Journal of Clinical Microbiology
September 2005; 43(9) : 4659-4664
http://dx.doi.org/10.1128/JCM.43.9.4659-4664.2005

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. Gallay[3], V. Vaillant[3], H. de Valk[3], R. Chikhi-Brachet[4], A. Flahaut[4], P. Pothier[1], and E. Kohli[1]

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

*: Corresponding author : 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.

Abstract: 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

Keywords: epidemiological, caliciviruses, gastroenteritis, cocirculation, genogroup

Introduction

Human caliciviruses (HuCVs, *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 to describe a great genetic diversity among the HuCVs which belong to two genera, *Sapovirus* and *Norovirus,* and to 8 different genogroups within these 2 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 reported 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 detect common sources of infection, is important to better understand these patterns.

In France, except for foodborne outbreaks, there is no systematic virological surveillance of acute gastroenteritis. However, we have recently conducted epidemiological studies including sporadic cases (6, not published 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 to 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.

Material and methods

Strains

Sporadic cases: 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), 54 in a regional study in the western region of France (Golfe du Morbihan, Bretagne, October 1999-May 2001, not published data). This study included **general practitioner** (GP) cases as well as children consulting an 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.

Outbreak strains: 72 calicivirus strains detected in 45 outbreaks investigated during the period December 1998-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 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 G1SKF/G1SKR (19) for genogroup I and Mon381/Mon383 (26) or G2SKF/G2SKR (19) for genogroup II. In addition, some genogroup I strains which were weakly or not amplified in the polymerase gene were detected and thus also characterized by amplification 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 by Wang *et al.* (33).

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

Sequence analysis

Sequence alignments with GenBank library were carried out by using the Fasta Version 3.3t06 and GCG software available on the national Service Infobiogen (http://www.infobiogen.fr).

Results

Sporadic case strains

One hundred sequence data were analysed during the period December 1998 to March 2002. Most of the strains (91%) had been detected during the winter seasons 1998-99 and 2000-01. **The number of norovirus positive cases collected during the winter 2000-01 may be explained by i) an important winter epidemic peak during this season, ii) a better participation of the GPs.**

The results showed a cocirculation of various caliciviruses with 18 different groups being observed **(Table I, Figure 1A)**. Noroviruses were predominant (93% versus 7% for sapoviruses) and among them, GII strains were the most frequently detected (66% versus 26% and 1 % for genogroups I and IV, respectively). 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): 1- the appearance in September 2000 in the western region of the new variant GGIIb strains. These strains which have been described elsewhere (3, 5, 28, 30) are recombinant viruses which show a unique polymerase gene associated to different capsids. They represented about 1/3 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 to Hawaii (GII-1) and 1 to Lordsdale (GII-4) genotypes; 2- the appearance of Norwalk-related strains **(GI-1)** which had the **characteristic** to be weakly or not detected in the polymerase gene despite many attempts. **These strains** were easily amplified in the capsid region where they showed 90% nucleotide and 96.7% amino acid identity with Norwalk virus (Accession n° 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 (Accession n° AY921623, 90% nucleotide identity with Norwalk virus, accession n° M87661). Both GGIIb and **Norwalk**-related strains which 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 among the 100 strains compiled here. Twenty five strains were found in the 0-2, 8 in the 2-5, 10 in the 5-15, 29 in the 15-65 and 4 in the > 65 **age-groups, respectively**. All the sapovirus strains (7) were detected in the 2-5 **age-group**. Of interest, GII noroviruses were the most frequent in all age groups except in the 15-65 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 0-2 **age-group** whereas this group represented 33% of the strains analysed.

Outbreak strains

Outbreak characteristics

The settings and transmission for the 45 outbreaks investigated are shown in figure 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 cocirculation of a great diversity of caliciviruses with 17 different groups being detected **(Table I,** Fig. 1B). Again, we observed a predominance of GII noroviruses (62% for the whole period), however,

GI strains became the most frequent between April 03 and February 04, representing 54% of the strains.

Concerning the different genotypes, it is **interesting** to note 1- the appearance of the recombinant GGIIb/Hawaii **(GII-1)** strains during the summer 2000 during a waterborne outbreak in a town (Gourdon, Lot) (3); these strains were detected in outbreaks until March 2002; GGIIb/Mexico **(GII-3)** strains were found from January 2001 to February 2004 whereas a GGIIb/Lordsdale **(GII-4)** and a GGIIb/Snow Mountain **(GII-2)** strains were also detected in outbreaks in March 2002 and February 2004 respectively; 2- the appearance in March 2000 of Norwalk-related strains similar to those described in sporadic strains. These strains were **not** detected in outbreaks after April 2001 although capsid primers for GI noroviruses were systematically used for detection; 3- the emergence of a new Lordsdale variant **(GII-4)** in April 02 whereas the previous Lordsdale strains were no more 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; 4- the recent increase of **Desert Shield** strains **(GI-3)**.

Strains detected according to the setting and transmission mode are shown in figure 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 person-to-person as well as in school outbreaks where the transmission was either person-to-person, foodborne 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 2 and 3 genogroup I strains (2 outbreaks), one or more GGI strains associated with one or more GGII strains (7 outbreaks), one sapovirus associated with 2 GGI strains and 4 GGII strains (1 outbreak).** Thus, 20 different strains were detected in 9 oyster-related outbreaks and 18 in 5 waterborne outbreaks, compared to 17

in 17 person-to-person transmitted outbreaks. 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%) belonging for most of them 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) among them 4 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 year old.**

Discussion

In this work, we have compiled sequences and epidemiological data from 172 caliciviruses detected in France from December 1998 to February 2004, among which 100 were from sporadic cases and 72 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 3 groups of strains which had not been detected before in our laboratory: in 2000 the new variant GGIIb and Norwalk-like strains which were difficult to detect as well as, in 2002, a new variant Lordsdale strain. Although these strains emerged during a period, other genotypes still cocirculated. Among these 3 groups of strains, the new variant GGIIb and the new Lordsdale 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 the conventional methods, it is probably difficult to conclude on their actual distribution. The recombinant GGIIb and Norwalk-related strains brought 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% of 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 2000-01 (overlapped period). **During this period, GGIIb and Norwalk-related viruses were predominant in both outbreaks and sporadic cases. In contast, no Lordsdale strain was detected in outbreaks whereas they accounted 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 waterborne 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 behaviour in the environment (decreased resistance, 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 6) 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 been already reported to be common (18, 20) and reflect a fecal contamination.

Finally, when considering the patient age in both epidemiological contexts, we observed that, whereas GII noroviruses were prominent in child sporadic cases less than 15 years, 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.* have described an outbreak caused by **recreational** water contaminated with a Birmingham strain (GI-**3**) (15) and Villena *et al.* 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 remain to be elucidated. From a

methodological point of view, the results presented here show that amplification and sequencing in the polymerase gene is not adequate in a significant number of cases both for detection and characterization and should be completed in this case by amplification and sequencing in the capsid gene.

Nucleotide sequence accession numbers.

The nucleotide sequence data of 3 different recombinant GGIIb strains and of the helicase region of a Norwalk-related strain has been deposited in Genbank nucleotide sequence databases with the following accession numbers: N°^{s}AY580335 (Hu/NLV/Gourdon78/2000/France); AY773210 (Hu/NLV/VannesL169/2000/France); AY682549 (Hu/NLV/Pont-de-Roide673/2004/France; AY921623 (helicase).

Acknowledgements: This work was supported by the Centre Hospitalier Universitaire de Dijon, France, 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.

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Table I : Distribution of human calicivirus strains detected in France in sporadic and outbreak cases from December 1998 to February 2004. The GGIIb recombinant strains showed 4 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.

Genogroup	Genotypes	No of strains detected in sporadic cases (%)	No of strains detected during outbreaks (%)
Noroviruses 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%)
Noroviruses 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%)
	GGII-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%)
Sapoviruses Genogroup I	Sapporo	4 (4%)	1 (1%)
Sapoviruses Genogroup II	London/92	3 (3%)	0 (0%)
	Total	100	72

Legend of the figures

Figure 1: Temporal distribution of human calicivirus strains circulating in France in sporadic cases (A) and in outbreaks (B) during a five-year period, from December 1998 to February 2004.

Figure 2: Setting and mode of transmission of 45 calicivirus outbreaks investigated during the period December 1998 - 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+, GI+GII: >1 strain belonging to genogroup I and/or II.

Figure 1

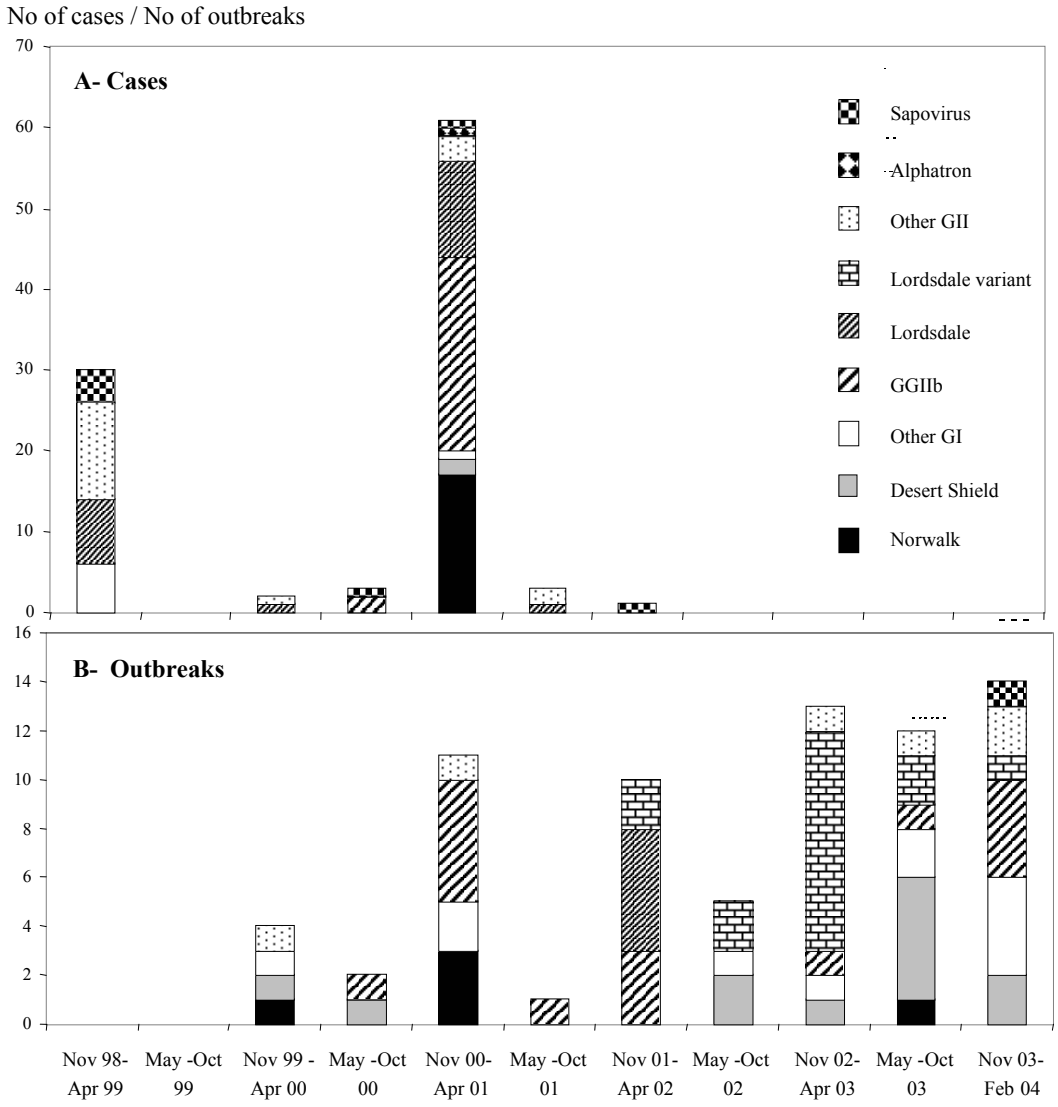
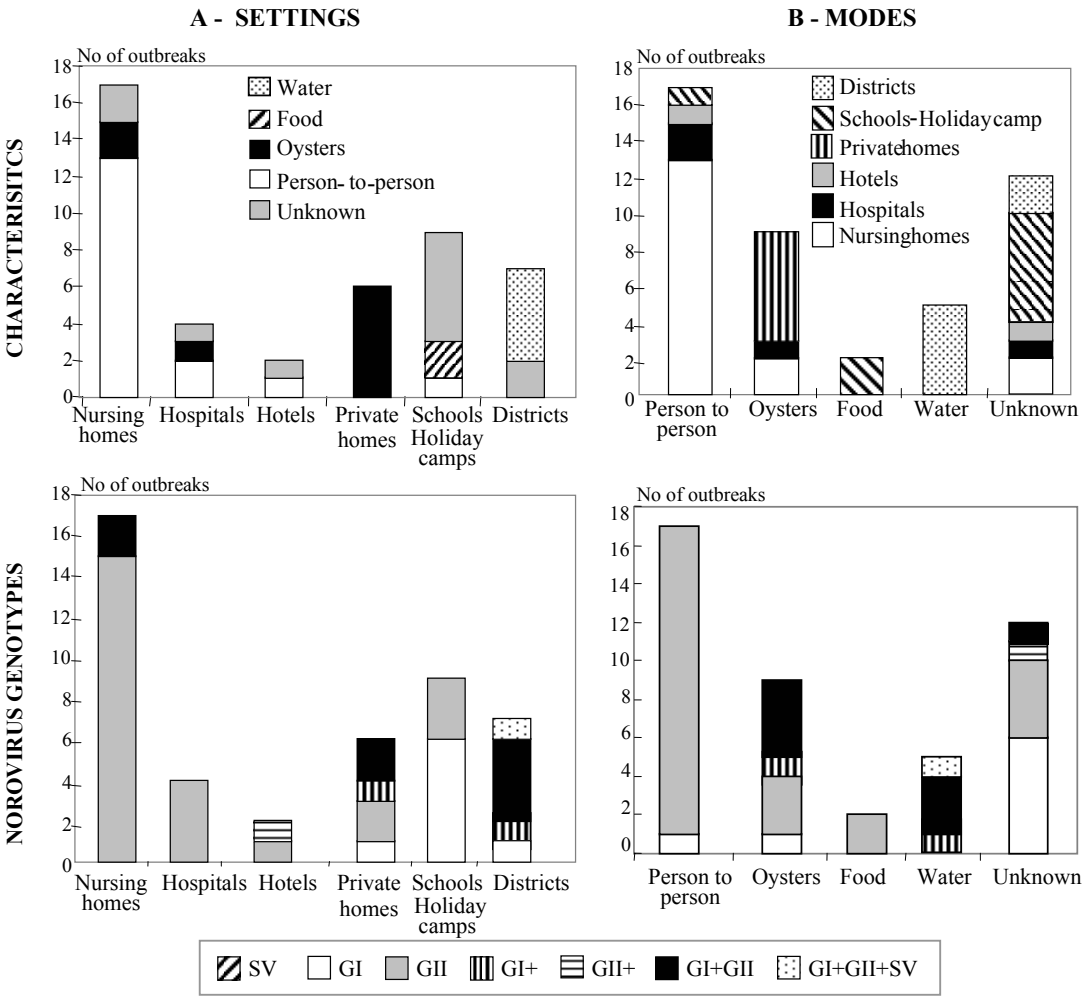

No of cases / No of outbreaks
A- Cases
B- Outbreaks
Sapovirus
Alphatron
Other GII
Lordsdale variant
Lordsdale
GGIIb
Other GI
Desert Shield
Norwalk
Nov 98- Apr 99
May -Oct 99
Nov 99 - Apr 00
May -Oct 00
Nov 00- Apr 01
May -Oct 01
Nov 01- Apr 02
May -Oct 02
Nov 02- Apr 03
May -Oct 03
Nov 03- Feb 04

Figure 2


A - SETTINGS
B - MODES
CHARACTERISITCS
NOROVIRUS GENOTYPES
No of outbreaks
Water
Food
Oysters
Person- to-person
Unknown
Districts
Schools-Holidaycamp
Privatehomes
Hotels
Hospitals
Nursinghomes
Nursing homes
Hospitals
Hotels
Private homes
Schools Holiday camps
Districts
Person to person
Oysters
Food
Water
Unknown
SV
GI
GII
GI+
GII+
GI+GII
GI+GII+SV