Journal of Microbiology, Immunology and Infection (2011) 44, 241—246

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.e-jmii.com

ORIGINAL ARTICLE

The relationship between health care and nonhealth care norovirus outbreak settings and norovirus genotype in Victoria, Australia, 2002—2005

Leesa Bruggink, John Marshall*

Gastroenteritis Section, Victorian Infectious Diseases Reference Laboratory, North Melbourne, Victoria, Australia

Received 1 February 2010; received in revised form 30 June 2010; accepted 12 August 2010

KEYWORDS
Genotype;
Norovirus;
Outbreak setting

Background: There is evidence that norovirus genotype is an important factor in determining norovirus epidemiology, but detailed information is lacking. This report examined this question by studying whether the mix of norovirus genotypes associated with norovirus outbreaks in health care settings was different to that in nonhealth care settings.
Methods: Norovirus outbreaks tested in Victoria, Australia, 2002—2005 were classified as either health care or nonhealth care. Open reading frame 1 nucleotide sequencing analysis was then used to determine the mix of norovirus genotypes in health care and nonhealth care norovirus outbreaks.
Results: For the three most common genotypes detected (GI.2, GII.4, and GIIb), the differences between health care and nonhealth care settings were significant. GII.4 was significantly more common in health care settings than in nonhealth care settings, whereas the genotypes GI.2 and GIIb were significantly more common in nonhealth care settings than in health care settings.
Conclusion: Norovirus genotype was found to be an important factor associated with norovirus outbreak setting.

Introduction

The noroviruses are single-stranded positive sense RNA viruses classified as the genus *Norovirus* within the family *Caliciviridae* and are considered the most common cause of outbreaks of nonbacterial gastroenteritis in humans. Noroviruses are currently classified into five genogroups, of

* Corresponding author. Victorian Infectious Diseases Reference Laboratory, 10 Wreckyn St, North Melbourne, Victoria 3051, Australia.
E-mail address: john.marshall@mh.org.au (J. Marshall).

1684-1182/$36 Copyright © 2011, Taiwan Society of Microbiology. Published by Elsevier Taiwan LLC. All rights reserved.
doi:10.1016/j.jmii.2010.08.001

which three, genogroup I (GI), genogroup II (GII), and genogroup IV (GIV), occur in human infections. Most noroviruses affecting humans belong to GI or GII. Within each genogroup one or more genotypes have been identified.[1]

Although there is little understanding of what factors control norovirus outbreak incidence, there is some evidence that outbreak setting is important.[2] In particular, it was found that norovirus outbreaks in health care settings showed a seasonal periodicity whereas norovirus outbreaks in nonhealth care settings did not.[2]

Recent studies in this laboratory have indicated that there appears to be a linkage between norovirus genotype and outbreak setting in some norovirus outbreaks.[3,4] The question, therefore, arises whether the mix of norovirus genotypes associated with norovirus outbreaks in health care settings is different to the mix of norovirus genotypes associated with norovirus outbreaks in nonhealth care settings.

The objective of this study was to use material from Victoria, Australia, 2002—2005 to examine whether the mix of norovirus genotypes associated with norovirus outbreaks in health care settings was different to the mix of norovirus genotypes associated with norovirus outbreaks in nonhealth care settings.

Methods

Outbreak definition and investigation

For the purposes of this study, an outbreak of gastroenteritis was defined as an incident, apparently associated with a common event or location, in which four or more individuals had symptoms of gastroenteritis.

The outbreaks included in this study were those for which specimens were sent to the Victorian Infectious Diseases Reference Laboratory for testing in the period 2002—2005. The Victorian Infectious Diseases Reference Laboratory is the main public health laboratory for viral identification in the state of Victoria, Australia and, as such, it receives faecal material from gastroenteritis outbreaks reported to the Victorian Department of Human Services where a viral etiology is suspected. Outbreak specimens are also occasionally sent by other entities. Only outbreaks that occurred in the state of Victoria were included in the study.

The date of an outbreak was taken as the onset date, if provided. If this was unavailable, the date taken was the date the outbreak was first notified to the Department of Human Services or the earliest date of collection of a specimen for testing. Outbreak documentation (including the setting of the outbreak) and management were carried out by staff of the Department of Human Services and/or staff at the affected setting.

For the purposes of analysis, norovirus outbreaks were divided into two groups, health care and nonhealth care, as follows. Health care settings included aged-care facilities, disabled care facilities, early parenting centers, hospitals, hospitals—geriatric ward, hospitals—pediatric ward, hospitals—plastic surgery unit, hospitals—psychiatric ward, and hospitals—rehabilitation unit. Nonhealth care settings included barracks, camps, camp—adult, camp—school, child care centers, children's activity centers, elderly activity centers, gatherings, a navy base, a prison, restaurants, and suspect food.

Only one faecal specimen per person per outbreak was tested for norovirus. A total of 3,788 faecal specimens from 655 gastroenteritis outbreaks were tested for norovirus during the course of this study. The number of specimens tested per outbreak varied from 1 to 34.

Faecal processing and reverse transcription-polymerase chain reaction

Faecal specimens were prepared as a 20% (vol/vol) suspension in Hanks' complete balanced salt solution and the suspension vigorously shaken and then centrifuged at 3,500*g* for 15 minutes. The supernatant fluid was collected, spun at 7,000*g* for 30 minutes, and an aliquot of the clarified fluid was then collected for testing for norovirus by reverse transcription-polymerase chain reaction (RT-PCR). Open reading frame (ORF) 1 RT-PCR testing for norovirus was carried out essentially as given by the method of Yuen et al.,[5] which both identifies and differentiates between GI and GII norovirus.

Norovirus ORF 1 nucleotide sequencing and phylogenetic analysis

Genotype definition of all norovirus outbreaks initially involved sequencing analysis of one norovirus specimen, chosen without bias, from each genogroup identified in that outbreak. The second-round PCR product of the ORF 1 RT-PCR test procedure was purified and sequenced essentially as given previously.[3,6] A region of 440 nucleotides within the RNA polymerase region, which corresponded to nucleotides 4,484—4,923 of Camberwell virus (AF145896), was then used for phylogenetic analysis and genotype definition (essentially as given previously[3,4,6]). A specimen, chosen without bias from each norovirus outbreak, was successfully sequenced in all but six norovirus outbreaks, where a usable sequence could not be obtained. (These six norovirus outbreaks were all in the health care category and all belonged to GII.)

Results

General

Of the 3,788 specimens tested from 655 gastroenteritis outbreaks for the period 2002—2005, one or more specimens were positive for norovirus in 430 outbreaks (i.e. 430 norovirus outbreaks were identified). The settings of the norovirus outbreaks are given in Table 1.

Norovirus genotypes and their relationship with norovirus outbreak setting

Based on the phylogenetic trees generated for each year of the study (Fig. 1), a total of 10 genotypes could be identified and the genotype(s) corresponding to each outbreak then determined. The relationship between norovirus outbreak setting and genotype is given in Table 2.

From Table 2 it can be seen that five GI genotypes (GI.2, GI.3b, GI.4, GI.?a, GI.?b), five GII genotypes (GII.1, GII.3b,

Table 1 Settings of norovirus outbreaks 2002—2005

Setting	*n* (%)
Health care	
Aged-care facility	283 (65.8)
Disabled care facility	3 (0.7)
Early parenting center	2 (0.5)
Hospital	30 (7.0)
Hospital—geriatric ward	23 (5.3)
Hospital—pediatric ward	3 (0.7)
Hospital—plastic surgery unit	1 (0.2)
Hospital—psychiatric ward	2 (0.5)
Hospital—rehabilitation unit	10 (2.3)
Nonhealth care	
Barracks	1 (0.2)
Camp	1 (0.2)
Camp—adult	1 (0.2)
Camp—school	4 (0.9)
Child care center	9 (2.1)
Children's activity center	4 (0.9)
Elderly activity center	1 (0.2)
Gathering	29 (6.7)
Navy base	1 (0.2)
Prison	1 (0.2)
Restaurant	9 (2.1)
Suspect food	12 (2.8)
Total	430 (100.0)

n = total number of norovirus outbreaks.

GII.3c, GII.4, GIIb), and one combination of GI and GII (GI.2/GII.4) were detected in the 424 sequenced norovirus outbreaks.

For eight genotype categories (GI.3b, GI.4, GI.?a, GI.?b, GII.1, GII.3b, GII.3c, GI.2/GII.4), the numbers of outbreaks were very small and the differences between the percentage of genotypes associated with health care and nonhealth care outbreaks were not significant ($p > 0.3$ in all cases; two-tailed Fisher's exact test).

For the three most common genotypes (GI.2, GII.4, GIIb), the differences between health care and nonhealth care settings were significant. In particular, GII.4 was significantly more common in health care settings than in nonhealth care settings ($p < 0.001$, χ^2 test). In contrast, GI.2 and GIIb were significantly more common in nonhealth care settings than in health care settings ($p < 0.002$, 0.00001, respectively; two-tailed Fisher's exact test).

Discussion

In a major study of norovirus outbreaks in England and Wales in 1992—2000, it was found that norovirus outbreaks in health care settings showed a seasonal peak whereas norovirus outbreaks in nonhealth care settings did not.[2] The aim of the current study was to examine the hypothesis that differences in epidemiology may be related to the fact that the mix of norovirus genotypes in norovirus outbreaks in health care settings is different to that in nonhealth care settings.

The results of the current study do indeed indicate that the mix of norovirus genotypes associated with norovirus outbreaks in health care as opposed to nonhealth care

Table 2 Genotypes of norovirus outbreaks in health care and nonhealth care settings[a]

Genotype	Number of health care outbreaks	% of health care outbreaks	Number of nonhealth care outbreaks	% of nonhealth care outbreaks
GI.2	4	1.1	6	9.0
GI.3b	1	0.3	1	1.5
GI.4	1	0.3	0	0.0
GI.?a[b]	1	0.3	0	0.0
GI.?b[a]	2	0.6	0	0.0
GII.1	1	0.3	0	0.0
GII.3a[c]	1	0.3	1	1.5
GII.3c[c]	1	0.3	0	0.0
GII.4	334	93.6	47	70.1
GIIb	9	2.5	12	17.9
GI.2/GII.4	2	0.6	0	0.0
Total	357	100.0	67	100.0

[a] For the three most common genotypes (GI.2, GII.4, and GIIb), the differences between health care and nonhealth care settings were significant. In particular, GII.4 was significantly more common in health care than in nonhealth care settings ($p < 0.001$, χ^2 test) and GI.2 and GIIb were significantly more common in nonhealth care than in health care settings ($p < 0.002$, 0.00001, respectively, two-tailed Fisher's exact test). For all the other genotype categories, the differences between health care and nonhealth care settings were not significant ($p > 0.3$, two-tailed Fisher's exact test).

[b] GI.?a and GI.?b did not match any well documented reference strains, but formed separate phylogenetic clusters.

[c] For GII.3 classification, five GII.3 reference strains were used to define the GII.3 genotype i.e. Toronto/1991/CAN (U02030), Mexico/1989/MEX (U22498), Sydney 2212/1998/AU (AY588132), Arg320/1995/AR (AF190817), and Osaka/2000/JP (AB071025). These reference strains fell into three distinct groups, not one, in the ORF 1 phylogenetic tree. These were designated as GII.3a (U02030), GII.3b (U22498), and GII.3c (AY588132, AF190817, AB071025).

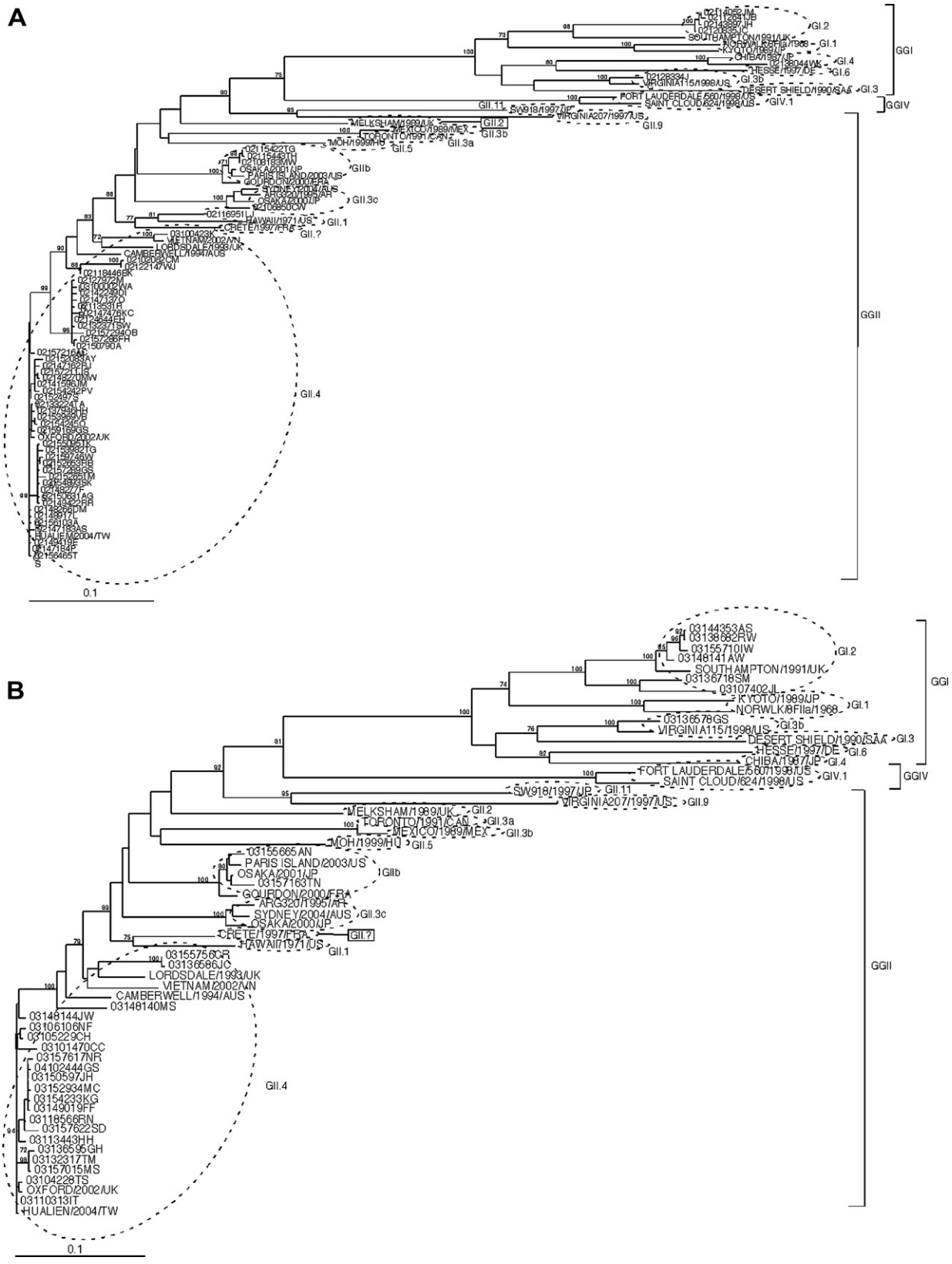

Figure 1. Phylogenetic trees showing unique sequences for all norovirus genotypes identified in each year of the study. Figures on branches represent bootstrap values (%) after resampling 1,000 data sets. Only bootstrap values $\geq$70% are shown. The scale markers represent nucleotide substitutions per site. (A) 2002; (B) 2003; (C) 2004; (D) 2005.

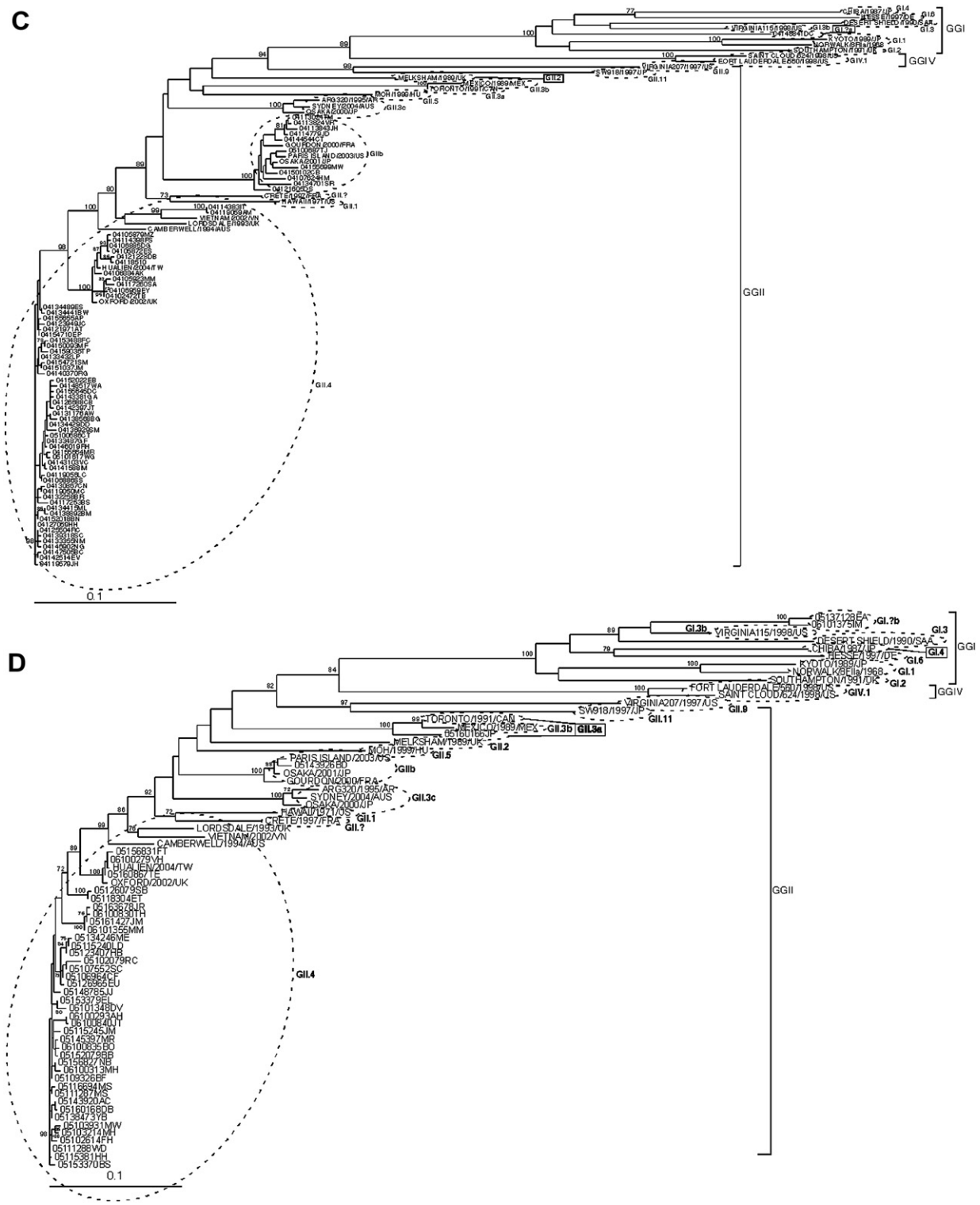

Figure 1. (*Continued*).

settings was different. GII.4 was found to be the predominant genotype in norovirus outbreaks in both health care and nonhealth care settings, but was significantly more common in health care settings (93.6%) than in nonhealth care settings (70.1%). Furthermore, norovirus outbreaks associated with the genotypes GI.2 and GIIb were significantly more common in nonhealth care settings than in health care settings. Thus, there is a link between norovirus outbreak setting and norovirus genotype. Further studies are needed to fully understand this relationship.

References

1. Marshall JA, Bruggink LD. Laboratory diagnosis of norovirus. *Clin Lab* 2006;**52**:571—81.
2. Lopman BA, Adak GK, Reacher MH, Brown DWG. Two epidemiologic patterns of *Norovirus* outbreaks: surveillance in England and Wales, 1992—2000. *Emerg Infect Dis* 2003;**9**:71—7.
3. Marshall JA, Dimitriadis A, Wright PJ. Molecular and epidemiological features of norovirus-associated gastroenteritis outbreaks in Victoria, Australia in 2001. *J Med Virol* 2005;**75**:321—31.
4. Bruggink LD, Marshall JA. Molecular and epidemiological features of GIIb norovirus outbreaks in Victoria, Australia, 2002—2005. *J Med Virol* 2009;**81**:1652—60.
5. Yuen LKW, Catton MG, Cox BJ, Wright PJ, Marshall JA. Heminested multiplex reverse transcription-PCR for detection and differentiation of Norwalk-like virus genogroups 1 and 2 in fecal samples. *J Clin Microbiol* 2001;**39**:2690—4.
6. Marshall JA, Hellard ME, Sinclair MI, Fairley CK, Cox BJ, Catton MG, et al. Incidence and characteristics of endemic Norwalk-like virus-associated gastroenteritis. *J Med Virol* 2003;**69**:568—78.