Annual reports

Australian Rotavirus Surveillance Program annual report, 2012

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Abstract

This report from the Australian Rotavirus Surveillance Program, together with collaborating laboratories Australia-wide, describes the rotavirus genotypes responsible for the hospitalisation of children with acute gastroenteritis during the period 1 January to 31 December 2012. During the survey period, 1,300 faecal samples were referred to the centre for rotavirus G and P genotype analysis, and of these 748 were confirmed as rotavirus positive. A total of 491 specimens were collected from children under 5 years of age, while 257 were from older children and adults. Genotype analysis revealed that G1P[8] was the dominant type in this reporting period, identified in 35% of strains nationally. Genotype G2P[4] was the second most common strain nationally, representing 28% of samples, followed by genotype G12P[8] (23%). This represents the first report where G12P[8] strains are a major cause of disease in this community. Fluctuations in genotype distribution were also observed based on the vaccine type in use. Genotype G2P[4] was more common in states and territories using Rotarix while G1P[8] was more common in states using RotaTeq. This survey of rotavirus strains circulating in 2012 highlights the continued fluctuations in rotavirus genotypes, with an annual change in dominant genotypes as well as emergence of a previously rare genotype, suggesting a dynamic wild-type population. *Commun Dis Intell* 2014;38(1):E29–E35.

Keywords: rotavirus, gastroenteritis, genotypes, disease surveillance

Introduction

Rotavirus is the major viral cause of severe diarrhoea in young children in all countries worldwide.[1] Rotaviruses belong to the Reoviridae family. They contain 11 segments of dsRNA that encode the 6 structural and 6 non-structural proteins. The significant morbidity and mortality associated with rotavirus infection led to the development of two live oral rotavirus vaccines Rotarix® (GlaxoSmithKline) and RotaTeq® (Merck). Large clinical trials with each vaccine have shown both to be safe and highly effective in the prevention of severe diarrhoea and hospitalisation due to rotavirus infections.[2,3]

Both rotavirus vaccines were included on the National Immunisation Program in Australia free of charge for all infants from 1 July 2007. Each state or territory selected one vaccine for use. RotaTeq is administered in Victoria, South Australia, Western Australia and Queensland, while Rotarix is administered in New South Wales, the Northern Territory, Tasmania and the Australian Capital Territory. Historically, rotavirus infection accounted for up to 10,000 childhood hospitalisations for diarrhoea each year in Australia.[4] The introduction of rotavirus vaccines has seen a significant impact on the disease burden, with national data showing a substantial decline in both rotavirus coded and non-rotavirus coded hospitalisations for diarrhoea since vaccine introduction.[5] State based studies in New South Wales, Queensland, South Australia and Victoria also reported major declines in hospitalisation and emergency room visits since vaccine introduction.[6–8]

The Australian Rotavirus Surveillance Program has reported annual changes in genotypes in the Australian population since 1997, with temporal and geographic changes observed each year.[9] The diversity of rotavirus strains capable of causing disease in children, and the patterns of emergence and circulation provide the baseline information vital to assist vaccine introduction and ongoing evaluation.

The introduction of rotavirus vaccines has increased population immunity to wild-type rotavirus strains. This in turn is likely to impact on the epidemiology of circulating strains. Therefore, investigation of circulating rotavirus genotypes will provide insight into whether vaccine introduction has impacted on virus epidemiology, and provide findings of the consequences of vaccination programs.

This report describes the genotype characterisation of rotavirus strains causing severe gastroenteritis in Australia for the period 1 January to 31 December 2012.

Methods

Rotavirus positive specimens detected by enzyme immunoassay (EIA) or latex agglutination in 21 collaborating laboratories across Australia were collected, stored frozen and forwarded to the National Rotavirus Reference Centre Melbourne, together with relevant age and sex details.

Viral RNA was extracted from a 10%–20% faecal extract prepared for each specimen using the QIAamp Viral RNA mini extraction kit (Qiagen) according to the manufacturer's instructions. The rotavirus G and P genotypes were determined for each sample by application of independent hemi-nested multiplex reverse transcription-polymerase chain reaction (RT-PCR) assays. The first round RT-PCR assays were performed using the Qiagen one step RT-PCR kit, using VP7 conserved primers VP7F and VP7R, or VP4 conserved primers VP4F and VP4R. The second round genotyping PCR reactions were conducted using specific oligonucleotide primers for G types 1, 2, 3, 4, 8, 9 and 12 or P types [4], [6], [8], [9], [10] and [11].[9–14] The G and P genotype of each sample was assigned using agarose gel analysis of second round PCR products.

Any samples that provided a discordant result between the initial antigen detection and genotype assay were further tested using the commercial rotavirus enzyme linked immunosorbent assay ProSpecT (Oxoid, UK), as per manufacturer's instructions to confirm the presence of rotavirus antigen.

Results

Number of isolates

During the period 1 January to 31 December 2012, a total of 1,300 specimens were received for analysis from 16 collaborating centres across Australia; located in Victoria, Western Australia, the Northern Territory, New South Wales, Queensland, South Australia, Tasmania and the Australian Capital Territory.

There were 748 samples confirmed as rotavirus positive by EIA (ProSpecT, OXOID) or RT-PCR analysis. Of these, 491 samples were collected from children under 5 years of age, and 257 samples were from older children and adults. An additional 552 specimens contained either insufficient specimen for genotyping (n=36), or the specimens were not confirmed to be positive for rotavirus (n=516) and were not analysed further.

Age distribution

In the current survey period, a total of 663 rotavirus positive specimens had patient age data available. In the cohort of children aged 5 years or less (n=402), 24.2% of cases were from infants 0–6 months of age, 12.3% were from infants 7–12 months of age, 29.1% from infants 13–24 months of age, 18.5% from infants 25–36 months of age, 6.2% from children 37–48 months of age and 8.9% from children 49–60 months of age.

There were 261 samples from older children and adults, 129 samples were obtained from children 5–10 years of age, 11 were from individuals 10–20 years of age, 91 were from individuals 21–80 years of age, and 30 were from individuals 80 years or older.

Genotype distribution

G1P[8] strains were the most common type identified nationally, representing 35% of all specimens (Table). This strain was identified in all states and territories and was the dominant type in the Northern Territory, Queensland and Victoria, representing between 33% and 65% of strains. It was also equally dominant in South Australia.

G2P[4] strains were the second most common genotype identified nationally, representing 28% of all specimens analysed. It was identified in 7 states and territories, and was the dominant type in New South Wales and the Australian Capital Territory and equal with G1P[8] in South Australia.

G12P[8] strains were the third most common genotype nationally, being identified in 4 states and territories representing 23% of strains. It was the dominant type in Western Australia, representing 43% of strains, and second most common type in the Northern Territory.

G3P[8], G4P[8] and G9P[8] strains each represented 5% or less of the total specimens genotyped (Table). Several rare or unusual genotypes were identified including a single G9P[4] strain identified in Queensland, a single G8P[nt] identified in South Australia. Strains which resembled a component of the RotaTeq vaccine were identified on 6 occasions from Western Australia, Victoria and South Australia. In addition, faecal specimens were received from 28 children who developed rotavirus gastroenteritis after being vaccinated with either RotaTeq or Rotarix. RotaTeq vaccine virus was identified in seven of these cases by RT-PCR and VP6 sequence analysis.

Sixteen samples contained multiple G and/or P genotypes, or a non-typeable G or P genotype. The non-typeable samples are likely to be samples that contain low virus levels, below the limits of our typing assays, or could have contained inhibitors in extracted RNA to prevent the function of the enzymes used in RT and/or PCR steps.

There were 261 confirmed rotavirus samples collected from older children and adults from 7 locations; New South Wales, the Northern Territory, Queensland, Western Australia, South Australia, Victoria and the Australian Capital Territory. The majority of these samples were collected from New

Table: Rotavirus G and P genotype distribution in Australian children ≤ 5 years, 1 January to 31 December 2012

Centre	Type total	G1P[8] %	G1P[8] n	G2P[4] %	G2P[4] n	G3P[8] %	G3P[8] n	G4P[8] %	G4P[8] n	G9P[8] %	G9P[8] n	G9P[4] %	G9P[4] n	G8P[14] %	G8P[14] n	G10P[14] %	G10P[14] n	G12P[8] %	G12P[8] n	Mix* %	Mix* n	G1P[6] %	G1P[6] n	Non-type† %	Non-type† n	Vaccine (RotaTeq) %	Vaccine (RotaTeq) n	Neg n	Insuff n
Australian Capital Territory																													
ACT	3	0	0	100	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	N/A	N/A	2	0
New South Wales																													
Sydney (POW)	20	10	2	80	16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	2	N/A	N/A	0	0
Sydney (Westmead)	58	7	4	93	54	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	N/A	N/A	1	0
Newcastle John Hunter	28	0	0	100	28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	N/A	N/A	19	0
Northern Territory																													
Alice Springs	23	30	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	61	14	0	0	0	0	9	2	N/A	N/A	18	6
Darwin	27	74	20	4	1	22	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	N/A	N/A	3	0
Western Diagnostic (NT)	2	50	1	0	0	50	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	N/A	N/A	0	0
PathWest WA	13	92	12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	1	0	0	0	0	0	0	N/A	N/A	0	1
Queensland																													
Pathology (Brisbane)	12	83	10	8	1	8	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	44	1
Qld Regional	10	50	5	20	2	10	1	0	0	10	1	0	0	0	0	0	0	10	1	0	0	0	0	0	0	0	0	63	0
Pathology – Townsville	7	71	5	0	0	0	0	0	0	0	0	14	1	0	0	0	0	14	1	0	0	0	0	0	0	0	0	11	0
Pathology – -Gold Coast	1	0	0	0	0	0	0	0	0	100	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7	0
South Australia																													
Adelaide	73	29	21	29	21	18	13	3	2	0	0	0	0	0	0	0	0	15	11	3	2	0	0	3	2	1	1	15	2
Tasmania																													
Hobart	1	100	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	N/A	N/A	3	0
Victoria																													
Melbourne Pathology	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14	1
RCH	10	30	3	20	2	10	1	20	2	0	0	0	0	0	0	0	0	0	0	10	1	0	0	0	0	10	1	74	12
Monash	6	50	3	33	2	17	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0
Western Australia																													
PathWest WA	161	40	64	4	7	2	3	4	6	2	3	0	0	0	0	0	0	42	67	1	2	0	0	3	5	2	4	8	2
Perth	36	42	15	3	1	0	0	3	1	3	1	0	0	0	0	0	0	50	18	0	0	0	0	0	0	0	0	60	0
Total	491	35	173	28	138	5	27	2	11	1	6	0	1	0	0	0	0	23	113	1	5	0	0	2	11	1	6	344	25

A total of 369 samples were omitted from analysis due to insufficient sample volume or sample was not confirmed as rotavirus positive.

Rotavirus vaccine, either Rotarix or RotaTeq, was identified in an additional 7 vaccinated infants, in whom samples were forwarded directly to ARSP directly from clinicians for analysis

Mix

PathWest, Western Australia: 1x G1/4P[8]; 1x G1/12P[8]

South Australia: 1x G1/3P[8]; 1x G3/9P[8]

RCH, Victoria: 1x G1/3/4P[8]

Non-typeables

Alice Springs, Northern Territory: 1x G1P[nt]; 1x GntP[nt]

PathWest, Western Australia: 3x G-ntP[8]; 2x G1P[nt],

Adelaide, South Australia: 1x G8P[nt]; 1x GntP[nt]

POW, New South Wales: 2x GntP[nt]

South Wales (n=72), Western Australia (n=77), South Australia (n=61) and Queensland (n=24). Genotype analysis of the rotavirus samples from older individuals showed a similar distribution to that observed in young children.

In New South Wales, the majority of the specimens were associated with G2P[4] in children 5–10 years of age. While in Western Australia and South Australia the majority of samples were G12P[8] or G2P[4] respectively, the same as the dominant type in children 5 years of age or younger in each location. A single G3P[14] strain was identified in an 11-year-old child from Victoria.

Analysis of G and P genotyping results revealed that in states where RotaTeq is in use, G1P[8] was the dominant genotype, identified in 40.6% of strains, while G12P[8] was second, identified in 31.6% of strains (Figure). G2P[4] was third most common representing 11.6% of strains. In states using Rotarix, G2P[4] strains were dominant (58.3%), while G1P[8] strains comprised 26.8% of specimens, and G12P[8] was identified in 8.6%. G3P[8] strains were identified at similar rates in both settings. G4P[8] and G9P[8] were only identified in states using RotaTeq vaccine, however, both represented minor types.

There appears to be consistency in genotype distribution within each vaccine type, for example, in 3 of the 4 RotaTeq states (Queensland, Victoria and South Australia), G1P[8] was the dominant type, and was second most common in the remaining location (Western Australia). Similarly, G2P[4] was dominant in 2 of the 3 states using Rotarix (New South Wales and the Australian Capital Territory).

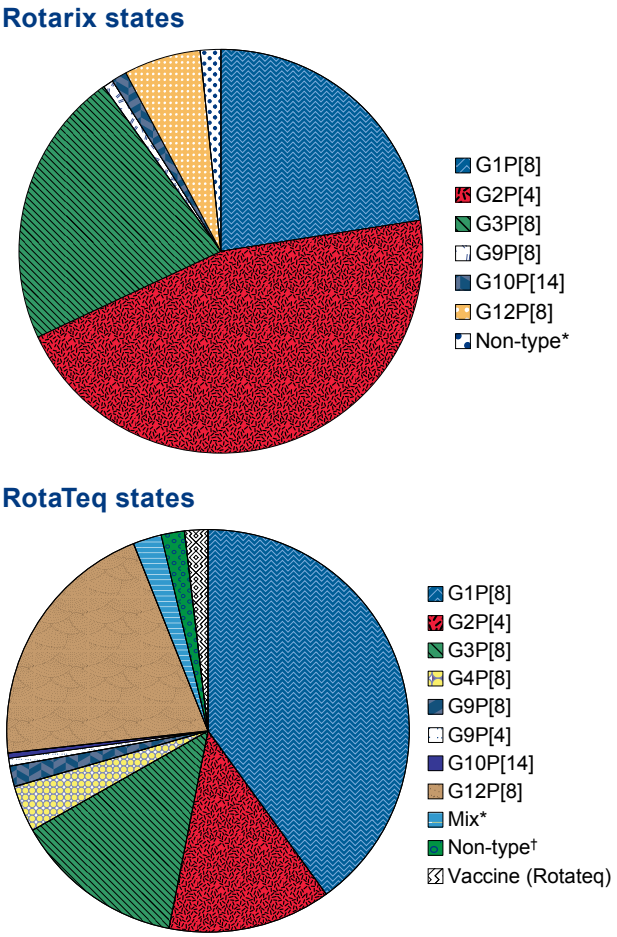
Discussion

The Australian Rotavirus Surveillance Program report for the period 1 January to 31 December 2012 describes the annual distribution of rotavirus genotypes and geographic differences in genotypes causing disease in Australian children.

The surveillance program identified that genotype G1P[8] emerged as the dominant genotype nationally, representing 35% of all strains. Genotype G2P[4] was the second predominant type nationally, comprising 28% of all strains. Genotype G12P[8] represented the third most common genotype, representing more than 23% of strains nationally. As observed in the previous post-vaccine years, the dominant genotype continued to fluctuate on a yearly basis, with G1P[8] and G2P[4] constantly alternating.[15–17] In the 11 years pre-vaccine introduction, G1P[8] was the dominant type in 8 of the 11 rotavirus seasons.[9] In each of the post vaccine years, the dominant type represented 50% of strains; however, this year the dominant genotype represented only 35% of strains across the country. This may be explained in part by the third most common genotype in this report representing more than 20% of strains (G12P[8]), when in past years the third most common strains fluctuated between 5% and 12%.

This report represents the first occasion that G12P[8] strains have been a major cause of disease

Figure: Overall distribution of rotavirus G and P genotypes identified in children, Australia, 1 January to 31 December 2012, by vaccine usage



* Mix

PathWest, Western Australia: 1x G1/4P[8]; 1x G1/12P[8]

South Australia: 1x G1/3P[8]; 1x G3/9P[8]

RCH, Victoria: 1x G1/3/4P[8]

† Non-typeables

Alice Springs, Northern Territory: 1x G1P[nt]; 1x GntP[nt]

PathWest, Western Australia: 3x G-ntP[8]; 2x G1P[nt],

Adelaide, South Australia: 1x G8 P[nt]; 1x GntP[nt]

POW, New South Wales: 2x GntP[nt]

Rotarix was used in New South Wales, Tasmania, and the Northern Territory. RotaTeq was used in Victoria, South Australia, Western Australia and Queensland.

in Australian children. Previously, G12 strains have been identified in a small outbreak in New South Wales during 2005, and as single isolates in Melbourne and Sydney in 2006–07.[18] The emergence of G12P[8] strains in 4 locations in 2012 represents the largest distribution of this genotype observed in Australia. Globally, G12 strains represent a minor type, identified in less than 2% of all strains genotyped from 2003 to 2007[19] G12 strains were recently identified in Belgium in 2007–08, the second season after vaccine introduction.[20] In a recent efficacy trial in South Africa and Malawi, Rotarix was shown to provide comparable protection against a range of circulating genotypes including G12 strains.[21] Both rotavirus vaccines are likely to be effective against the emergence of G12P[8] strains.

The use of different vaccines in Australian states and territories provides a unique opportunity to compare the effect of each vaccine on the circulating wild-type strains. Differences in genotype distribution have been observed during the past 5 years. During years 1 and 2, G2P[4] strains were more common in states and territories using Rotarix, while G3P[8] were more common in RotaTeq locations. During years 3 and 4, the pattern changed such that G2P[4] strains were more common in states using RotaTeq, while G1P[8] strains were more common in locations using Rotarix. G3P[8] remained more common in RotaTeq states only in year 3, after which they occurred at similar rates in years 4 and 5. In the current survey, (year 5), the occurrence of G2P[4] reverted to that observed in years 1 and 2, being more common in locations using Rotarix.[15,17] Thus differences were evident in genotype distribution, but there was no consistent genotype distribution linked to a particular vaccine.

The worldwide interest in uncommon rotavirus genotypes continues because of the possible impact they could have on rotavirus vaccine programs. In previous years, uncommon VP7/VP4 genotype combinations have been identified, and this year the emergence of G12 further highlights this emergence of different genotypes. In 2011, G10P[14] strains were identified in the Northern Territory causing acute gastroenteritis in 5 infants and 1 adult. This represented the first report of this genotype combination in Australia. Full genome sequence analysis identified that the virus was likely the result of an *Artiodactyl*-to-human interspecies transmission.[22] Whether the introduction of vaccine is exerting an increase on immune pressure or simply natural variation is still unclear, but the identification of G10 and G12 strains strengthens the need to continue rotavirus surveillance in both humans and animals.

This report again details a significant number of rotavirus positive samples in older children and adults. A large rotavirus outbreak caused by G2P[4] occurred in New South Wales in 2012, occurring predominantly in children aged 5–9 years. The rates of gastroenteritis in this age group were significantly higher levels than in previous years (J Musto, personal communication). This report also continues the previous reports of an increase in adult rotavirus cases observed in South Australia and Western Australia, as well as in other locations.[23] A reduction in circulating virus in the post-vaccine era may have led to a decrease in protection from rotavirus in older unvaccinated children or adults.

This survey of rotavirus strains causing disease between 1 January and 31 December 2012 highlights the continued fluctuations in rotavirus genotypes across Australia. However, the genotype patterns continue to change on an annual basis and illustrate a more dynamic wild-type population than observed in the pre-vaccine era. This suggests that vaccine pressure may be speeding up the selection process. This is supported by the observation of G12P[8] strains and cases in older children and adults. Therefore, on-going surveillance of the wild-type strains circulating in Australia is required to monitor any changes that may emerge and impact vaccine effectiveness.

Acknowledgements

The Rotavirus Surveillance Program is supported by grants from the Australian Government Department of Health, GlaxoSmithKline and CSL. Dr Kirkwood is supported by a CDA Fellowship, National Health and Medical Research Centre.

We thank H Tran for providing technical assistance.

Rotavirus positive specimens were collected from numerous centres throughout Australia. The significant time and effort involved in the collection, storage, packaging, compiling data and forwarding of specimens was much appreciated.

The participating laboratories for this sampling period were:

- Princess Margaret Hospital for Children, Subiaco, Western Australia
- Division of Microbiology, PathWest LM, Western Australia
- Queen Elizabeth Medical Centre, Nedlands, Western Australia
- Microbiology Department, Royal Darwin Hospital, Casuarina, Northern Territory

- Department of Microbiology, Western Diagnostic Pathology, Northern Territory and Western Australia
- Microbiology Department, Alice Springs Hospital, Alice Springs, Northern Territory
- Virology Division, SEALS, Prince of Wales Hospital, New South Wales
- Microbiology Department, The Children's Hospital at Westmead, New South Wales
- Centre for Infectious Diseases and Microbiology, Sydney, New South Wales
- Microbiology Department, John Hunter Hospital, Newcastle, New South Wales
- Forensic and Scientific Services, Queensland Health Herston, Queensland
- Pathology Queensland, Herston, Queensland
- Queensland Paediatric Infectious Diseases Laboratory, Queensland
- Royal Children's Hospital, Brisbane, Queensland
- Queensland Health laboratories in Townsville, Cairns and Gold Coast, Queensland
- Virus Laboratory Institute of Medical and Veterinary Science, Adelaide, South Australia
- Royal Hobart Hospital and the Communicable Disease Prevention Unit, Tasmania
- Department of Health and Human Services, Hobart, Tasmania
- ACT Pathology, Canberra, Australian Capital Territory
- Virology Department, Royal Children's Hospital, Parkville, Victoria
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