

# Epidemic viral gastroenteritis in Queensland coincides with the emergence of a new norovirus variant

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## Abstract

Norovirus infections cause widespread morbidity and have significant economic impact on the community. An increase in outbreaks of norovirus gastroenteritis in hospitals, nursing homes and in the community was observed in Queensland in 2004. Molecular analysis of positive samples indicated the emergence of a single strain of norovirus. A 252 nucleotide sequence from the polymerase region (POL) was compared to sequences of the new variant genotype GII.4 that has caused epidemics in the Northern Hemisphere in 2002 and 2003. Sequence analysis indicated greater than 95 per cent similarity in the POL between the Queensland strain and the Northern Hemisphere 2002/3 GII.4 variant. Phylogenetic analysis revealed that the Queensland strain forms a branch within the GII.4 genotype separate from the 2002 variant from Europe and North America. Although norovirus genotype GII.4 had circulated in Queensland in the past, the 2004 strain was characterised specifically by three nucleotides not present in any other sequences held in our database covering the years 2002–June 2004. *Commun Dis Intell* 2005;29:370–373.

*Keywords: norovirus, epidemic, new variant*

## Introduction

It has long been recognised that noroviruses (previously called Norwalk-like viruses) are one of the common causes of outbreaks of acute non-bacterial gastroenteritis.<sup>1</sup> Members of the Family *Caliciviridae*, noroviruses are non-enveloped positive strand RNA viruses.<sup>2,3,4</sup> Noroviruses that infect humans cannot, presently, be grown in tissue culture or cultivated in suitable animal models. Noroviruses have a low infectious dose, are able to withstand harsh environmental conditions and can be spread by a variety of routes.<sup>5,6</sup> In 1993, Jiang and colleagues published the full length sequence of Norwalk virus.<sup>3</sup> Since then a myriad of different molecular detection and molecular analytical techniques have been developed and applied to a number of epidemiological studies.<sup>7,8</sup> The genome of 7.2–7.7 kb can be divided into three open reading frames; ORF 1, ORF 2 and ORF 3. Open reading frame 1 contains a RNA dependent RNA polymerase, whilst ORF 2 and ORF 3 code for a capsid protein and a minor structural protein respectively.

Norovirus strains have been traditionally named according to the geographical location from which they were first identified (e.g. Bristol, Toronto, Hawaii).<sup>9</sup> It has been proposed that noroviruses associated with human infection be divided into a number of genogroups namely GI, GII and GIV. These genogroups can then be further divided into at least 31 genotypes.<sup>10</sup>

The last decade has seen genogroup II, genotype 4 (GII.4) activity on a number of continents. In 1995–1996 a Lordsdale-like norovirus variant was demonstrated to have circulated worldwide. This strain predominated in different locations within the United States of America (USA) and was also demonstrated to have circulated in seven countries on five continents.<sup>11</sup> In 2002 and 2003 a large number of outbreaks of non-bacterial gastroenteritis occurred in North America and in Europe. The aetiological agent of the outbreaks was identified as norovirus.<sup>12,13</sup> This increase in norovirus activity occurred at the same time as the emergence of a new variant of GII.4 was identified. This variant had a major economic impact on cruise ships, hotels,

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nursing homes, hospitals and other public health facilities with a large number of staff, guests and residents of these facilities affected.<sup>14</sup>

At the beginning of 2004, Queensland experienced a large epidemic of viral gastroenteritis comprised of numerous outbreaks. These outbreaks were characterised by high attack rates and rapid spread throughout the community (Table). The virus was first observed circulating in the heavily populated south east corner of the state in January before spreading north to Cairns by June 2004. Virus activity was also reported in the west of the state with isolated outbreaks identified in Toowoomba and Biloela in June 2004. Outbreaks occurred in northern New South Wales including Lismore and Tweeds Heads in May 2004.

Institutional spread occurred despite the efforts of public health officials who provided advice on food handling and infection control procedures. The virus was responsible for ward closures in six Brisbane metropolitan public hospitals as well as a number of the larger private hospitals. Aged-care facilities were particularly hard hit. Sequence analysis of a proportion of samples revealed a single GII.4 variant was responsible for the recent outbreaks.

## Methods

### Sample collection

Eight hundred and fifty samples from private pathology laboratories,<sup>14</sup> public health units and public hospitals throughout Queensland were submitted to this laboratory for norovirus analysis between January 2004 and June 2004 representing 159 outbreaks of community acquired and institutional viral gastroenteritis (Table). Fifty-seven of these norovirus isolates separated chronologically and geographically were then selected for sequence analysis.

### RNA extraction and reverse transcriptase polymerase chain reaction protocol

Faecal samples were prepared as a 10 per cent suspension in phosphate buffered saline and centrifuged for one minute at 20,000 *g* to pellet solid material. Viral RNA was then extracted from 140  $\mu$ l

of the supernatant using the QIAamp Viral RNA Mini Kit (QIAGEN) according to the manufacturer's instructions. Reverse transcriptase polymerase chain reaction (RT-PCR) was used to amplify a 319 bp section of ORF 1 using primer pair p289/290, based on a modified method by Jiang, *et al.*<sup>15</sup> (personal communication). All products were analysed by gel electrophoresis.

### Nucleotide sequencing and phylogenetic analysis

Sequence of the amplified product generated from isolates was used to characterise the strain(s) responsible for the recent outbreaks. Fifty-seven positive samples from 41 hospital and nursing home outbreaks, and 11 community-acquired outbreaks were subjected to sequence analysis. Amplified PCR products of 252 nucleotides of the polymerase region (POL) were purified using QIAquick<sup>®</sup> Gel Extraction Kit according to the manufacturer's instructions (QIAGEN). Products were sequenced in both directions using Applied Biosystems ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kits. Sequences were compared to databases at the Centers for Disease Control and Prevention (USA) and the National Institute of Public Health and the Environment (Netherlands). Sequences were aligned using ClustalW. Phylogenetic analysis was performed and unrooted trees constructed using neighbour-joining.<sup>16</sup>

## Results

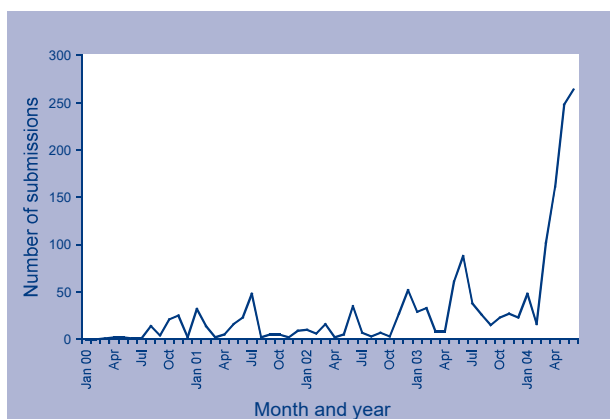
Queensland experienced higher rates of norovirus-related gastrointestinal illness in the first six months of 2004 than was usually the case for the previous three years (Table, Figure 1). This affected six major metropolitan public hospitals, six regional hospitals, a further 29 aged-care facilities, and, in addition, there was evidence of community spread. In the interest of public health we initiated a molecular epidemiological study of the epidemic.

A representative proportion of the norovirus PCR-positive samples were sequenced (57 of 97 positive samples). Analysis of the sequences obtained from a fragment of the POL region indicated that 95 per cent (55 of the 57 representative samples) belonged

**Table. Details of outbreaks of gastroenteritis linked to norovirus between 2002 and 2004**

Year	Number of samples	Number of samples positive for norovirus	Number positive (%)	Number of samples sequenced	Number of GII.4	Number of Queensland strain, 2004
2002	156	57	37	27	10	0
2003	387	121	31	61	32	0
January–June 2004	850	362	43	57	55	54

**Figure 1. Number of samples submitted for norovirus analysis between January 2000 and June 2004**



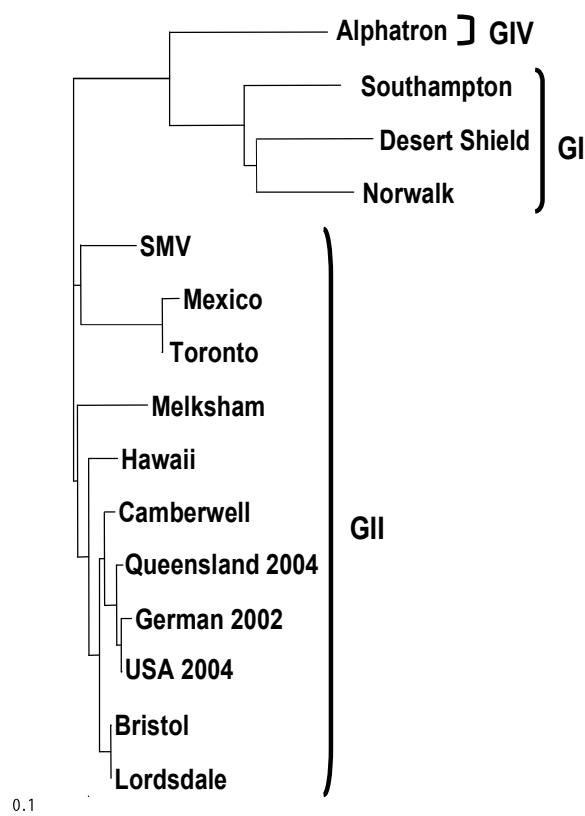
to genotype GII.4 (Figure 2). Two isolates were distinctly different from GII.4 and grouped in GII.8 and GI.2, respectively (data not shown). All of the 55 GII.4 isolates sequenced had sequence similarity of 99 per cent. More significantly, when these sequences were compared to GII.4 strains circulating in 2002 and 2003, differences were observed with these earlier strains. A three nucleotide signature sequence was identified which distinguished the recent strain from all previous GII.4 noroviruses isolated in Queensland. The Queensland strain had 95 per cent sequence identity to a strain circulating in the United States in 2002 (GenBank accession number AY502023). Phylogenetic analysis revealed that the Queensland strain forms a branch within the GII.4 genotype separate from the 2002 variant from Europe and North America (Figure 2).

**Discussion**

Norovirus infections will continue to cause significant illness in Australia and worldwide. This latest Queensland epidemic is significant because, by comparison with our in-house norovirus sequence database of previous years, it is apparent that a single genotypically homogenous strain has predominated. This observation is not due to increased reporting as surveillance activities by health authorities in 2004 have been no different to previous years.

Experience in the past has shown that generally outbreaks of norovirus infections have a variety of genotypic and phenotypic presentations. The Northern Hemisphere strain, with which the Queensland strain is closely related, has been associated with a severe pathology.<sup>13,14</sup> Interestingly, anecdotal evidence from clinicians and public health physicians indicated that the severity of symptoms of the recent Queensland strain was greater than has been previously seen (Dr Brad McCall, personal communication); however, we have no clinical data to support this observation.

**Figure 2. Phylogram indicating the relationship between norovirus strains\* based on 252 nucleotides in the RNA POL gene**



\* Strain identification and GenBank accession number in parentheses for human noroviruses listed according to a recently proposed classification system.<sup>(2,17)</sup> Proposed Genogroup IV Hu/NLV/Alphatron (personal communication Harry Vennema), Genogroup 1, Hu/NLV/Southampton 1991/UK (L07418), Hu/NLV/Desert Shield 395/1990/SA (U04469), Hu/NLV/Norwalk/8FIIa/1968/JP (M87661), Genogroup II, Hu/NLV/Snow Mountain/2003/US (AY134748), Hu/NLV/Mexico/1989/Mx (U22498), Hu/NLV/Melksham/1989/UK (X81879), Hu/NLV/Hawaii/1971/US (U07611), Hu/Camberwell/1994/AU (U46500), Hu/NoV/Brisbane/01/2004/AU (AY780432), Hu/NoV/Farmington Hills/2002/US (AY502023), Hu/NLV/GII/Langen1061/2002/DE (AY485642), Hu/NLV/Bristol/1993/UK (X76716), Hu/NLV/Lordsdale/1993/UK (X86557).

The cost to the community in terms of hospital ward closures, deferred medical interventions, extensive and repeated decontamination of aged facilities as well as lost productivity has been substantial.

In terms of improvements to future methodology, analyses including sequences from both the conserved capsid and POL regions would allow for a more accurate analysis in genotyping studies. In addition, we suggest epidemiological studies of norovirus outbreaks would be enhanced by standardisation of detection and genotyping procedures nationally, and mandatory reporting to a central database. This would be particularly useful for detection

of foodborne norovirus from foods such as imported oysters. Recently Vinjé and colleagues<sup>17</sup> reported on an international collaborative study to compare RT-PCR assays for the detection and genotyping of noroviruses. The laboratories participating in this study were located in the United States of America, the United Kingdom and Europe. Collaboration and standardisation of testing procedures with laboratories in the Southern Hemisphere and a central database could link researchers to provide an enhanced global surveillance system.

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