Journal of Clinical Virology 49 (2010) 265–271

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Norovirus GII.4 variant 2006b caused epidemics of acute gastroenteritis in Australia during 2007 and 2008

John-Sebastian Eden[a], Rowena A. Bull[a], Elise Tu[a,c], Christopher J. McIver[a,b,c], Michael J. Lyon[d], John A. Marshall[e], David W. Smith[f], Jennie Musto[g], William D. Rawlinson[a,b,c], Peter A. White[a,*]

[a] School of Biotechnology and Biomolecular Sciences, Faculty of Science, University of New South Wales, Sydney 2052, New South Wales, Australia
[b] School of Medical Sciences, Faculty of Medicine, University of New South Wales, Sydney, New South Wales, Australia
[c] Virology Division, Department of Microbiology, SEALS, Prince of Wales Hospital, Randwick, New South Wales, Australia
[d] Public Health Virology, Queensland Health Scientific Services, Brisbane, Queensland, Australia
[e] Victorian Infectious Diseases Reference Laboratory, North Melbourne, Victoria, Australia
[f] PathWest Laboratory Medicine, Queen Elizabeth II Medical Centre, Sir Charles Gairdner Hospital, Nedlands, Western Australia, Australia
[g] Communicable Diseases Branch, New South Wales Department of Health, North Sydney, New South Wales, Australia

ARTICLE INFO

Article history:
Received 17 May 2010
Received in revised form 27 August 2010
Accepted 1 September 2010

Keywords:
Norovirus (NoV)
Molecular epidemiology
Gastroenteritis
Reverse transcriptase polymerase chain reaction (RT-PCR)

ABSTRACT

Background: Over the last decade, four epidemics of norovirus-associated gastroenteritis have been reported in Australia. These epidemics were characterized by numerous outbreaks in institutional settings such as hospitals and nursing homes, as well as increases in requests for NoV testing in diagnostic centers. During 2007 and 2008, widespread outbreaks of acute gastroenteritis were once again seen across Australia, peaking during the winter months.
Objectives: The primary objective of this study was to characterize two winter epidemics of NoV-associated gastroenteritis in 2007 and 2008 in Australia. Following this, we aimed to determine if these epidemics were caused by a new GII.4 variant or a previously circulating NoV strain.
Study design: NoV-positive fecal samples ($n = 219$) were collected over a 2-year period, December 2006 to December 2008, from cases of acute gastroenteritis in Australia. NoV RNA was amplified from these samples using a nested RT-PCR approach targeting the 5′ end of the capsid gene, termed region C. Further, characterization was performed by sequence analysis of the RdRp and capsid genes and recombination was identified using SimPlot.
Results: From 2004 to 2008, peaks in the numbers of NoV-positive EIA tests from the Prince of Wales Hospital Laboratory correlated with the overall number of gastroenteritis outbreaks reported to NSW Health, thereby supporting recent studies showing that NoV is the major cause of outbreak gastroenteritis. The predominant NoV GII variant identified during the 2007–2008 period was the GII.4 pandemic variant, 2006b (71.51%, 128/179), which replaced the 2006a variant identified in the previous Australian epidemic of 2006. Four novel GII variants were also identified including the three GII.4 variants: NoV 2008, NoV Osaka 2007 and NoV Cairo 2007, and one novel recombinant NoV designated GII.e/GII.12.
Conclusion: The increase in acute gastroenteritis outbreaks in 2007 and 2008 were associated with the spread of the NoV GII.4 variant 2006b.

1. Background

Norovirus (NoV) is the leading cause of outbreaks of viral gastroenteritis worldwide[1,2] and is also considered a significant cause of sporadic cases of diarrhea in the community.[3,4] NoV transmission occurs primarily from person-to-person, however, transmission through contaminated food and water are also well-documented.[5–7] The high number of NoV-associated outbreaks of gastroenteritis in hospitals and nursing homes highlights the increased risk of transmission within semi-closed environments.[8,9] Furthermore, viral shedding is not limited to the symptomatic phase of the illness and has been reported in pre-symptomatic, post-symptomatic and even asymptomatic individuals.[10–12]

NoV, a member of the *Caliciviridae* family, was originally identified by its small round virion of approximately 27–35 nm in diameter.[13,14] NoV has a single-stranded, positive-sense, polyadenylated RNA genome of approximately 7.5 kilobases, which is organized into three open-reading frames (ORF).[15] The first ORF encodes for six non-structural proteins including the viral RNA-dependent RNA polymerase (RdRp), whilst the second ORF

* Corresponding author. Tel.: +61 9385 3780, fax: +61 9385 1483.
E-mail address: p.white@unsw.edu.au (P.A. White).

1386-6532/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.jcv.2010.09.001

Table 1
Summary of NoV-positive specimens analyzed in this study.

Location[a]	Date of collection	No. of confirmed outbreaks	No. of NoV specimens tested	No. sequenced[b]	NoV genotype(s) and prevalence[c]	
					Genotype[d]	% of isolates from region
NSW	December 2006–December 2008	28	127	100	GII.4 2006b	73.0
					GII.6	7.0
					GII.4 2008	6.0
					GII.b/GII.3	5.0
					GII.4 2006a	3.0
					GII.7	3.0
					Other GII.4	2.0
					GII.e/GII.12	1.0
QLD	January–September 2007	30	30	30	GII.4 2006b	73.3
					GII.4 2006a	23.3
					GII.b/GII.3	3.3
VIC	October–November 2007	21	21	20	GII.4 2006b	90.0
					GII.4 2006a	10.0
WA	January–August 2007	2	41	29	GII.4 2006b	51.7
					GII.4 2006a	41.4
					GII.b/GII.3	3.4
					Other GII.4	3.4
	Total	81	219	179		

[a] NSW, New South Wales; QLD, Queensland; VIC, Victoria; WA, Western Australia.
[b] Region sequenced included 266 bp of the capsid gene (see Section 3).
[c] Genotype classification based on Zheng et al. 2006.[17]
[d] For recombinant NoVs. RdRp genotype shown on left and capsid genotype on right as Bull et al. 2007.[34]

encodes VP1, the capsid protein and the third ORF encodes a minor structural protein, VP2.[16] NoVs are classified into five genogroups (GI–GV), but only GI, GII and GIV are known to infect humans, with GII the most prevalent and diverse.[17] Genogroups are further divided into more than 30 genotypes and are designated, for example, as GII.1 (genogroup II, genotype 1).[17,18] There is typically 15% divergence between genotypes and 45% between genogroups, based on full-length capsid amino acid sequences.[17]

In the last decade, increasing frequencies of epidemics of NoV-associated gastroenteritis have been described.[19–21] A single genotype, GII.4, has emerged as the major cause of NoV pandemics, with five distinct pandemic variants identified, including US-95/96,[20] Farmington Hills,[22] Hunter,[19] and the 2006a and 2006b variants.[21] Overwhelming evidence now demonstrates that gastroenteritis pandemics are preceded with the emergence of a new GII.4 variant.[19,21,23]

2. Objectives

In the winter periods, June–August, of 2007 and 2008, increases in gastroenteritis activity were once again reported across Australia. Therefore, in this study we investigated the cause of the 2007 and 2008 NoV epidemics in Australia to determine if a new GII.4 variant had once again emerged from the dominant GII.4 pandemic lineage.

3. Study design

3.1. Identification of NoV-associated outbreaks of acute gastroenteritis in NSW, Australia

The number of institutional gastroenteritis outbreaks reported to NSW Health, NSW Department of Health, was collated for the period January 2004–December 2008. The reported institutional gastroenteritis outbreak data was then compared to the total number of NoV-positive samples detected by the South Eastern Area Laboratory Service (SEALS), Prince of Wales Hospital (POWH), Sydney for the same time period using GraphPad Prism v5 (GraphPad Software, San Diego, CA, USA).

3.2. Stool specimens

In total, 219 NoV-positive stool specimens from cases of acute gastroenteritis were collected from diagnostic public health laboratories in NSW, Queensland (QLD), Victoria (VIC), and Western Australia (WA) during the period from December 2006 through December 2008 (see Table 1 for details).

3.3. Sample preparation, RNA extraction and detection of NoV GII RNA

Stool specimens were prepared as 20% suspensions as described previously.[21] Viral RNA was extracted using the QIAamp Viral RNA Mini kit (Qiagen, Hilden, Germany). A 311 bp region of the NoV GII capsid gene (VP1), was amplified from extracted RNA with a real-time, nested RT-PCR approach using a MyiQ Single-Color real-time PCR detection system (Bio-Rad, Hercules, CA, USA) as described previously[21] for all samples except those from QLD which were amplified using the primers p289/290.[24]

3.4. Amplification of the full-length RdRp and capsid genes

Reverse transcription (RT) was performed on 10 μl of extracted RNA using the Superscript VILO cDNA synthesis kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Full-length capsid genes were PCR amplified with the primers NV2oF2[19] and GV132.[21] Full-length RdRp encoding regions were also amplified with the primers GV22 and GV6.[19] For each RT-PCR, 35 cycles of amplification was performed with High Fidelity Platinum Taq DNA polymerase (Invitrogen) according to the manufacturer's instructions.

3.5. Recombinant identification

Potential recombinants were investigated by amplifying the region across the ORF1–ORF2 overlap using a nested RT-PCR approach. The first round primers were Hep170[19] and NV2oR,[19] and then second round primers were Hep172[19] and G2SKR.[25]