associated outbreaks on cruise ships, 2 had a related but different region B sequence, and 1 (ship outbreak 5) was attributed to a norovirus belonging to a genetic cluster not reported previously.

During 2002, the CDC confirmed that 33 land-based outbreaks were attributable to noroviruses; 10 (30%) were associated with the Farmington Hills strain, and all of them occurred between May 2002 and December 2002, comprising 45% of the 22 norovirus-confirmed outbreaks occurring during that period (figure 2). The 10 Farmington Hills strain–associated outbreaks occurred in a total of 8 states and were associated with restaurants and catered events, retirement and nursing homes, and a day-care center (table 3).

To determine whether strains that had identical region B sequences were, in fact, the same, we further sequenced, in

Table 1. Summary of laboratory testing for norovirus and sapovirus, in 12 outbreaks (OBs) of gastroenteritis on 10 cruise ships—United States, 2002.

OB number	Ship	No. (%) of stool samples tested for calicivirus		Etiology	No. of different nucleotide sequences detected	Genogroup or genetic cluster[a]	Farmington Hills strain
		Total	Positive				
1	A	14	12 (86)	Sapovirus	2	GI and GII	Absent
2	B	17	6 (35)	Norovirus	4	GII/4	Present
3	C	12	11 (92)	Norovirus	1	GII/4	Absent
4	D	9	6 (67)	Norovirus	1	GII/4	Present
5	E	26	6 (23)	Norovirus	2	GII/8 and GII/4	Absent
6	A	5	4 (80)	Norovirus	1	GI/3	Absent
7	F	13	13 (100)	Norovirus	1	GII/4	Present
8	G	26	15 (58)	Norovirus	1	GII/4	Present
9	G	15	8 (53)	Norovirus	3	GII/4	Present
10	H	29	25 (86)	Norovirus	4	GII/4	Present
11	I	10	3 (30)	Norovirus	2	GII/4 and GI/3	Present
12	J	14	5 (36)	Norovirus	1	GII[b]	Absent

[a] Norovirus classification according to the system of Ando et al. [2]; sapovirus classification according to the system of Schuffenecker et al. [11].

[b] Strain not well resolved into cluster by a 172-bp region of the norovirus polymerase gene (region B) sequence alone.

Table 2. Summary of epidemiologic features of 12 outbreaks (OBs) of acute gastroenteritis attributable to calicivirus (norovirus or sapovirus), on 10 cruise ships—United States, 2002.

OB number	Ship	Month	Itinerary	Duration of cruise, days	Passengers		Crew		Case-control or cohort study?	Mode of transmission[b]	Ship removed from service?[c]	Farmington Hills strain
					Total no.	No. (%) ill[a]	Total no.	No. (%) ill[a]				
1	A	Mar	US Atlantic coast	7	93	32 (34)	36	6 (17)	No	**WB**	Yes	Absent
2[d]	B	May	Alaska	7	1895	11 (<1)	874	3 (<1)	No	PP	No	Present
3[d]	C	Jun	Alaska	7	2103	48 (2)	850	7 (<1)	No	PP	No	Absent
4	D	Jul	Alaska	7	1318	**167 (13)**	564	**9 (2)**	No	PP, Env	Yes	Present
			Alaska	7	1336	**189 (14)**	571	**30 (5)**				
5	E	Oct	Caribbean	7	1984	**356 (18)**	941	**13 (1)**	Yes	**FB, PP**	No	Absent
6	A	Oct	Caribbean	10	77	4 (5)	35	7 (2)	No	**WB**	No	Absent
7	F	Oct	Pacific-Caribbean	21	1336	**399 (30)**	600	23 (4)	Yes	**PP, FB,** Env	Yes	Present
			Caribbean	7	1269	33 (3)	596	7 (1)				
			Caribbean	7	1273	155 (12)	586	18 (3)				
			Caribbean	7	1232	53 (4)	562	16 (3)				
8	G	Nov	Caribbean	7	2318	**416 (18)**	988	17 (2)	Yes	**FB,** PP, Env	Yes	Present
			Caribbean	7	2456	195 (8)	999	23 (2)				
9[d]	G	Nov	Caribbean	7	2153	55 (3)	1026	6 (<1)	No	PP, Env	No	Present
			Caribbean	7	2474	30 (1)	1020	6 (<1)				
			Caribbean	7	2579	32 (1)	1024	2 (<1)				
10	H	Dec	Caribbean	14	1861	288 (15)	868	28 (3)	Yes	**PP**	No	Present
11	I	Dec	Caribbean	7	3154	224 (7)	1184	39 (3)	Yes	PP, Env	No	Present
			Caribbean	7	3072	63 (2)	1181	31 (3)				
12[d]	J	Dec	Caribbean	3	2604	41 (2)	821	2 (<1)	No	PP, Env	No	Absent
			Caribbean	4	2605	64 (2)	821	10 (1)				

NOTE. Env, environmental; FB, foodborne; PP, person to person; WB, waterborne.

[a] Bold indicates that a survey of all passengers or crew was conducted; otherwise, data refer to persons reporting illness at the ship infirmary.

[b] Bold indicates statistical or microbiologic evidence.

[c] Ships were removed from service for 1 week of extensive cleaning.

[d] Fits the definition of an outbreak as defined by the Viral Gastroenteritis Section, Centers for Disease Control and Prevention (CDC), but not that as defined by the Vessel Sanitation Program, CDC (≥3% of passengers/crew ill).

region C, the Farmington Hills strains and also examined outbreak reports to investigate possible connections between these outbreaks (figure 3). Seven outbreaks (4 on cruise ships and 3 on land) shared a Farmington Hills strain with identical region C sequences; 2 cruise-ship outbreaks (ship outbreaks 4 and 7) occurred on ships (D and F) that belonged to the same company and that had launched at the same location (on the west coast of Canada) 3 months apart. Ship D bunkered municipal tap water, whereas ship F produced drinking water from seawater, by evaporation; crew transfer also could not explain transmission of the strain from one ship to the other. The third outbreak (ship outbreak 8) that was associated with an identical region C sequence occurred on ship G, which belonged to a different company and which sailed from Florida to the Caribbean. No common food suppliers for the provisioning of ships D, F, and G were identified.

In a fourth outbreak (ship outbreak 9), the Farmington Hills strain from 1 stool sample had a region C sequence that was identical to those of strains from stool samples from ship outbreaks 4, 7, and 8. This outbreak occurred on the same ship (ship G) as did ship outbreak 8 but on a cruise immediately following 1 week of intensive cleaning undertaken in an attempt to control the outbreak. However, sequences of strains from 3 other stool samples from ship outbreak 9 were different from the sequence of the strain responsible for ship outbreak 8, a result that suggests that, although 1 strain persisted from ship outbreak 8 (despite cleaning), the predominant strain in ship outbreak 9 was, likely, newly introduced.

Three land-based outbreaks were caused by the Farmington Hills strain with a region C sequence that was identical to the Farmington Hills strain that was detected in the 4 cruise-ship outbreaks occurring during the same period. In 1 of these land-based outbreaks (land outbreak 8), the index case was a resident of a nursing home who returned ill from ship outbreak 8 on ship G. No common epidemiologic link was found between the 2 other land-based outbreaks and the 4 cruise-ship outbreaks.

Finally, in the outbreak on ship H (ship outbreak 10), Farmington Hills strains with identical region B sequences were detected in all 25 stools; however, analysis of region C found that the outbreak was associated with 4 sequence types that were different from each other by 1 or 2 nucleotides. The 2 predominant sequences types (the first found in 16 stool samples and the second in 5 stool samples) were from persons who had become ill early in the outbreak and who had boarded the ship after taking a common airline flight, which suggests that 2 separate viruses had been introduced onto the ship.

To determine whether infectiousness and environmental persistence differed among norovirus strains, we compared both the attack rates and the number of affected cruises in outbreaks of gastroenteritis caused by the Farmington Hill strain with those in outbreaks of gastroenteritis caused by other norovirus strains. For cruise ships, we included data only on outbreaks in which no survey of passengers or crew had been conducted. The median attack rate for the 10 land-based outbreaks caused by the Farmington Hills strain was 35%, compared with 50% for the 19 outbreaks caused by other norovirus strains for which data had been collected ($P > .05$, by Wilcoxon rank sum test). Among cruise-ship outbreaks, those caused by the Farmington Hills strain infected more people (median attack rate, 3%) than did those caused by non–Farmington Hills strains (median attack rate, 2%). In addition, cruise-ship outbreaks caused by the Farmington Hills strain were more likely to persist (5 [63%] of 8 outbreaks) than were cruise-ship outbreaks caused by a non–Farmington Hills strain (1 [33%] of 3 outbreaks). Neither the difference in attack rates nor the difference in persistence was statistically significant.

DISCUSSION

In 2002, 12 (86%) of the 14 outbreaks of gastroenteritis on cruise ships for which stool samples were submitted to the CDC were due to caliciviruses, a finding consistent with the role of these viruses as the most common cause of outbreaks of gastroenteritis. Overall, these outbreaks shared 3 distinct characteristics: they affected a large number of persons, they were spread by multiple routes, and they continued on contiguous cruises despite intensive efforts at control. The continuation of outbreaks associated with the same virus sequence on consecutive cruises with new passengers and the detection of the identical outbreak sequence even after 1 week of cleaning (ship G, outbreaks 8 and 9) suggest that viral environmental contamination and/or prolonged and significant shedding by crew play a major role in outbreaks. However, we cannot exclude the possibility that a virus that was identical in regions B and C was reintroduced onto ship G immediately after the cleaning. These 12 outbreaks represented a sharp increase versus the 4 and 2 confirmed calicivirus outbreaks in 2001 and 2000, respectively [8]. Of the 11 norovirus outbreaks, 7 (64%) were associated with the newly identified Farmington Hills strain, which is a member of GII/4. From May 2002 to December 2002, this strain also was detected in 45% of all land-based outbreaks of gastroenteritis, which occurred in diverse settings and were confirmed by us to be attributable to noroviruses.

Although an increase in norovirus outbreaks during the fall and winter seasons has been well documented [12, 13], local health authorities throughout the United States noted a particularly large increase in the number of confirmed and suspected norovirus outbreaks (especially in nursing homes) during the second half of 2002 [8]. Our data on land-based norovirus outbreaks suggest that the emergence of the Farmington Hills strain contributed substantially to the burden of norovirus-associated gastroenteritis among persons in nursing homes, a group

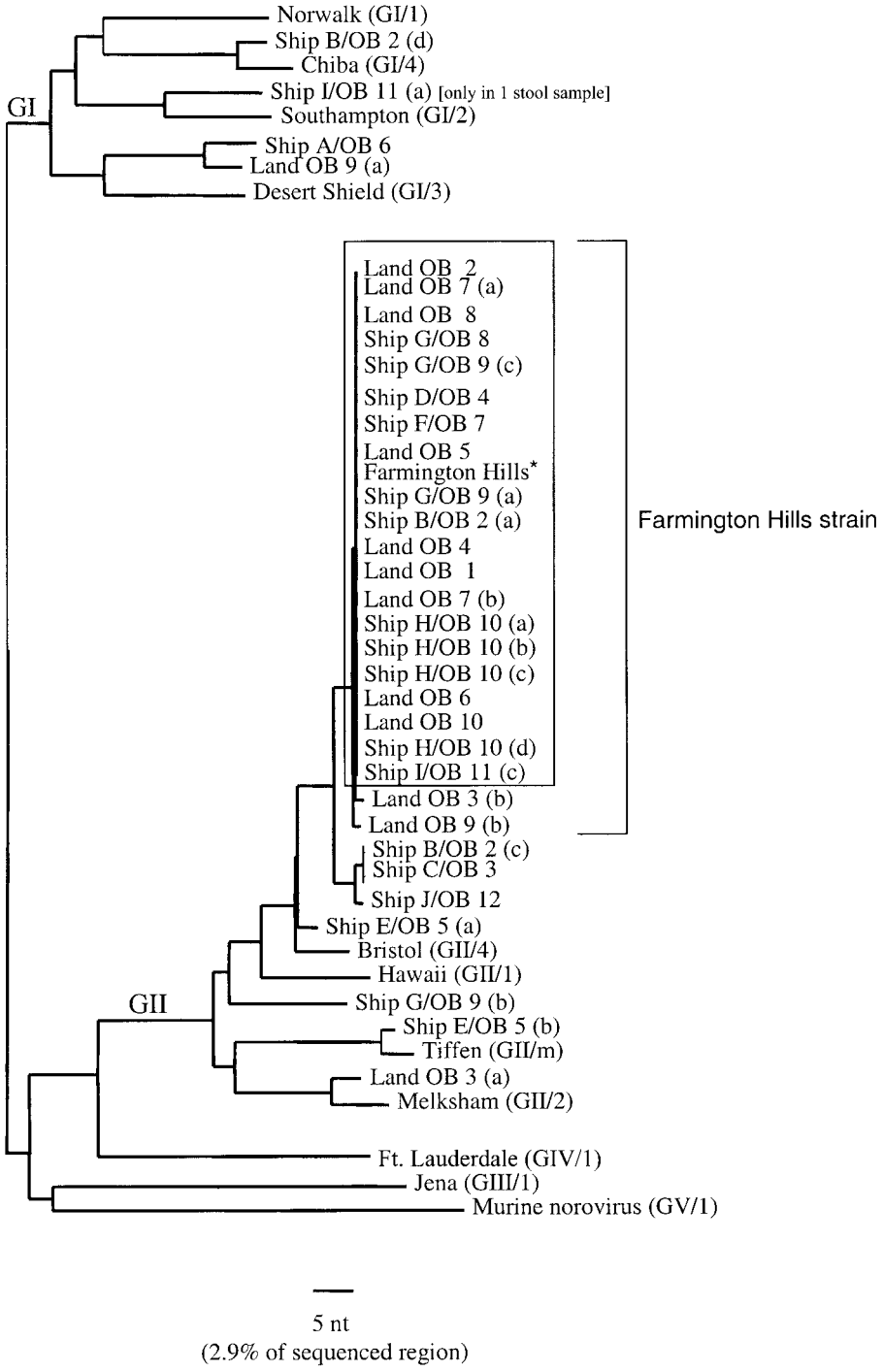

Figure 1. Genetic relationship of sequences of a 172-bp region of the norovirus polymerase gene (region B) associated with cruise-ship and land-based outbreaks of gastroenteritis—United States, 2002. Phylogram consists of 20 norovirus sequence types associated with 11 cruise-ship outbreaks, 13 sequence types associated with 10 land-based outbreaks, 11 reference sequences from GenBank, and the Farmington Hills strain sequence type (*). The tree was created by use of the DISTANCES program (with uncorrected distances) followed by analysis by use of the GROWTREE program (version 10.3). Suffixes (a), (b), (c), and (d) refer to the relative predominance of sequences, distinguishable in either region B or in a 277-bp region of the norovirus capsid gene (region C), in stool samples from the same outbreak. Unless marked otherwise, sequences suffixed (a) were found in $\geq$2 stool samples and sequences marked (b), (c), or (d) in only 1 stool sample. The lack of a suffix indicates that only 1 sequence type was associated with an outbreak. The box highlights sequences types that belong to the Farmington Hills strain and that are identical in region B. GenBank accession nos. for reference strains in this figure are as follows: Norwalk virus, M87661; Chiba virus, AB022679; Southampton virus, L07418; Desert Shield virus, U04469; Bristol virus, X76716, Hawaii virus, U07611; Tiffen virus, AF493209; Melksham virus, X818879; Ft. Lauderdale virus, AF414426; Jena virus, AJ011099; murine norovirus, AY228235. OB, outbreak.

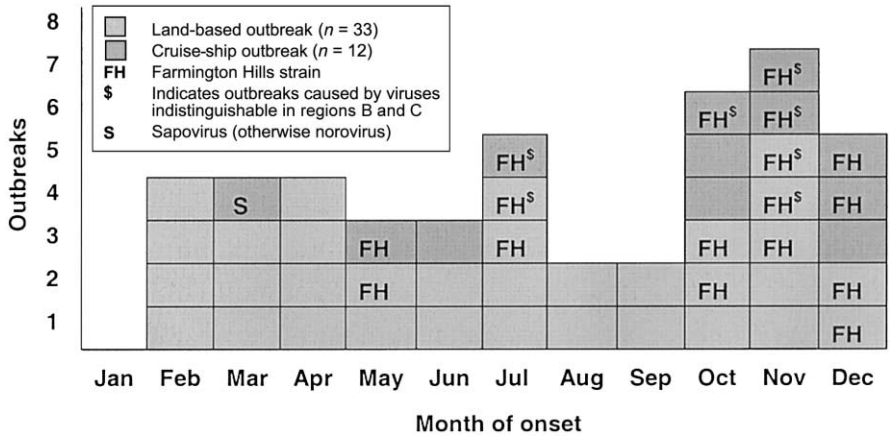

Figure 2. Outbreaks of gastroenteritis on land and on cruise ships attributed to either norovirus or sapovirus—United States, 2002. Region B, a 172-bp region of the norovirus polymerase gene; region C, a 277-bp region of the norovirus capsid gene.

at greater risk for severe morbidity and mortality from gastroenteritis [14, 15].

Nucleotide-sequence analysis provided insights into the transmission and epidemiology of noroviruses and permitted the documentation of the seeding of 1 outbreak in a nursing home by a patient from a cruise ship, the introduction of 2 outbreak strains from passengers who had taken a common flight, and the persistence of a strain on a ship despite 1 week of cleaning and disinfecting. However, the emergence of a common strain of norovirus did complicate the interpretation of the molecular data. Outbreaks that occurred at different times of the year, in different settings, and in geographically different locations (on land and at sea) were found to be caused by noroviruses that have both identical region B sequences and identical region C sequences, but no obvious common source of infection was found. It remains possible that a common source of infection (e.g., food) linked the outbreaks with indistinguishable sequence types. It is more likely, however, that these outbreaks occurred because of the introduction, by different sources, of a common strain circulating on land. Conversely, when the epidemiology and region B sequence analysis suggested either a common source of infection or the continuation of an outbreak after extensive ship cleaning, analysis of the more variable capsid region (region C) demonstrated the presence of distinct sequence types that differed by only a few base pairs. The natural rate of the mutation of noroviruses is not known, but the discovery of indistinguishable or closely related sequences associated with outbreaks at different times and places suggests that the rate is relatively low, compared with that for other RNA enteric viruses, such as poliovirus [16].

Table 3. Summary of 10 land-based outbreaks (OBs) of gastroenteritis associated with the Farmington Hill strain of norovirus—United States, 2002.

OB number	Month	State	No. (%) of persons exposed and ill		Mode of transmission	Setting	No. (%) of stools tested for norovirus		No. of different nucleotide sequences detected
			Total exposed	Total ill			Total	Positive	
1	May	Kentucky	136	42 (31)	PP	Retirement home	4	3 (75)	1
2	Jul	North Carolina	23	11 (48)	FB	Catered birthday	5	3 (60)	1[a]
3	Jul	Wyoming	56	17 (30)	FB	Catered meal	4	3 (75)	2
4	Oct	Alaska	171	22 (13)	PP	Day-care center	4	2 (50)	1
5	Oct	Georgia	170	85 (50)	FB	Wedding	8	5 63)	1
6	Nov	Utah	300	50 (17)	FB	Restaurant	13	7 (54)	1
7	Nov	Alaska	10,000	7150 (72)	WB	Community	8	6 (75)	1[a]
8	Nov	Pennsylvania	673	126 (19)	PP	Nursing home	5	2 (40)	1[a]
9	Dec	Georgia	57	25 (44)	PP	Nursing home	8	4 (50)	2
10	Dec	West Virginia	65	26 (40)	PP	Nursing home	6	5 (83)	1

NOTE. FB, foodborne; PP, person to person; WB, waterborne.

[a] Sequences in a 277-bp region of the norovirus capsid gene (region C) identical both to each other and to sequences associated with 3 cruise-ship outbreaks (figure 1).

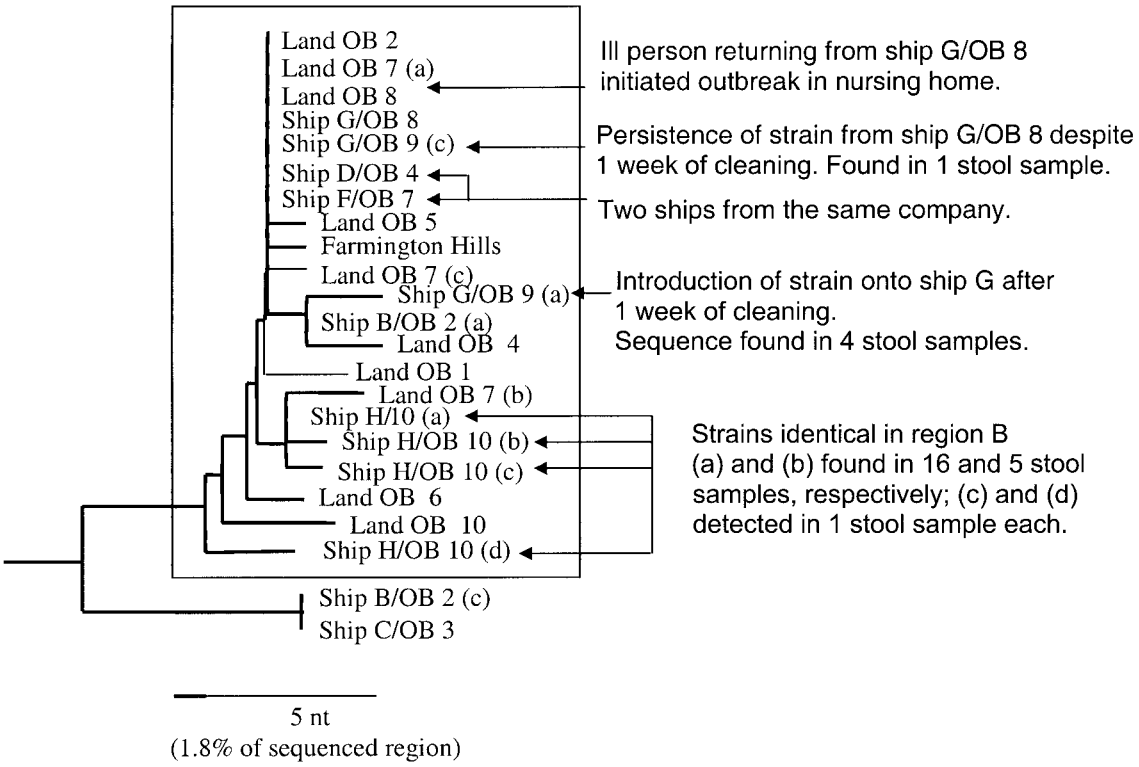

Figure 3. Genetic relationship of sequences of a 277-bp region of the norovirus capsid gene (region C) associated with cruise-ship and land-based outbreaks of gastroenteritis—United States, 2002. Phylogram consists of 12 norovirus sequence types associated with 7 cruise-ship outbreaks, 10 sequence types associated with 8 land-based outbreaks, and the Farmington Hills strain sequence type. The tree was created by use of the DISTANCES program (with uncorrected distances) followed by analysis by use of the GROWTREE program. Suffixes (a), (b), (c), and (d) refer to different sequences, distinguishable in either a 172-bp region of the norovirus polymerase gene (region B) or region C, detected in stool samples from the same outbreak. Unless marked otherwise, sequences suffixed (a) were found in >2 stool samples, and sequences suffixed (b), (c), and (d) were found in only 1 stool sample. The lack of a suffix indicates that only 1 sequence type was associated with an outbreak. The box highlights sequence types that belong to the Farmington Hills strain and that are indistinguishable in region B. No region C sequence was available for strain ship I/OB 11 (b), and no region B sequence was available for strain land OB 7 (c). Region C sequence was available for strain ship B/OB 2 (b), but is not shown here. OB, outbreak.

The detection of several different sequences in cases from 6 cruise-ship outbreaks suggests that there was multiple introductions of noroviruses by crew and/or by each new cohort of passengers and is consistent with evidence suggesting that noroviruses are the most common etiologic agent of sporadic gastroenteritis [17, 18].

Noroviruses of GII/4, to which the Farmington Hills strain belongs, are some of the most common strains worldwide and have been associated with outbreaks in closed settings in the United States, Europe, and Australia [15, 19–21]. During 1995–1996, a different GII/4 strain (95/96-US) was reported to be globally predominant and to be responsible for >50% of the outbreaks (most in health-care settings) in the United States during the 1995–1996 winter season [22]. It is possible that the clinical manifestations (e.g., increased vomiting) of GII/4 strain infections or the physical characteristics (e.g., environmental persistence) of this strain facilitate spread in closed settings. Though not of statistical significance, cruise-ship outbreaks of gastroenteritis caused by the Farmington Hills strain featured higher attack rates and persisted longer on successive cruises than did outbreaks caused by other norovirus strains. Of interest, the herd immunity that would be expected to develop to some degree after the exposure of a population to an emergent GII/4 strain (i.e., 95/96-US) does not seem to have prevented the emergence of a related GII/4 strain (i.e., Farmington Hills); this finding is consistent with reports that strain-specific protective immunity may last only 6 months [23].

Large outbreaks of viral gastroenteritis have been reported for successive sailings on both cruise and military ships, aboard which bunkered drinking water, close living quarters, common food galleys, and the mixing of people for several days facilitate norovirus transmission via multiple routes [24–30]. The successful detection of noroviruses on environmental swab samples during recently reported outbreaks has confirmed that environmental contamination is a major source of norovirus infection [31, 32]. However, decontamination is complicated by the probable resistance of noroviruses to common disinfectants [33–35] and by a lack of a norovirus cell-culture system by

which to assess the efficacy of alternative disinfection compounds. Also, crew members may unwittingly act as a reservoir of infection between cruises—studies using volunteers have suggested that >30% of norovirus infections may be asymptomatic [36] and that shedding can occur for ⩾14 days after onset of disease [37]. Consistent with our results, other investigations have found that, during outbreaks, crew are often less affected than passengers [24–27]. This finding is partly caused by underreporting (due to issues of job security), but perhaps it is also due to the crew being younger than the passengers, having different exposures (e.g., different foods from different galleys), and, in many cases, being from varied countries and so possibly having different genetic factors that lead either to resistance to infection or to disease, as described recently [38].

Our experience suggests that all possible routes of norovirus transmission should be addressed immediately when infection-control measures are implemented on ships. Aggressive disinfection should be complemented by the implementation of basic measures, including washing of hands, paid sick leave for ill workers, ongoing training of foodhandlers, and isolation of sick persons. Effective measures to prevent seeding of outbreaks can be implemented rapidly when a common source is identified [39], but, to better uncover links between outbreaks, both the further development of existing national and global surveillance systems for norovirus outbreaks [13, 19] and the collection of sequence data are required. Efforts are now focused on ensuring that differences between primers and between computer software do not complicate the interpretation of information or the smooth transfer of information between systems [40]. Full genetic characterization of emergent strains (such as the Farmington Hills strain) may identify consistent nucleotide differences that translate to particular antigens associated with pathogenicity and immunity. This would help to pave the way for future vaccine-development efforts.

Acknowledgments

We thank Andy Mullins, Jay Varma, Vincent Hsu, Cynthia Stover, Jaret Ames, Jon Schnoor, Julia Chervoni, John Sarisky, Charles Otto, Daniel Harper, and Don Ackerman, for assistance in the investigation of outbreaks; Laura Mosher and Virginia Leykam, for reverse-transcription polymerase chain reaction (RT-PCR) and sequence analysis performed at the Michigan Department of Community Health (Lansing); Howard White, for RT-PCR performed at the Centers for Disease Control and Prevention (Atlanta, Georgia); Claudia Chesley, for editorial comments; and anonymous reviewers, for constructive comments.

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