Australian Rotavirus Surveillance Program annual report, 2015

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Abstract

The Australian Rotavirus Surveillance Program, together with collaborating laboratories Australia-wide, reports the rotavirus genotypes responsible for the hospitalisation of children with acute gastroenteritis during the period 1 January to 31 December 2015. During the survey period, 1,383 faecal samples were referred for rotavirus G and P genotype analysis, and of these, 1,031 were confirmed as rotavirus positive. A total of 634 specimens had been collected from children under 5 years of age, while 397 were from older children and adults. Genotype analysis of samples from both children and adults revealed that G12P[8] was the dominant genotype in this reporting period, identified in 48.2% of strains nationally. Genotype G3P[8] was the second most common strain nationally, representing 22.8% of samples, followed by G2P[4] and G1P[8] (9% and 8% respectively). G3P[8] was further divided as equine-like G3P[8] (13.2% of all strains) and other wild-type G3P[8] (9.6%). This report highlights the continued predominance of G12P[8] strains as the major cause of disease in this population. Genotype distribution was distinct between jurisdictions using RotaTeq and Rotarix vaccines. Genotype G12P[8] was more common in states using RotaTeq, while equine-like G3P[8] and G2P[4] were more common in the states and territories using Rotarix. This survey highlights the dynamic change in rotavirus genotypes observed since vaccine introduction, including the emergence of a novel equine-like G3P[8] as a major strain. The prolonged dominance of G12P[8] for a 4th consecutive year further illustrates the unexpected trends in the wild type rotaviruses circulating in the Australian population since vaccine introduction. *Commun Dis Intell* 2016;40(4):E527–E538.

Keywords: rotavirus, gastroenteritis, genotypes, disease surveillance

Introduction

Rotaviruses belong to the Reoviridae family and are triple layered dsRNA viruses that contain a segmented genome, consisting of 11 gene segments that encode 6 structural proteins and 6 non-structural proteins.[1] Rotaviruses are the most common cause of severe diarrhoea in young children worldwide, and are estimated to cause up to 453,000 deaths annually.[2] The significant morbidity and mortality associated with rotavirus infection has led to the development of vaccines, such as Rotarix® (GlaxoSmithKline) and RotaTeq® (Merck). These 2 oral live attenuated rotavirus vaccines have been shown to be safe and highly effective in the prevention of severe diarrhoea due to rotavirus infection,[3,4] leading to both vaccines being licensed in over 125 countries and included in the national vaccination schedules of 63 predominantly high and middle-income countries worldwide.[5] In Australia, rotavirus vaccines have been included in the National Immunisation Program since 1 July 2007, with excellent uptake in subsequent years. RotaTeq is administered in Queensland, South Australia, Victoria and Western Australia, while Rotarix is administered in the Australian Capital Territory, New South Wales, the Northern Territory and Tasmania.[6]

In Australia prior to vaccination being introduced, rotavirus infection had accounted for up to 10,000 childhood hospitalisations for diarrhoea each year.[7] A significant impact on the disease burden has been observed since vaccine introduction, with studies showing a substantial decline in both rotavirus coded and non-rotavirus coded hospitalisation and emergency room visits.[6,8–12]

The Australian Rotavirus Surveillance Program has characterised the G- and P- genotypes of rotavirus strains causing severe disease in Australian children since 1997. Data from this surveillance has shown that strain diversity as well as temporal and geographic changes occur each year; providing critical baseline data.[13] Ongoing characterisation of circulating rotavirus genotypes in the vaccine era will provide insight into whether vaccine introduction has impacted on virus epidemiology, and altered circulating strains, which could have ongoing consequences for the success of current and future vaccination programs.

This report describes the genotype distribution of rotavirus strains causing severe gastroenteritis in Australia for the period 1 January to 31 December 2015.

Methods

Rotavirus positive specimens detected by enzyme immunoassay (EIA) or latex agglutination in collaborating laboratories across Australia were

collected, stored frozen and forwarded to the Australian Rotavirus Reference Centre Melbourne, together with relevant age and sex details. The laboratories contributing samples were:

- ACT Pathology, Canberra, Australian Capital Territory
- The Virology Division, South Eastern Area Laboratory Services, Prince of Wales Hospital, New South Wales
- Virology Department, The Children's Hospital at Westmead, New South Wales
- Centre for Infectious Diseases and Microbiology, Westmead, New South Wales
- The Microbiology Department, John Hunter Hospital, Newcastle, New South Wales
- The Microbiology Department, Royal Darwin Hospital, Casuarina, Northern Territory
- The Microbiology Department, Alice Springs Hospital, Alice Springs, Northern Territory
- Forensic and Scientific Services, Queensland Health, Herston, Queensland
- Microbiology division, Pathology Queensland, Herston, Queensland
- The Queensland Paediatric Infectious Diseases laboratory, Royal Children's Hospital, Brisbane, Queensland
- Queensland Health laboratory in Townsville, Queensland
- Microbiology and Infectious Diseases Laboratory, SA Pathology, Adelaide, South Australia
- The Serology Department, Royal Children's Hospital, Parkville, Victoria
- Department of Microbiology, Monash Medical Centre, Clayton, Victoria
- Division of Microbiology, PathWest LM, The Queen Elizabeth Medical Centre, Nedlands, Western Australia.

Viral RNA was extracted from 10% to 20% faecal extracts using the QIAamp Viral RNA mini extraction kit (Qiagen) according to the manufacturer's instructions. Rotavirus G and P genotypes were determined using an in-house hemi-nested multiplex reverse transcription polymerase chain reaction (RT-PCR) assay. The first round RT-PCR reactions 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].[14–19] The G and P genotype of each sample was assigned using agarose gel analysis of second round PCR products.

First round amplicons for VP7 were also purified for sequencing by using a Wizard SV Gel for PCR Clean-Up System (Promega), according to the manufacturer's protocol. Purified DNA together with oligonucleotide primers (VP7F/R) were sent to the Australian Genome Research Facility, Melbourne, and sequenced using an ABI PRISM BigDye Terminator Cycle Sequencing Reaction Kit (Applied Biosystems, Foster City, CA, USA) in an Applied Biosystems 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA, USA). Sequences were edited with Sequencher v.4.10.1. The genotype assignment was accomplished using BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and RotaC v2.0 (http://rotac.regatools.be).[20]

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

Results

Number of isolates

A total of 1,383 faecal specimens were collected during the period 1 January to 31 December 2015 for analysis from 15 collaborating centres across Australia. For this reporting period, no samples were received from Tasmania.

A total of 1,031 samples were confirmed as rotavirus positive. Of these, 634 had been collected from children under 5 years of age, and 397 were from older children and adults. An additional 352 specimens contained either insufficient specimen for genotyping (n= 7), were duplicates of samples already analysed (n=77) or the specimen was not confirmed to be positive for rotavirus (n= 268) and were thus not analysed further.

Age distribution

During the 2015 reporting period, 61.5% of samples were obtained from children under 5 years of age (Table 1). Of these, one-third of all samples (33.3%) were identified in children aged 13–24 months, while the next most common age group was 25–36 months (19.7%).

Genotype distribution

All of the 1,031 confirmed rotavirus samples collected from children and adults underwent

Table 1: Age distribution of gastroenteritis cases

Age range (months)	Age range (years)	Number	% of total	% under 5 years
0–6		120	11.6	18.9
7–12	≤1	82	8.0	12.9
13–24	1–2	211	20.5	33.3
25–36	2–3	125	12.1	19.7
37–48	3–4	53	5.1	8.4
49–60	4–5	43	4.2	6.8
Sub-total		634	61.5	–
61–120	5–10	139	13.5	
121–240	10–20	48	4.7	
241–960	20–80	165	16.0	
961+	>80	45	4.4	
Total		1,031	–	

genotype analysis (Table 2). G12P[8] strains were the most common genotype identified nationally, representing 48.2% of all specimens analysed. This genotype was identified as the dominant type in 4 states, Queensland, Victoria, Western Australia and South Australia, representing 53.8%, 80.2%, 65.4% and 50.8% of strains respectively.

G3P[8] strains were the second most common genotype identified nationally, representing 22.8% of all specimens. These were further divided as equine-like G3P[8] (13.2%) and other wild-type G3P[8] (9.6%). Equine-like G3P[8] were identified in all 7 states or territories that submitted samples, and was the dominant type in the Australian Capital Territory and New South Wales, where it represented 96% and 67.2% respectively. In the Northern Territory, equine-like G3P[8] was the second most common type after G2P[4], representing 36.4% and 48.6% of strains respectively. No other wild-type G3P[8] strains were detected in Rotarix states.

G2P[4] and G1P[8] strains were the third and fourth most common genotypes nationally, representing 8.6% and 8.1% of all specimens respectively. G1P[8] was identified in all locations except the Australian Capital Territory, and was the third most common strain in Queensland and South Australia, representing 11.3% and 13.2% of strains respectively.

Thirty samples (3% of all strains) were categorised into 9 uncommon G- and P- genotype combinations (Table 2). Six G3P[3], 5 G8P[14], 4 G9P[4], and 4 G12P[6] strains were identified. Three G1P[4] strains were also noted, as were 3 G8P[8] strains. A further 2 G8P[14], 2 G12P[4] and a single G10P[14] strain were identified.

A G- or P- genotype could not be assigned to 35 samples, of which 23 (65.7%) were observed in Western Australia. Of the 35 samples, 31 were G- non-typeable, 2 were P- non-typeable and 2 had no G- or P- genotype assigned, although they were positive by EIA. The partially non-typeable samples could be strains that contain unusual or uncommon G- or P- genotypes and would not be typeable with the primers used. While this can also be suggested for the EIA positive/G- and P- non-typeable samples, another possible explanation is that the extracted RNA for these samples contained inhibitors that could have prevented the function of the enzymes used in the RT and/or PCR steps.

Thirty-eight samples were identified that contained a strain that was a component of the RotaTeq or Rotarix vaccine. Such Rotarix strains were found in New South Wales (n=1), and the Northern Territory (n=2), while RotaTeq strains were identified in South Australia (n=5), Victoria (n=1) and Western Australia (n=29). In each instance, a vaccine component was determined by RT-PCR and confirmed by sequence analysis of the VP6 and VP7 gene.

Genotypes identified in samples from children less than 5 years of age

In total, 634 rotavirus samples were collected from children under 5 years of age (Table 3). In this cohort, genotype G12P[8] strains were the most commonly identified, found in 41.6% of samples. G3P[8] was the second most common genotype (23.5%), comprising of 14.8% equine-like G3P[8] and 8.7% other wild-type G3P[8] (Figure 1). G2P[4] strains were the third most common genotype (10.1%). G1P[8] and G9P[8] strains represented minor genotypes in this cohort, and were identified in 8.8% and 2.2% of samples respectively (Table 3).

Figure 1: Wild-type G3P[8] occurrences in infants and children under 5 years of age

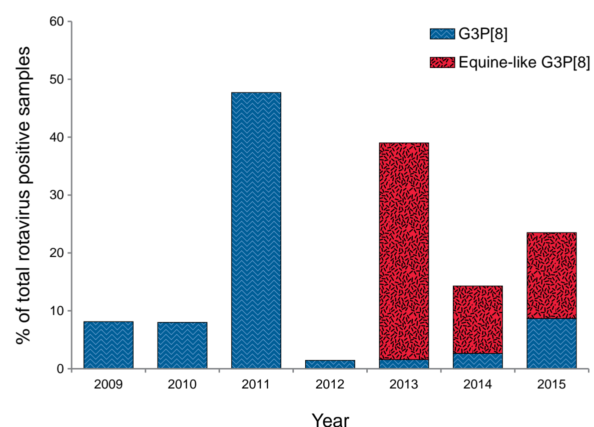


Table 2: Rotavirus G and P genotype distribution in infants, children and adults, 1 January to 31 December 2015

Centre	Type total	G1P[8]		G2P[4]		G3P[8]		G3P[8]*		G4P[8]		G9P[8]		G12P[8]		Non-type†		Vaccine‡		Other		Neg	Insuff
		%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	n	n
Australian Capital Territory																							
ACT	25	–	0	–	0	–	0	96	24	–	0	–	0	–	0	–	0	–	0	4	1	1	0
New South Wales																							
POW	0	–	0	–	0	–	0	–	0	–	0	–	0	–	0	–	0	–	0	–	0	0	0
Westmead	35	3	1	9	3	–	0	71	25	–	0	3	1	6	2	3	1	–	0	6	2	2	0
John Hunter	20	10	2	–	0	–	0	60	12	–	0	15	3	5	1	–	0	5	1	5	1	6	0
NSW subtotal:	55	5	3	5	3	–	0	67	37	–	0	7	4	5	3	2	1	2	1	5	3	8	0
Northern Territory																							
Alice Springs	15	–	0	93	14	–	0	–	0	–	0	–	0	–	0	–	0	–	0	7	1	7	0
Darwin	48	–	0	35	17	–	0	54	26	–	0	10	5	–	0	–	0	–	0	–	0	3	0
Western Diagnostic	12	–	0	–	0	–	0	100	12	–	0	–	0	–	0	–	0	–	0	–	0	0	0
Other§	31	3	1	68	21	3	1	–	0	–	0	–	0	–	0	6	2	6	2	13	4	10	2
Northern Territory subtotal:	106	1	1	49	52	1	1	36	38	–	0	5	5	–	0	2	2	2	2	5	5	20	2
Queensland																							
Pathology Brisbane	61	10	6	11	7	7	4	15	9	–	0	5	3	46	28	3	2	–	0	3	2	9	0
Qld Regional	0	–	0	–	0	–	0	–	0	–	0	–	0	–	0	–	0	–	0	–	0	3	0
Pathology Townsville	19	16	3	–	0	–	0	–	0	–	0	5	1	79	15	–	0	–	0	–	0	3	0
Queensland subtotal:	80	11	9	9	7	5	4	11	9	–	0	5	4	54	43	3	2	–	0	3	2	15	0
South Australia																							
Adelaide	418	13	55	5	22	21	89	3	12	<1	1	<1	1	51	212	2	7	1	5	3	14	100	5
Victoria																							
RCH	80	6	5	–	0	–	0	9	7	–	0	1	1	80	64	–	0	1	1	3	2	30	0
Monash	1	–	0	–	0	–	0	–	0	–	0	–	0	100	1	–	0	–	0	–	0	0	0
Victoria subtotal:	81	6	5	–	0	–	0	9	7	–	0	1	1	80	65	–	0	1	1	2	2	30	0

Table 2 *continued*: Rotavirus G and P genotype distribution in infants, children and adults, 1 January to 31 December 2015

Centre	Type total	G1P[8]		G2P[4]		G3P[8]		G3P[8]*		G4P[8]		G9P[8]		G12P[8]		Non-type†		Vaccine‡		Other		Neg	Insuff
		%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	n	n
Western Australia																							
PathWest	266	4	10	2	5	2	5	3	9	–	0	3	8	65	174	9	23	11	29	1	3	92	0
Total	1031	8	83	9	89	10	99	13	136	<1	1	2	23	48	497	3	35	4	38	3	30	266	7

* Equine-like G3P[8]

† A specimen where G and/or P genotype was not determined.

‡ A specimen where a vaccine component from RotaTeq or Rotarix was found.

§ Faecal specimens from the Northern Territory which were processed in Adelaide.

Neg = Negative for rotavirus

Insuff = Insufficient sample for testing

POW Prince of Wales Hospital

RCH Royal Children's Hospital

Non-typeable† samples:

Northern Territory: 1x G-nt P[8]; 1x G-nt P[4]

New South Wales: 1x G-nt P[8]

Queensland: 1x G-nt P[8]; 1x G12P[nt]

South Australia: 4x G-nt P[8]; 1x G-nt P[6]; 1x G4P[nt] and 1x nt (EIA positive)

Western Australia: 22x G-nt P[8]; 1x nt (EIA positive)

Other samples:

Australian Capital Territory 1x G12P[6]

New South Wales 1x G8P[8], 1x G8P[14], 1x G9P[14]

Northern Territory 1x G1P[4], 4x G3P[3]

Queensland 1x G8P[8], 1x G8P[14]

South Australia 2x G1P[4], 1x G3P[3], 2x G8P[4], 2x G8P[14], 2x G9P[4], 1x G10P[14], 2x G12P[14], 2x G12P[6]

Victoria 1x G9P[4], 1x G12P[6]

Western Australia 1x G3P[3], 1x G8P[8], 1x G8P[14]

Table 3: Rotavirus G and P genotype distribution in infants and children under 5 years of age, 1 January to 31 December 2015

Centre	Type total	G1P[8]		G2P[4]		G3P[8]		G3P[8]*		G4P[8]		G9P[8]		G12P[8]		Non-type†		Vaccine‡		Other		Neg	Insuff
		%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	n	n
Australian Capital Territory																							
ACT	14	–	0	–	0	–	0	93	13	–	0	–	0	–	0	–	0	–	0	7	1	1	0
New South Wales																							
POW	0	–	0	–	0	–	0	–	0	–	0	–	0	–	0	–	0	–	0	–	0	0	0
Westmead	23	4	1	4	1	–	0	78	18	–	0	4	1	–	0	4	1	–	0	4	1	0	0
John Hunter	10	–	0	–	0	–	0	60	6	–	0	20	2	–	0	–	0	10	1	10	1	1	0
NSW subtotal:	33	3	1	3	1	–	0	73	24	–	0	9	3	–	0	3	1	3	1	6	2	1	0
Northern Territory																							
Alice Springs	14	–	0	93	13	–	0	–	0	–	0	–	0	–	0	–	0	–	0	7	1	7	0
Darwin	44	–	0	34	15	–	0	55	24	–	0	11	5	–	0	–	0	–	0	–	0	3	0
Western Diagnostic	12	–	0	–	0	–	0	100	12	–	0	–	0	–	0	–	0	–	0	–	0	0	0
Other§	27	4	1	64	18	–	0	–	0	–	0	–	0	–	0	7	2	7	2	15	4	7	2
Northern Territory subtotal:	97	1	1	47	46	–	0	37	36	–	0	5	5	–	0	2	2	2	2	5	5	17	2
Queensland																							
Pathology Brisbane	24	17	4	4	1	4	1	17	4	–	0	–	0	54	13	4	1	–	0	–	0	7	0
Qld Regional	0	–	0	–	0	–	0	–	0	–	0	–	0	–	0	–	0	–	0	–	0	3	0
Pathology Townsville	8	13	1	–	0	–	0	–	0	–	0	13	1	75	6	–	0	–	0	–	0	2	0
Queensland subtotal:	32	16	5	3	1	3	1	13	4	0	0	3	1	59	19	3	1	–	0	–	0	12	0
South Australia																							
Adelaide	238	15	36	6	15	22	52	3	8	<1	1	<1	1	45	107	2	4	2	5	4	9	39	3
Victoria																							
RCH	55	7	4	–	0	–	0	7	4	–	0	2	1	78	43	–	0	2	1	4	2	17	0
Monash	1	–	0	–	0	–	0	–	0	–	0	–	0	100	1	–	0	–	0	–	0	0	0
Victoria subtotal:	56	7	4	–	0	–	0	7	4	–	0	2	1	79	44	0	0	2	1	4	2	17	0
Western Australia																							
PathWest	164	5	9	1	1	1	2	3	5	–	0	2	3	57	94	12	19	18	29	1	2	55	0
Total	634	9	56	10	64	9	55	15	94	<1	1	2	14	42	264	4	27	6	38	3	21	142	5

* Equine-like G3P[8].

† A specimen where G and/or P genotype was not determined.

‡ A specimen where a vaccine component from RotaTeq or Rotarix was found.

§ Faecal specimens from the Northern Territory which were processed in Adelaide.

Neg Negative for rotavirus

Insuff Insufficient sample for testing

POW Prince of Wales Hospital

RCH Royal Children's Hospital

Other samples:

Australian Capital Territory	1x G12P[6]
New South Wales	1x G8P[14], 1x G9P[4]
Northern Territory	1x G1P[4], 4x G3P[3]
South Australia	1x G1P[4], 1x G3P[3], 2x G8P[4], 2x G9P[4], 1x G10P[14], 2x G12P[4]
Victoria	1x G9P[4], 1x G12P[6]
Western Australia	1x G3P[3], 1x G8P[14]

Genotypes identified in samples from individuals greater than 5 years of age

A total of 397 rotavirus samples were collected from children over the age of 5 years, and adults (Table 4). In this cohort, genotype G12P[8] strains were the most common, found in 58.4% of samples. G3P[8] strains (including equine-like G3P[8]) were the second most common genotype (21.7%), and G1P[8] and G2P[4] strains were equal third most common genotypes, identified in 6.8% and 6.3% respectively. Genotype G9P[8] strains were identified in 2.3% of samples.

Distribution of genotypes according to vaccine type

G and P genotypes from the rotavirus samples collected from infants and children under 5 years of age by vaccine usage were compared (Figure 2). In states where RotaTeq is in use, G12P[8] strains were the dominant genotype in children less than 5 years of age, identified in 54% of samples, while G3P[8] were the second most common, identified in 15.5% of strains. These G3 were further divided as equine-like G3P[8] (4.3%) or other wild type G3P[8] (11.2%). G1P[8] strains were the third most common genotype representing 11% of samples. In locations using Rotarix, equine-like G3P[8] strains were dominant, identified in 50.3% of strains, followed by G2P[4], identified in 32.4% of samples.

Consistency in genotype distribution among states using RotaTeq vaccine was observed, with all 4 RotaTeq states (Queensland, Victoria, South Australia and Western Australia) having G12P[8] as the dominant genotype. However, in states and territories using Rotarix (New South Wales, the Australian Capital Territory and the Northern Territory), the dominant genotype differed, with G2P[4] dominant in Northern Territory and equine-like G3P[8] dominant in New South Wales and the Australian Capital Territory.

Discussion

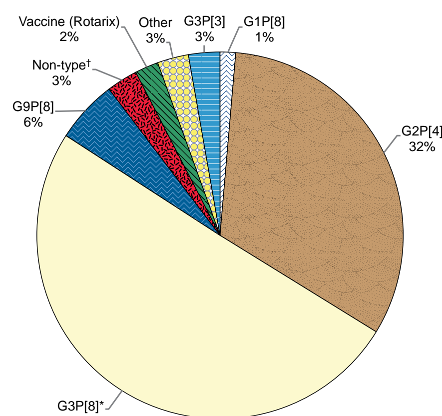
This 2015 Australian Rotavirus Surveillance report describes the annual distribution of rotavirus genotypes and geographic differences in genotypes causing disease in Australia for the period 1 January to 31 December 2015. Genotype G12P[8] remained the dominant genotype nationally, representing 48.2% of all strains from all age groups. Genotype G3P[8] was the second most common genotype nationally, comprising 23% of all strains, but was the dominant genotype in New South Wales and the Australian Capital Territory only. Genotype G2P[4] and G1P[8] were the third and fourth most common, representing 8.6% and 8.1% of all strains respectively.

In the samples collected from infants and children under 5 years of age, genotype G12P[8] continued to remain as the dominant genotype nationally, identified in 41.6% of samples. The second most common genotype was G3P[8], identified in 23.5% of samples, while the third most common was G2P[4], identified in 10% of samples.

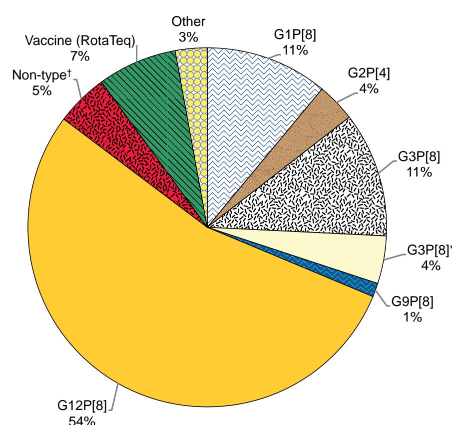
In Australia, G12P[8] emerged in 2012 and subsequently became the dominant strain in 2013 and 2014, representing 33% and 29.6% of all strains respectively.[19,22,23] This figure increased in 2015 to encompass 48.2% of all strains, and has been identified as the dominant strain in all 4 states that use the RotaTeq vaccine (South Australia, Queensland, Victoria and Western Australia). Similar observations have been observed in

Figure 2: Overall distribution of rotavirus G and P genotypes identified in Australian children based on vaccine usage, Australia, 1 January to 31 December 2015

Rotarix states†



RotaTeq states‡



* Equine-like G3P[8][21]

† The Australian Capital Territory, New South Wales, and the Northern Territory

‡ Queensland, South Australia, Victoria, and Western Australia

Table 4: Rotavirus G and P genotype distribution in children over 5 years of age and adults, 1 January to 31 December 2015

Centre	Type total	G1P[4]		G1P[8]		G2P[4]		G3P[8]		G3P[8]*		G8P[8]		G8P[14]		G9P[8]		G12P[6]		G12P[8]		Non-type†		Neg	Insuff
		%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	n	n
Australian Capital Territory																									
ACT	11	–	0	–	0	–	0	–	0	100	11	–	0	–	0	–	0	–	0	–	0	–	0	0	0
New South Wales																									
POW	0	–	0	–	0	–	0	–	0	–	0	–	0	–	0	–	0	–	0	–	0	–	0	0	0
Westmead	12	–	0	–	0	17	2	–	0	58	7	8	1	–	0	–	0	–	0	17	2	–	0	2	0
John Hunter	10	–	0	20	2	–	0	–	0	60	6	–	0	–	0	10	1	–	0	10	1	–	0	5	0
NSW subtotal	22	–	0	9	2	9	2	–	0	59	13	5	1	–	0	4	1	–	0	14	3	–	0	–	0
Northern Territory																									
Alice Springs	1	–	0	–	0	100	1	–	0	–	0	–	0	–	0	–	0	–	0	–	0	–	0	0	0
Darwin	4	–	0	–	0	50	2	–	0	50	2	–	0	–	0	–	0	–	0	–	0	–	0	0	0
Western Diagnostic	0	–	0	–	0	–	0	–	0	–	0	–	0	–	0	–	0	–	0	–	0	–	0	0	0
Other‡	4	–	0	–	0	75	3	25	1	–	0	–	0	–	0	–	0	–	0	–	0	–	0	3	0
Northern Territory subtotal	9	–	0	–	0	67	6	11	1	22	2	–	0	–	0	–	0	–	0	–	0	–	0	3	0
Queensland																									
Pathology Brisbane	37	–	0	5	2	16	6	8	3	14	5	3	1	3	1	8	3	–	0	41	15	3	1	2	0
Qld regional	0	–	0	–	0	–	0	–	0	–	0	–	0	–	0	–	0	–	0	–	0	–	0	0	0
Pathology Townsville	11	–	0	18	2	–	0	–	0	–	0	–	0	–	0	–	0	–	0	73	8	9	1	1	0
Queensland subtotal:	48	–	0	8	4	13	6	6	3	10	5	2	1	2	1	6	3	–	0	48	23	4	2	3	0
South Australia																									
Adelaide	180	1	1	11	19	4	7	21	37	2	4	–	0	1	2	–	0	1	2	58	105	2	3	61	2
Victoria																									
RCH	25	–	0	4	1	–	0	–	0	12	3	–	0	–	0	–	0	–	0	84	21	–	0	13	0
Monash	0	–	0	–	0	–	0	–	0	–	0	–	0	–	0	–	0	–	0	–	0	–	0	0	0
Victoria subtotal:	25	–	0	4	1	–	0	–	0	12	3	–	0	–	0	–	0	–	0	84	21	–	0	13	0
Western Australia																									
PathWest	102	–	0	1	1	4	4	3	3	4	4	1	1	–	0	5	5	–	0	78	80	4	4	37	0
Total	397	<1	1	7	27	6	25	11	44	11	42	1	3	1	3	2	9	1	2	58	232	2	9	124	2

* Equine-like G3P[8]

† A specimen where G and/or P genotype was not determined.

‡ Faecal specimens from the Northern Territory which were processed in Adelaide.

Neg Negative for rotavirus

Insuff Insufficient sample for testing

POW Prince of Wales Hospital

RCH Royal Children's Hospital.

Nicaragua, where RotaTeq was added to the infant schedule in 2006; more than a 2-fold increase in rotavirus cases in children under 5 years of age was attributed to a G12 outbreak in the 2012/2013 season.[24] Since 2000, over 30 countries have reported an emergence or re-emergence of G12 rotavirus strains.[25–27] Furthermore, G12P[8] have been identified as the dominant strain in multiple countries since 2010.[24,28–31] During the 2010-2011 season, 65% of all strains in Basque Country, Spain were identified as G12P[8].[31] At this time, vaccine coverage was estimated to be below 30%, even though both vaccines were available in Basque Country since 2006–2007.[31] In 2013, G12P[8] represented 82% of all strains in Nicaragua; 86% in St Louis, Missouri and 89% in Atlanta, Georgia.[24,29,30] In addition, G12P[8] emerged as a dominant strain in Brazil, from 22.7%–27.3% in 2012 to 86.6% in 2014, although G12 had decreased to represent only 2.7% of all strains in 2013.[28,32,33]

Whilst not yet dominant, G12 has been identified as an important genotype in countries such as Italy, Saudi Arabia, Cameroon, and North and South America.[34–38] In Delhi, India, a study investigating strain diversity of samples collected from 2007–2012, established that G12 represented 14.8% of all genotypes identified. The majority of G12 strains found in the study had VP4 genotype P[6] (67.4%), while P[8] represented only 7% of all G12 cases. Another combination, G12P[4], was identified and represented 14% of all G12 strains identified.[39] G12P[4] and G12P[6] have also been identified in Australia in 2013 and 2015, however they were uncommon and represented less than 1% for each surveillance period. Taken together, these data demonstrate that G12 strains are an important cause of rotavirus disease both in Australian and global settings.

This survey reports the persistence of G3P[8] strains as the second most common genotype across Australia. Previously, G3P[8] strains generally represented 4% to 11% of strains over a 1-year reporting period. However, in 2013, G3P[8] emerged as the second most common genotype, representing 31% of all strains.[19] Full genome analysis of these G3P[8] samples revealed that in 2013, a novel inter-genogroup reassortant strain (denoted as equine-like G3P[8], or G3P[8]* in Figure 2) had emerged, which had a distinct VP7 antigenic profile compared to other wild-type Australian G3P[8] strains.[21] The genome constellation of equine-like G3P[8] contained an equine-like G3 VP7, a P[8] VP4 and a genogroup 2 backbone I2-R2-C2-M2-A2-N2-T2-E2-H2.[21] This strain continued to persist, representing 37% of all strains in 2013, 12% in 2014 and 15% of 2015 samples from infants and children under 5 years of age (Figure 1).

The state and territory based tender process has resulted in the use of either RotaTeq or Rotarix vaccines in states and territories in Australia and provides a unique opportunity to observe and compare rotavirus strain diversity. Since vaccine introduction, significant differences in genotype distribution in infants and children under 5 years of age have been observed between jurisdictions using each vaccine.[40] In the current survey, equine-like G3P[8] was dominant in locations using Rotarix (50.3%), although no other wild-type G3P[8] strains were identified; whereas in RotaTeq states, other wild-type G3P[8] were more common (11.25%) compared with equine-like G3P[8] (4.3%).

The emergence of G12P[8] and novel genotypes globally have raised concerns regarding vaccine efficacy; however a review of vaccine trials and surveillance studies up to 2012 demonstrated that in high income countries such as Belgium and the United States of America, the monovalent Rotarix (G1P[8]) and pentavalent RotaTeq (G1-4, P[8]) vaccines continue to be highly efficacious against commonly circulating rotavirus strains.[41] The pooled effectiveness of Rotarix was 94% against homotypic strains, to 87% against fully heterotypic strains.[41] RotaTeq exhibited similar effectiveness; from 83% against homotypic strains to 75% against single-antigen non-vaccine type strains.[41] Nevertheless, a recent study from Belgium reported that multiple deduced amino acid differences existed between the VP7 and VP4 antigenic epitopes of vaccine and currently circulating strains, such as G12 and G9.[42] The VP7 proteins of G12P[8] strains alone contained 9 amino acid differences compared with the strains of RotaTeq, and 16 amino acid differences to Rotarix strain.[42] These differences, together with the high evolutionary rate of the G12 VP7 gene (1.66×10^{-3} substitutions/site/year), raise the concern that this strain could ultimately escape the rotavirus neutralising antibody response induced by vaccines.[27,42] Vaccine effectiveness studies are required to determine if current vaccines are able to protect successfully against novel strains, such as the equine-like G3P[8] and G12P[8].

The continued dominance of G12P[8] over a 4-year period in states using RotaTeq support the concept hypothesis that vaccine-related selective pressure may be arising. Vaccine-related selective pressure have also been implicated in the persistence of G2P[4] in countries such as Belgium, where Rotarix provides high vaccine coverage.[43,44] This observation is of particular interest, as the 2015 Australian data described in this report found that in infants and children under 5 years of age, there was a marked increase in G2P[4] cases to 32% in jurisdictions using Rotarix, reflecting a trend also observed in 2012.[45]

In this reporting period, over 1,000 rotavirus positive samples were collected from across Australia. Caution in interpreting the data is recommended, as the increase in the number of samples received may not necessarily reflect the number of cases being greater, since not all samples are forwarded by collaborating hospitals and diagnostic laboratories. Regardless, a robust increase in the number of samples sent from South Australia, Western Australia and Victoria have been noted, particularly in 2013 and 2015 in which the majority of samples from these states were genotyped as G12P[8]. The emergence of G12 and novel strains such as the equine-like G3P[8], G3P[3], G10P[14] and G8 strains demonstrate a dynamic virus population in the current post vaccine era. Therefore, the continued variations in the wild type strain population will remain a challenge to vaccine effectiveness and will require continued monitoring.

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