Molecular Epidemiology of Caliciviruses Detected in Sporadic and Outbreak Cases of Gastroenteritis in France from December 1998 to February 2004

F. Bon,^{1*} K. Ambert-Balay,¹ H. Giraudon,¹ J. Kaplon,¹ S. Le Guyader,² M. Pommepuy,²
A. Gally,³ V. Vaillant,³ H. de Valk,³ R. Chikhi-Brachet,⁴ A. Flahaut,⁴
P. Pothier,¹ and E. Kohli¹

*Laboratoire de Virologie and CNR des Virus Entériques, EA562, CHU de Dijon and UFRs Médecine-Pharmacie, Dijon,¹
Laboratoire de Microbiologie, IFREMER, Nantes,² Institut National de Veille Sanitaire, Paris,³ and Institut National
de la Santé et de la Recherche Médicale Unité U 444, Paris,⁴ France*

Received 23 February 2005/Returned for modification 13 April 2005/Accepted 11 June 2005

We compiled sequence and epidemiological data from 172 caliciviruses detected in France from December 1998 to February 2004 in sporadic and outbreak cases. The results showed a cocirculation of strains with a majority of genogroup II (GII) noroviruses. Three groups of noroviruses, not detected before in our laboratory, emerged and spread during the period: the recombinant GGIIb and Norwalk-related strains not amplified in the polymerase gene in 2000 and a new Lordsdale variant in 2002. We observed that (i) GII-4 noroviruses were predominant in nursing home and hospital outbreaks but rare in oyster- and water-related outbreaks despite continuous circulation in the population; (ii) at the opposite, genogroup I strains were detected in the majority of environmental outbreaks; (iii) several strains were frequently found in oyster- and water-linked outbreaks (up to seven), whereas one single strain was detected when transmission was from person to person; and (iv) whereas GII noroviruses were predominant in sporadic cases where patients were under 15 years of age, GI strains were more frequent in outbreaks occurring in this age group. Finally, from a methodology point of view, this compilation shows that detection and characterization in the polymerase gene are not adequate in a significant number of cases and should be completed by amplification and sequencing in the capsid gene.

Human caliciviruses (*Caliciviridae* family) are a major cause of acute gastroenteritis outbreaks in industrialized countries (9, 24) and are also now recognized as a frequent agent of sporadic gastroenteritis in all age groups (6–8, 29). The development of molecular methods for their detection allowed description of a great genetic diversity among the human caliciviruses, which belong to two genera, *Sapovirus* and *Norovirus*, and to eight different genogroups within these two genera. Each genogroup is further divided into genotypes (2, 11, 12, 35). The follow-up of molecular epidemiology of caliciviruses during a given period has shown the prominence of one type during a given period in small or wide geographic areas and a cocirculation of different strains during other periods (20, 22, 27). The factors governing these epidemiological patterns are probably related to both the host and the viral strain, but until now, they have not been elucidated. Surveillance of calicivirus molecular epidemiology in sporadic cases and outbreaks during a given period and comparison between different countries, in addition to detection of common sources of infection, are important to better understand these patterns.

In France, except for food-borne outbreaks, there is no systematic virological surveillance of acute gastroenteritis. However, we have recently conducted epidemiological studies including sporadic cases (6; unpublished data), and we have been investigating an increasing number of outbreaks as a

national reference center. Caliciviruses, especially noroviruses, were detected as a major cause of acute gastroenteritis in all these situations. With the aim to have a global view of molecular epidemiology of caliciviruses detected in France between December 1998 and February 2004 and to underline possible characteristics in terms of period, age of the patients, or settings, we have compiled sequences and epidemiological data for all the caliciviruses characterized in our laboratory during this period.

MATERIALS AND METHODS

Strains. (i) Sporadic cases. A total of 100 calicivirus strains detected in symptomatic sporadic cases during the period of the study were included. Among them, 30 had been detected during a physician-based study of acute diarrhea nested in the Sentinelles French network during the winter of 1998 to 1999 (6), and 54 were detected in a regional study in the western region of France (Golfe du Morbihan, Bretagne, October 1999 to May 2001 [unpublished data]). This study included cases reported by general practitioners as well as those of children consulting a hospital pediatric department in Vannes. Both studies included patients of all age groups. The 16 remaining strains had been detected in stool samples which had been sent to our laboratory by different hospital-based laboratories.

(ii) Outbreak strains. A total of 72 calicivirus strains detected in 45 outbreaks investigated during the period of December 1998 to February 2004 all over France, in the context of our national reference center activity, were included.

Sequencing. All the strains were amplified and sequenced in the polymerase gene using one or more of the primers described previously by Chikhi-Brachet et al. (6). However, some norovirus strains which could not be firmly classified into one genetic type were further characterized by sequencing in the capsid gene using primers SRI-1/SRI-2 (13) or GISKF/GISKR (19) for genogroup I (GI) and Mon381/Mon383 (26) or G2SKF/G2SKR (19) for genogroup II. In addition, some genogroup I strains which were weakly amplified or not amplified in the polymerase gene were detected and were thus also characterized by amplification

* Corresponding author. Mailing address: Laboratoire de Virologie, Hôpital du bocage, 2 Bd Maréchal de Lattre de Tassigny 21079 Dijon, France. Phone: 33 3 80 29 34 37. Fax: 33 3 80 29 36 04. E-mail: fabienne.bon@chu-dijon.fr.

TABLE 1. Distribution of human calicivirus strains detected in France in sporadic and outbreak cases from December 1998 to February 2004^a

Genogroup	Genotype	No. of strains detected in sporadic cases (%)	No. of strains detected during outbreaks (%)
Norovirus genogroup I	GI-1 Norwalk	17 (17)	5 (7)
	GI-2 Southampton	6 (6)	4 (6)
	GI-3 Desert Shield	2 (2)	12 (17)
	GI-4 Chiba	1 (1)	4 (6)
	GI-6 Hesse	0 (0)	2 (3)
Norovirus genogroup II	GII-1 Hawaii	2 (2)	2 (3)
	GII-2 Melksham	5 (5)	1 (1)
	GII-3 Mexico	4 (4)	1 (1)
	GII-4 Lordsdale	22 (22)	21 (29)
	GII-6 Seacroft	0 (0)	1 (1)
	GII-7 Utrecht/Leeds	2 (2)	1 (1)
	Amsterdam	1 (1)	1 (1)
	GII non assigned	1 (1)	0 (0)
	Arg320	3 (3)	0 (0)
	GGIIB*	5 (5)	4 (6)
	GGIIB/Hawai	1 (1)	4 (6)
	GGIIB/Lordsdale	1 (1)	1 (1)
	GGIIB/Mexico	19 (19)	6 (8)
	GGIIB/Snow Mountain	0 (0)	1 (1)
Norovirus genogroup IV	Alphatron	1 (1)	0 (0)
Sapovirus genogroup I	Sapporo	4 (4)	1 (1)
Sapovirus genogroup II	London/92	3 (3)	0 (0)
Total		100	72

^a The GGIIB recombinant strains showed four different capsids close to Mexico (GII-3), Hawaii (GII-1), Lordsdale (GII-4), and Snow Mountain (GII-2) viruses. For some strains (GGIIB*), the capsid was not characterized.

and sequencing in the capsid gene using SRI-1/SRI-2. For these strains, a region of the helicase gene was also sequenced using primers described previously by Wang et al. (33).

On the whole, the polymerase region was analyzed for most of the strains (86%), and a capsid region was analyzed for 48% of the strains.

Sequence analysis. Sequence alignments with the GenBank library were carried out by using Fasta software, version 3.3t06, and GCG software, available on the national service Infobiogen (<http://www.infobiogen.fr>).

Nucleotide sequence accession numbers. The nucleotide sequence data of three different recombinant GGIIB strains and of the helicase region of a Norwalk-related strain have been deposited in GenBank nucleotide sequence databases under the following accession numbers: AY580335 (Hu/NLV/Gourdon78/2000/France), AY773210 (Hu/NLV/VannesL169/2000/France), AY682549 (Hu/NLV/Pont-de-Roide673/2004/France), and AY921623 (helicase).

RESULTS

Sporadic case strains. One hundred sequence data were analyzed during the period December 1998 to March 2002. Most of the strains (91%) had been detected during the winter seasons of 1998 to 1999 and 2000 to 2001. The number of norovirus-positive cases detected during winter 2000 to 2001 may be explained by (i) an important winter epidemic peak during this season and (ii) a better participation of the general practitioners.

The results showed a cocirculation of various caliciviruses with 18 different groups observed (Table 1 and Fig. 1A). Noroviruses were predominant (93% versus 7% for sapoviruses), and among them, GII strains were most frequently detected (66% versus 26% and 1% for genogroups I and IV, respec-

tively). Whereas coinfections including other enteric viruses were observed for some samples (6), we did not observe coinfections including more than one calicivirus strain.

Considering the different genotypes, two remarkable features could be observed (Fig. 1A). One feature was the appearance in September 2000 of the new variant GGIIB strains in the western region of France. These strains, which have been described elsewhere (3, 5, 28, 30), are recombinant viruses that show a unique polymerase gene associated to different capsids. They represented about one-third of all the strains characterized from November 2000 to April 2001. Among them, 15 capsids were characterized: 13 could be assigned to Mexico (GII-3), 1 could be assigned to Hawaii (GII-1), and 1 was assigned to Lordsdale (GII-4) genotypes. The other feature was the appearance of Norwalk-related strains (GI-1) that had the characteristic of being weakly detected or not detected in the polymerase gene despite many attempts. These strains were easily amplified in the capsid region, where they showed 90% nucleotide identity and 96.7% amino acid identity with Norwalk virus (GenBank accession no. M87661). The different attempts to amplify a region in the polymerase gene were carried out by using multiple primers in different combinations (1, 17, 21, 32–34) as well as by decreasing annealing temperatures. Finally, the helicase region was also sequenced for three of these strains, and the results confirmed that they were Norwalk related (GenBank accession no. AY921623; 90% nucleotide identity with Norwalk virus, [accession no. M87661]).

FIG. 1. Temporal distribution of human calicivirus strains circulating in France in sporadic cases (A) and in outbreaks (B) during a 5-year period from December 1998 (98) to February 2004 (04).

Both GGIIb and Norwalk-related strains that had not been detected during the first study throughout France (6) accounted for 56% of all the strains detected in sporadic cases from November 2000 to April 2001.

The patient age was available for 76 of the 100 strains compiled here. Twenty-five strains were found in the 0- to 2-year age group, 8 were found in the 2- to 5-year age group, 10 were found in the 5- to 15-year age group, 29 were found in the 15- to 65-year age group, and 4 were found in the >65-year age group. All the sapovirus strains (7) were detected in the 2- to 5-year age group. Of interest, GII noroviruses were the most frequent in all age groups except in the 15- to 65-year group, for which GI noroviruses were most frequent (52 and 46% for GI and GII, respectively). Moreover, 76% of the GI strains were found in this age group. Finally, the majority of GGIIb strains (58%) were detected in the 0- to 2-year age group, whereas this group represented 33% of the strains analyzed.

Outbreak characteristics. The settings and transmission for the 45 outbreaks investigated are shown in Fig. 2.

Outbreak strains. As for sporadic cases, analysis of 72 sequence data generated during the investigation of 45 calicivirus outbreaks from December 1999 to March 2004 showed a co-circulation of a great diversity of caliciviruses, with 17 different groups being detected (Table 1 and Fig. 1B). Again, we observed a predominance of GII noroviruses (62% for the whole period); however, GI strains became the most frequent be-

tween April 2003 and February 2004, representing 54% of the strains.

Concerning the different genotypes, it is interesting to note the following. (i) There was an appearance of the recombinant GGIIb/Hawaii (GII-1) strains during summer 2000 during a water-borne outbreak in a town (Gourdon, Lot) (3), and these strains were detected in outbreaks until March 2002; GGIIb/Mexico (GII-3) strains were found from January 2001 to February 2004, whereas GGIIb/Lordsdale (GII-4) and GGIIb/Snow Mountain (GII-2) strains were also detected in outbreaks in March 2002 and February 2004, respectively. (ii) There was an appearance of Norwalk-related strains similar to those described in sporadic strains in March 2000 (these strains were not detected in outbreaks after April 2001, although capsid primers for GI noroviruses were systematically used for detection). (iii) There was an emergence of a new Lordsdale variant (GII-4) in April 2002, whereas the previous Lordsdale strains were no longer detected after December 2002 (this new strain was recently reported by Lopman et al. [22] to have diffused at the European level and may have caused an atypical spring and summer peak of gastroenteritis outbreaks in 2002). (iv) There was a recent increase of Desert Shield strains (GI-3).

Strains detected according to the setting and transmission mode are shown in Fig. 2. Whereas a single strain was detected in the majority of the cases in elderly nursing home and hospital outbreaks, where the transmission mode was mainly per-

FIG. 2. Setting and mode of transmission of 45 calicivirus outbreaks investigated during the period of December 1998 to February 2004 and distribution of human calicivirus strains according to setting (A) and transmission (B). SV, sapovirus; GI, genogroup I norovirus; GII, genogroup II norovirus; GI+, GII+, and GI+GII, >1 strain belonging to genogroup I and/or genogroup II.

son to person, as well as in school outbreaks, where the transmission was either person to person, food borne, or unknown, two or more strains were commonly detected in private homes and districts where the transmission was mainly either oyster or water related. The oyster- and water-related outbreaks with several strains involved two and three genogroup I strains (two outbreaks), one or more GGI strains associated with one or more GGII strains (seven outbreaks), and one sapovirus associated with two GGI strains and four GGII strains (one outbreak). Thus, 20 different strains were detected in 9 oyster-related outbreaks and 18 strains were detected in 5 waterborne outbreaks, compared to 17 in 17 outbreaks where the transmission mode was person to person. In addition, the only outbreaks in the elderly where more than one strain was detected had been oyster related. Finally, a clear predominance of GII noroviruses (94%) where most of the strains belonged to the Bristol-Lordsdale genotype (GII-4) (79%) and the GGIIb new variant (11.8%) was observed in nursing home and hospital outbreaks but rarely in schools. In fact, during the same period, the outbreaks in schools involved mainly GI strains (6/9), and among them, four were Desert Shield-related strains (GI-3). The nursing home and hospital outbreaks involved people more than 65 year old, whereas the outbreaks in schools involved children less than 15 years old.

DISCUSSION

In this work, we have compiled sequences and epidemiological data from 172 caliciviruses detected in France from De-

cember 1998 to February 2004, among which 100 were from sporadic cases and 72 were from outbreaks. A great diversity of strains was observed during the whole period, with a predominance of GII noroviruses as previously reported in other countries (12, 24).

The temporal distribution showed the appearance of three groups of strains which had not been detected before in our laboratory: the new variant, GGIIb, and Norwalk-like strains, which were difficult to detect, in 2000 as well as a new variant Lordsdale strain in 2002. Although these strains emerged during a period, other genotypes still cocirculated. Among these three groups of strains, the new variant, GGIIb, and the new Lordsdale strain but not the Norwalk-like strains had a large distribution in Europe (5, 22, 28, 30) and were still regularly detected until recently. However, considering the difficulty to detect the Norwalk-related strains described here with conventional methods, it is probably difficult to speculate on their actual distribution. The recombinant GGIIb and Norwalk-related strains led us to consider the capsid gene both for detection, when the results were negative in the polymerase gene, and for further characterization, when the polymerase region presented less than 90 and 85% identity with reference strains for GII and GI strains, respectively.

Although strains from sporadic cases and from outbreaks were included here, a comparison could be done only during the winter season of 2000 to 2001 (overlapped period). During this period, GGIIb and Norwalk-related viruses were predominant in both outbreaks and sporadic cases. In contrast, no Lordsdale strain was detected in outbreaks, whereas they ac-

counted for 20% of sporadic cases. This could be explained by the fact that all the outbreaks reported during this period were oyster related. Indeed, considering all the outbreaks reported here, we observed that whereas Lordsdale strains were the most frequent strains detected in nursing home and hospital outbreaks (76%) as previously reported (23), they were rarely detected in oyster outbreaks (11%) or water-borne outbreaks (environmental outbreaks). This observation contrasts with the continuous and high circulation of these strains in the population and may be explained by a particular behavior in the environment (decreased resistance or adsorption on inorganic or organic particulate matter), their transmission being mainly from person to person. In contrast, GI strains may be more resistant in the environment; indeed, they were detected in 78% of the environmental outbreaks, whereas they were not predominant in sporadic cases.

Oyster- and water-linked outbreaks were also clearly characterized by the fact that several strains (up to six) were detected in one single outbreak, whereas one single strain was detected when transmission was from person to person. These multiple contaminations of oysters and water have already been reported to be common (18, 20) and reflect a fecal contamination.

Finally, when the patient age in both epidemiological contexts is considered, we observed that whereas GII noroviruses were prominent in sporadic cases involving children less than 15 years of age, GI strains were more frequent in outbreaks occurring among this age group (6/9 [67%]). The high frequency of GII noroviruses in children has often been reported (4, 5, 10, 14, 16, 25). However, few data are available concerning the distribution of norovirus strains in outbreaks occurring in children. Recently, Hoebe et al. described an outbreak caused by recreational water contaminated with a Birmingham strain (GI-3) (15), and Villena et al. described an outbreak involving noroviruses from both genogroups I and II (31).

In conclusion, this period was remarkable for the diversity of calicivirus strains circulating in France and by the emergence of strains presenting a high potential of diffusion. The molecular basis for such a selective advantage remains to be elucidated. From a methodological point of view, the results presented here show that amplification and sequencing in the polymerase gene are not adequate in a significant number of cases for both detection and characterization and should be completed in this case by amplification and sequencing in the capsid gene.

ACKNOWLEDGMENTS

This work was supported by the Centre Hospitalier Universitaire de Dijon, Dijon, France, and the Conseil Régional de Bourgogne and by grants LITEAU1 LT98036 and QLK-CT-1999-00594 from the European Community.

We thank the physicians and their patients for participation in this study.

REFERENCES

1. Ando, T., S. S. Monroe, J. R. Gentsch, Q. Jin, D. C. Lewis, and R. I. Glass. 1995. Detection and differentiation of antigenically distinct small round-structured viruses (Norwalk-like viruses) by reverse transcription-PCR and Southern hybridization. *J. Clin. Microbiol.* **33**:64–71.
2. Ando, T., J. S. Noel, and R. L. Fankhauser. 2000. Genetic classification of "Norwalk-like viruses." *J. Infect. Dis.* **181**(Suppl. 2):S336–S348.
3. Bon, F., K. Balay, L. Le Coustumier, P. Megraud, P. Le Cann, A. Gallay, P. Pothier, and E. Kohli. Waterborne outbreak of acute gastroenteritis in France caused by multiple pathogens: *Campylobacter coli*, group A rotaviruses and two noroviruses including a new genogroup II variant (GGIIb). Manuscript in preparation.
4. Bon, F., P. Fascia, M. Dauvergne, D. Tenenbaum, H. Planson, A. M. Petion, P. Pothier, and E. Kohli. 1999. Prevalence of group A rotavirus, human calicivirus, astrovirus, and adenovirus type 40 and 41 infections among children with acute gastroenteritis in Dijon, France. *J. Clin. Microbiol.* **37**:3055–3058.
5. Buesa, J., B. Collado, P. López-Andújar, R. Abu-Mallouh, J. R. Díaz, A. G. Díaz, J. Prat, S. Guix, T. Llovet, G. Prats, and A. Bosch. 2002. Molecular epidemiology of caliciviruses causing outbreaks and sporadic cases of acute gastroenteritis in Spain. *J. Clin. Microbiol.* **40**:2854–2859.
6. Chikhi-Brachet, R., F. Bon, L. Toubiana, P. Pothier, J. C. Nicolas, A. Flahault, and E. Kohli. 2002. Virus diversity in a winter epidemic of acute diarrhea in France. *J. Clin. Microbiol.* **40**:4266–4272.
7. de Wit, M. A., M. P. Koopmans, L. M. Kortbeek, N. J. van Leeuwen, A. I. Bartelds, and Y. T. van Duynhoven. 2001. Gastroenteritis in sentinel general practices, The Netherlands. *Emerg. Infect. Dis.* **7**:82–91.
8. de Wit, M. A., M. P. Koopmans, L. M. Kortbeek, W. J. Wannet, J. Vinje, F. van Leusden, A. I. Bartelds, and Y. T. van Duynhoven. 2001. Sensor, a population-based cohort study on gastroenteritis in The Netherlands: incidence and etiology. *Am. J. Epidemiol.* **154**:666–674.
9. Fankhauser, R. L., S. S. Monroe, J. S. Noel, C. D. Humphrey, J. S. Bresee, U. D. Parashar, T. Ando, and R. I. Glass. 2002. Epidemiologic and molecular trends of "Norwalk-like viruses" associated with outbreaks of gastroenteritis in the United States. *J. Infect. Dis.* **186**:1–7.
10. Farkas, T., X. Jiang, M. L. Guerrero, W. Zhong, N. Wilton, T. Berke, D. O. Matson, L. K. Pickering, and G. Ruiz-Palacios. 2000. Prevalence and genetic diversity of human caliciviruses (HuCVs) in Mexican children. *J. Med. Virol.* **62**:217–223.
11. Farkas, T., W. M. Zhong, Y. Jing, P. W. Huang, S. M. Espinosa, N. Martinez, A. L. Morrow, G. M. Ruiz-Palacios, L. K. Pickering, and X. Jiang. 2004. Genetic diversity among sapoviruses. *Arch. Virol.* **149**:1309–1323.
12. Green, K. Y., R. M. Chanock, and A. Z. Kapikian. 2001. Human caliciviruses, p. 841–874. In D. M. Knipe, P. M. Howley, et al. (ed.), *Fields virology*, 4th ed., vol. 1. Lippincott Williams & Wilkins, Philadelphia, Pa.
13. Haftiger, D., M. Gilgen, J. Luthy, and P. Hubner. 1997. Seminested RT-PCR systems for small round structured viruses and detection of enteric viruses in seafood. *Int. J. Food Microbiol.* **37**:27–36.
14. Hansman, G. S., K. Katayama, N. Maneekarn, S. Peerakome, P. Khamrin, S. Tonusin, S. Okitsu, O. Nishio, N. Takeda, and H. Ushijima. 2004. Genetic diversity of norovirus and sapovirus in hospitalized infants with sporadic cases of acute gastroenteritis in Chiang Mai, Thailand. *J. Clin. Microbiol.* **42**:1305–1307.
15. Hoebe, C. J., H. Vennema, A. M. de Roda Husman, and Y. T. van Duynhoven. 2004. Norovirus outbreak among primary schoolchildren who had played in a recreational water fountain. *J. Infect. Dis.* **189**:699–705.
16. Iritani, N., Y. Seto, H. Kubo, T. Murakami, K. Haruki, M. Ayata, and H. Ogura. 2003. Prevalence of Norwalk-like virus infections in cases of viral gastroenteritis among children in Osaka City, Japan. *J. Clin. Microbiol.* **41**:1756–1759.
17. Jiang, X., P. W. Huang, W. M. Zhong, T. Farkas, D. W. Cubitt, and D. O. Matson. 1999. Design and evaluation of a primer pair that detects both Norwalk- and Sapporo-like caliciviruses by RT-PCR. *J. Virol. Methods* **83**:145–154.
18. Kageyama, T., M. Shinohara, K. Uchida, S. Fukushi, F. B. Hoshino, S. Kojima, R. Takai, T. Oka, N. Takeda, and K. Katayama. 2004. Coexistence of multiple genotypes, including newly identified genotypes, in outbreaks of gastroenteritis due to norovirus in Japan. *J. Clin. Microbiol.* **42**:2988–2995.
19. Kojima, S., T. Kageyama, S. Fukushi, F. B. Hoshino, M. Shinohara, K. Uchida, K. Natori, N. Takeda, and K. Katayama. 2002. Genogroup-specific PCR primers for detection of Norwalk-like viruses. *J. Virol. Methods* **100**:107–114.
20. Koopmans, M., C. H. von Bonsdorff, J. Vinje, D. de Medici, and S. Monroe. 2002. Foodborne viruses. *FEMS Microbiol. Rev.* **26**:187–205.
21. Le Guyader, F., M. K. Estes, M. E. Hardy, F. H. Neill, J. Green, D. W. Brown, and R. L. Atmar. 1996. Evaluation of a degenerate primer for the PCR detection of human caliciviruses. *Arch. Virol.* **141**:2225–2235.
22. Lopman, B., H. Vennema, E. Kohli, P. Pothier, A. Sanchez, A. Negro, J. Buesa, E. Schreier, M. Reacher, D. Brown, J. Gray, M. Iturriza, C. Gallimore, B. Bottiger, K. O. Hedlund, M. Torven, C. H. von Bonsdorff, L. Maunula, M. Poljsak-Prijatelj, J. Zimsek, G. Reuter, G. Szucs, B. Melegh, L. Svensson, Y. van Duynhoven, and M. Koopmans. 2004. Increase in viral gastroenteritis outbreaks in Europe and epidemic spread of new norovirus variant. *Lancet* **363**:682–688.
23. Lopman, B. A., G. K. Adak, M. H. Reacher, and D. W. Brown. 2003. Two epidemiologic patterns of norovirus outbreaks: surveillance in England and Wales, 1992–2000. *Emerg. Infect. Dis.* **9**:71–77.
24. Lopman, B. A., D. W. Brown, and M. Koopmans. 2002. Human caliciviruses in Europe. *J. Clin. Virol.* **24**:137–160.
25. Martinez, N., C. Espul, H. Cuello, W. Zhong, X. Jiang, D. O. Matson, and T. Berke. 2002. Sequence diversity of human caliciviruses recovered from chil-

- dren with diarrhea in Mendoza, Argentina, 1995-1998. *J. Med. Virol.* **67**: 289-298.
26. Noel, J. S., T. Ando, J. P. Leite, K. Y. Green, K. E. Dingle, M. K. Estes, Y. Seto, S. S. Monroe, and R. I. Glass. 1997. Correlation of patient immune responses with genetically characterized small round-structured viruses involved in outbreaks of nonbacterial acute gastroenteritis in the United States, 1990 to 1995. *J. Med. Virol.* **53**:372-383.
 27. Noel, J. S., R. L. Fankhauser, T. Ando, S. S. Monroe, and R. I. Glass. 1999. Identification of a distinct common strain of "Norwalk-like viruses" having a global distribution. *J. Infect. Dis.* **179**:1334-1344.
 28. Nygard, K., M. Torven, C. Ancker, S. B. Knauth, K. O. Hedlund, J. Giesecke, Y. Andersson, and L. Svensson. 2003. Emerging genotype (GGIIB) of norovirus in drinking water, Sweden. *Emerg. Infect. Dis.* **9**:1548-1552.
 29. Tompkins, D. S., M. J. Hudson, H. R. Smith, R. P. Eglin, J. G. Wheeler, M. M. Brett, R. J. Owen, J. S. Brazier, P. Cumberland, V. King, and P. E. Cook. 1999. A study of infectious intestinal disease in England: microbiological findings in cases and controls. *Commun. Dis. Public Health* **2**:108-113.
 30. Vennema, H., E. Kohli, D. Brown, E. Schreier, C. H. S. L. von Borsdorff, F. Hanon, and M. Koopmans. 2002. International spread in 7 countries in Europe of new pol-type calicivirus associated with 3 different capsids facilitated by food- and waterborne transmission. Presented at the XIIth International Congress of Virology, Paris, France, 27 July to 1 August 2002.
 31. Villena, C., R. Gabrieli, R. M. Pinto, S. Guix, D. Donia, E. Buonomo, L. Palombi, F. Cenko, S. Bino, A. Bosch, and M. Divizia. 2003. A large infantile gastroenteritis outbreak in Albania caused by multiple emerging rotavirus genotypes. *Epidemiol. Infect.* **131**:1105-1110.
 32. Vinje, J., and M. P. Koopmans. 1996. Molecular detection and epidemiology of small round-structured viruses in outbreaks of gastroenteritis in the Netherlands. *J. Infect. Dis.* **174**:610-615.
 33. Wang, J., X. Jiang, H. P. Madore, J. Gray, U. Desselberger, T. Ando, Y. Seto, I. Oishi, J. F. Lew, K. Y. Green, and M. K. Estes. 1994. Sequence diversity of small, round-structured viruses in the Norwalk virus group. *J. Virol.* **68**:5982-5990.
 34. Yuen, L. K. W., M. G. Catton, B. J. Cox, P. J. Wright, and J. A. Marshall. 2001. Heminested multiplex reverse transcription-PCR for detection and differentiation of Norwalk-like virus genogroups 1 and 2 in fecal samples. *J. Clin. Microbiol.* **39**:2690-2694.
 35. Zheng, D., T. Ando, R. Glass, and S. Monroe. 2004. Norovirus classification and proposed strain nomenclature. Presented at the Second International Calicivirus Conference, Dijon, France, 6 to 10 November 2004.