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SHORT REPORT
Norovirus genotype diversity associated with gastroenteritis outbreaks in aged-care facilities

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SUMMARY

Noroviruses are a major cause of gastroenteritis. Vaccine strategies against norovirus are currently under consideration but depend on a detailed knowledge of the capsid genotypes. This study examined the incidence of norovirus outbreaks in residential aged-care facilities in Victoria, Australia over one year (2013) and documented the (capsid) norovirus genotypes associated with these outbreaks. It was found that 65·0% of 206 outbreaks tested were associated with norovirus infection, thereby showing norovirus to be the major cause of viral gastroenteritis in residential aged-care facilities. Fifteen capsid (open reading frame 2) genotypes were identified as follows: GI.2 (0·9%), GI.3 (1·8%), GI.4 (3·7%), GI.6 (0·9%), GI.7 (0·9%), GI.8 (0·9%), GII.1 (0·9%), GII.2 (0·9%), GII.3 (1·8%), GII.4 (2009-like) (0·9%), GII.4 (2012) (48·6%), GII.4 (2012-like) (16·5%), GII.4 (unknown) (9·2%), GII.5 (2·8%), GII.6 (0·9%), GII.7 (0·9%), GII.13 (6·4%) and an as yet unclassified GII genotype (0·9%). Although GII.4 was the most common norovirus capsid genotype detected, the great diversity of norovirus genotypes in the elderly indicates vaccination strategies for this demographic are not straightforward.

Key words: Aged care, genotypes, norovirus, outbreaks, vaccine strategies.

Noroviruses, which are now recognized as a major cause of gastroenteritis [1], are single-stranded positive-sense RNA viruses classified as the genus *Norovirus* within the family Caliciviridae [2]. The norovirus genome comprises three open reading frames (ORFs) [2]. ORF 1 encodes the non-structural polyprotein, ORF 2 the major structural capsid protein and ORF 3 the minor structural protein [2].

Noroviruses are classified into a number of genogroups [3] three of which, genogroups I, II and IV (GI, GII and GIV), occur in human infections [3], although little is known concerning the incidence and clinical significance of GIV noroviruses in human infections [4]. Norovirus classification at the next level has been referred to as classification at the genotype level [3]. The GII.4 genotype appears to be the most common in outbreaks in humans [1] and has been further subdivided into 'variants' [5].

Norovirus infections are an important cause of gastroenteritis in the elderly [1, 6] and in this regard Bartsch *et al.* [7] noted that norovirus vaccination should be considered for those aged $\geqslant 65$ years. The development of effective vaccine strategies is dependent, at least in part, on a detailed knowledge of the range of norovirus capsid genotypes in the demographic being targeted for vaccination.

Despite acknowledgement of the public health significance of norovirus infections in the elderly, the proportion of gastroenteritis outbreaks linked to norovirus

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and the diversity of norovirus genotypes associated with gastroenteritis in aged-care facilities have not been extensively investigated. The current study examines the incidence of norovirus outbreaks in residential aged-care facilities in Victoria, Australia over one calendar year (2013) and documents the ORF 2 (capsid) norovirus genotypes associated with these outbreaks. The findings are compared with the results of related studies in other countries.

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. This study is based on the outbreaks which occurred in 2013 for which faecal specimens were sent to the Victorian Infectious Diseases Reference Laboratory (VIDRL) for norovirus testing. A calendar year was chosen as a suitable time period for a meaningful analysis of norovirus incidence, as this time period normally corresponds to a baseline period as well as an epidemic peak in Victoria, Australia [8, 9]. The VIDRL is the main public health laboratory for viral identification in the state of Victoria, Australia. As such, it receives faecal material from gastroenteritis outbreaks reported to the Victorian Health Department. Only outbreaks which occurred in Victoria were included in the study.

The date of an outbreak was taken as the onset date. If this was not available, the date the outbreak was first notified or the earliest date of collection of a specimen from the outbreak was taken as the date of the outbreak. Only one faecal specimen per person per outbreak was tested.

Aged-care facilities were identified on the basis of the description of the facility provided by the Victorian Health Department in the outbreak investigation report. All aged-care facilities in the current study were residential in nature.

Three two-round reverse transcription–polymerase chain reaction (RT–PCR) protocols were used in the study. For the first round of each of the three protocols a commercial kit, the Qiagen OneStep RT–PCR kit (Qiagen GmbH, Germany), that combined the RT step and the first round of the PCR, was utilized. The second-round PCR was performed using the Qiagen *Taq* DNA polymerase kit.

Initially all specimens received for norovirus identification were tested by an ORF 1 RT–PCR for GI and GII norovirus. This was conducted with a two-round RT–PCR protocol using primers NV 4562, NV 4611, NV 4692, NV 5296, NV 5298 and NV 5366, as given previously [10]. Nucleotide sequencing analysis of the PCR product derived from this protocol utilized a 440 bp fragment corresponding to nucleotides 4484–4923 (relative to Camberwell virus, AF145896).

One specimen from every outbreak was then tested by an ORF 2 RT–PCR protocol directed to region C of GI noroviruses. This two-round RT–PCR protocol was performed using primers COG1F and G1SKR as given previously [10]. Nucleotide sequencing analysis of the PCR product derived from the GI protocol utilized a 198 bp fragment corresponding to nucleotides 5415–5612 (relative to Norwalk virus, M87661).

One specimen from every outbreak was also tested by an ORF 2 RT–PCR protocol directed to region C of GII noroviruses. This two-round RT–PCR protocol was performed using primers G2F3 and G2SKR as given previously [10], except that these two primers were used in both rounds. Nucleotide sequencing analysis of the PCR product derived from the GII protocol utilized a 195 bp fragment corresponding to nucleotides 5133–5327 (relative to Camberwell virus, AF145896).

Nucleotide sequencing and phylogenetic analysis were performed essentially as described previously [11]. Genotype analysis also made use of the norovirus automated genotyping tool (http://www.rivm.nl/mpf/norovirus/typingtool) [12].

GII.4 variant status was determined in a three-step process. First, norovirus ORF 2 sequences were tested by the norovirus automated genotyping tool. If the genotyping tool assigned a variant form, it was accepted. Second, if the typing tool classified a variant as 'unknown' it was classified as a 'like' variant if the ORF 2 sequence of 195 bases used was no more than two bases different from an accepted variant reference strain; this approach yielded the various '2009-like' and '2012-like' GII.4 forms found in the study. Third, if an ORF 2 sequence had more than two base changes from a known variant reference strain it was classified as 'GII.4 (unknown)'.

In the calendar year 2013, 206 gastroenteritis outbreaks were received for testing from residential aged-care facilities. Of these 123 were positive by the ORF 1 PCR and a further 11 were negative by the ORF 1 PCR but positive by one of the ORF 2 PCRs. Thus a total of 134 (65·0%) of all outbreaks from aged-care facilities were positive for norovirus, indicating it was the major cause of viral gastroenteritis outbreaks in residential aged-care facilities. The average age of 134 norovirus-positive individuals (one from each

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Table 1. *Norovirus ORF 2 (capsid) genotypes detected in outbreaks from aged-care facilities in 2013*

ORF 2 genotype	No. of outbreaks (% of total)
GI.2	1 (0·9)
GI.3	2 (1·8)
GI.4	4 (3·7)
GI.6	1 (0·9)
GI.7	1 (0·9)
GI.8	1 (0·9)
GII.1	1 (0·9)
GII.2	1 (0·9)
GII.3	2 (1·8)
GII.4 (2009-like)	1 (0·9)
GII.4 (2012)	53 (48·6)
GII.4 (2012-like)	18 (16·5)
GII.4 (unknown)	10 (9·2)
GII.5	3 (2·8)
GII.6	1 (0·9)
GII.7	1 (0·9)
GII.13	7 (6·4)
GII.unclassified	1 (0·9)
Total	109 (100)

norovirus-positive outbreak) was 84·9 ± 8·3 years (mean ± standard deviation), with a range of 61–98 years.

A total of 14 different ORF 1 norovirus genotypes were identified in the outbreaks, including GI.2, GI.3, GI.4, GI.7, GI.8, GI.a, GI.b, GII.3, GII.4 (2009), GII.16, GII.22, GII.b, GII.e and GII.g. The most common ORF 1 genotype detected was GII.e (83·7% of outbreaks of known ORF 1 genotype).

A total of 15 different ORF 2 norovirus genotypes were identified in the outbreaks (Table 1). Fourteen could be typed and, in addition, a GII norovirus sequence, not typable by the norovirus automated genotyping tool, was detected in a 90-year-old individual associated with a gastroenteritis outbreak in November 2013. A BLAST search indicated the sequence had 99% nucleotide identity with a 2013 Korean strain KF774001. The current strain has been lodged in GenBank as KM025343. The most common norovirus ORF 2 genotype detected was GII.4 (75·2% of outbreaks of known ORF 2 genotype) and a number of GII.4 variant forms were noted (Table 1, Fig. 1). The broad range of GI and GII norovirus capsid (ORF 2) genotypes identified in the study is represented visually in Fig. 1.

Although norovirus infection is recognized as an important cause of gastroenteritis in the elderly [1, 6] estimates of the incidence of norovirus infection in gastroenteritis outbreaks in aged-care facilities can vary widely. In the current study, which focused specifically on this issue, a figure of 65·0% was obtained. However, in a related study on gastroenteritis outbreaks (2010–2012) in aged-care facilities in France, Barret *et al.* [13] only found norovirus in 36·2% of the outbreaks tested. It is possible differences in sampling protocols between the current study and that of Barret *et al.* [13] may have been a factor; Barret *et al.* [13] tended to investigate outbreaks in larger institutions and also outbreaks with greater attack rates, whereas in the current study there was no such bias. It is worth noting that in a study of norovirus incidence in outbreaks in long-term care facilities in the USA in the period 2009–2010, Wikswo & Hall [14] found norovirus in 86% of outbreaks studied. Although this study did not specifically target aged-care facilities, Wikswo & Hall [14] suggested the outbreaks did include the elderly. In summary the combined data from these three studies suggest norovirus infection is an important factor in gastroenteritis outbreaks in aged-care facilities and therefore norovirus vaccination in the elderly could make an important contribution to reducing the burden of illness in this group.

A number of studies have recently examined the norovirus genotypes associated with gastroenteritis outbreaks in long-term care facilities, which would be expected to include the elderly. A consistent trend emerges – the GII.4 norovirus genotype predominates although a large number of other genotypes can be detected. For example Rosenthal *et al.* [15] examined norovirus genotypes in outbreaks in long-term care facilities in the USA in the period 2003–2006. Eight ORF 2 norovirus genotypes were detected, including GI.1, GI.4, GI.6, GII.3, GII.4, GII.5, GII.6, and GII.10, with GII.4 accounting for 84% of the outbreaks with known genotype. Vega *et al.* [16] examined the norovirus genotypes associated with gastroenteritis outbreaks in long-term care facilities in the USA for the period 2009–2013. Twenty-one norovirus genotypes were detected including GI.1, GI.2, GI.3, GI.4, GI.5, GI.6, GI.7, GI.9, GII.1, GII.2, GII.3, GII.4, GII.5, GII.6, GII.7, GII.12, GII.13, GII.14, GII.16, GII.17 and GII.21, with GII.4 accounting for 84·1% of outbreaks tested.

The current study differs from these two studies in that it focused exclusively on outbreaks in aged-care facilities, it was conducted in a different continent (Australia) and over a relatively short time period (one year). Nevertheless the findings are similar. Fifteen ORF 2 genotypes were detected including

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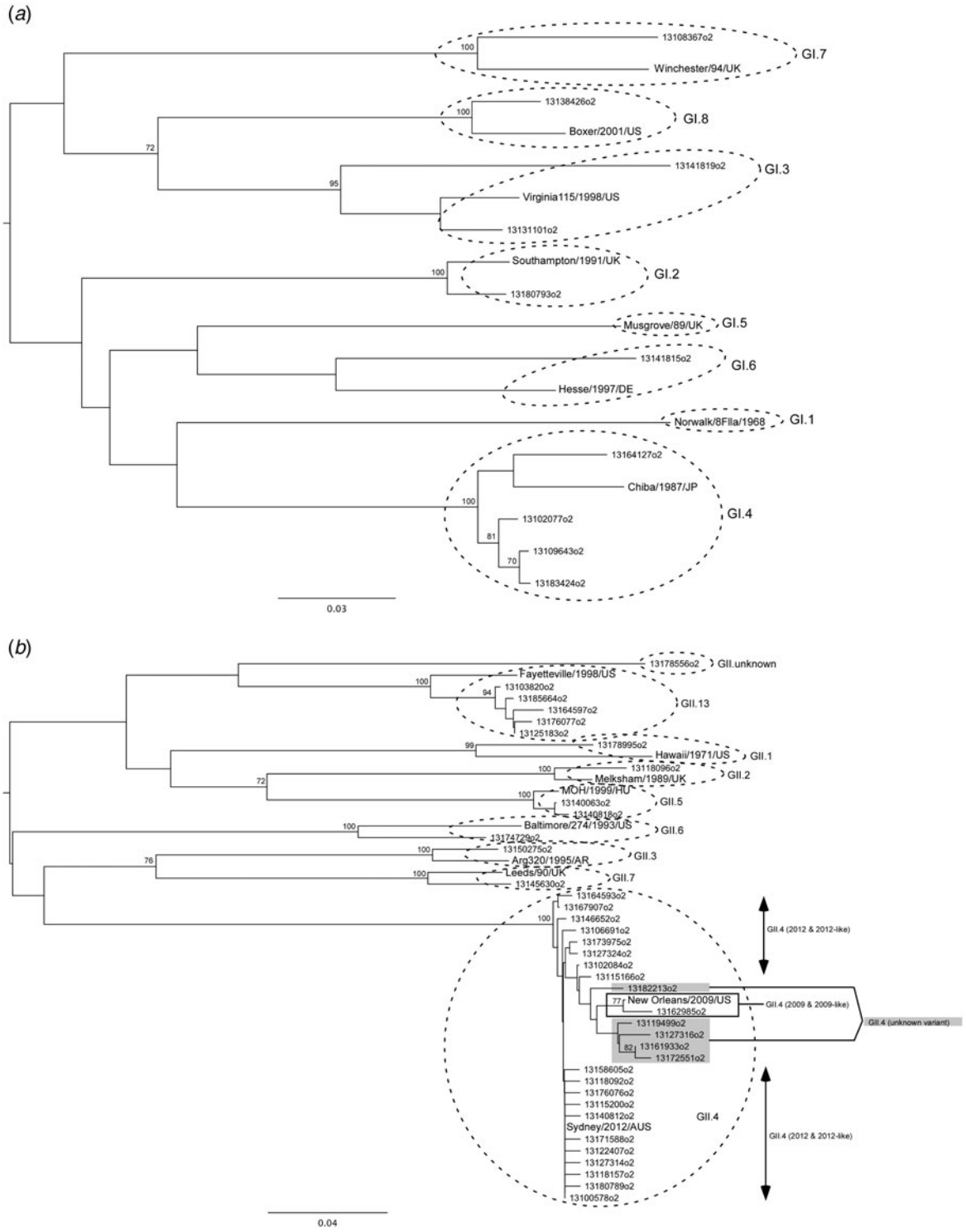

Fig. 1. DNA (dist) Kimura phylogenetic trees showing the relationship of ORF 2 GI (*a*) and GII (*b*) genotypes detected. The GI tree was based on an ORF 2 sequence 198 bp long and the GII tree was based on an ORF 2 sequence 195 bp long. Only unique sequences were included in the trees. The reference strains included in the trees were as follows: Norwalk/8FIIa/1968 (M87661), Southampton/1991/UK (L07418), Virginia115/1998/US (AY038598), Chiba/1987/JP (AB042808), Musgrove/89/UK (AJ277614), Hesse/1997/DE (AF093797), Winchester/94/UK (AJ277609), Boxer/2001/US (AF538679), Hawaii/1971/US (U07611), Melksham/1989/UK (X81879), Arg320/1995/AR (AF190817), MOH/1999/HU (AF397156), Baltimore/274/1993/US (AF414408), Leeds/90/UK (AJ277608), Fayetteville/1998/US (AY113106), NewOrleans/2009/US (GU445325), and Sydney/2012/AUS (JX459908). The figures on the branches represent bootstrap values (%) after resampling 1000 datasets. Only bootstrap values $\geqslant 70\%$ are shown. The scale represents substitutions per site.

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GI.2, GI.3, GI.4, GI.6, GI.7, GI.8, GII.1, GII.2, GII.3, GII.4, GII.5, GII.6, GII.7, GII.13, and an as yet unclassified GII genotype. As with the US studies, GII.4 was the predominant genotype, accounting for 75·2% of outbreaks with known genotype. Furthermore, the relatively short time period of the study emphasized the fact that many norovirus genotypes circulate throughout the community at roughly the same time.

Although GII.4 norovirus was the most common norovirus genotype detected in this and related studies [15, 16], the collective findings of the three reports indicate that norovirus vaccination strategies that target only GII.4 could exclude numerous other norovirus genotypes that infect the elderly. It is conceivable these other noroviruses could become more prevalent in the absence of GII.4, as vaccine-induced selective pressure may drive an increase in the non-vaccine genotypes. Ongoing surveys of norovirus genotypes in different settings are important for the development of meaningful vaccine strategies.

DECLARATION OF INTEREST

None.

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