

# AUSTRALIAN ROTAVIRUS SURVEILLANCE PROGRAM ANNUAL REPORT, 2013

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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 2013. During the survey period, 1,035 faecal samples were referred for rotavirus G and P genotype analysis. Of these 828 were confirmed as rotavirus positive. A total of 503 specimens were collected from children under 5 years of age, while 325 were from older children and adults. Genotype analysis of the 828 rotavirus samples collected from both children and adults revealed that G12P[8] was the dominant genotype in this reporting period, identified in 33% of strains nationally. Genotype G3P[8] was the second most common strain nationally, representing 31% of samples, followed by genotype G2P[4] (14%). This represents the first report where G12P[8] strains are the major cause of disease in this population. The genotype distribution was slightly altered when the analysis was restricted to samples collected from children under 5 years of age, with G3P[8] being the dominant genotype (39.2%) followed by G12P[8] as the second most common genotype (31%). Fluctuations in genotype distribution were also observed based on the vaccine type in use. Genotype G12P[8] was more common in states and territories using RotaTeq, while G3P[8] was more common in the locations using Rotarix. This survey highlights the yearly fluctuations in rotavirus genotypes observed since vaccine introduction, with changes in dominant genotypes an annual event. The emergence of G12P[8] as the dominant genotype further illustrates the ongoing changes in the wild type rotavirus population evident in the Australian population since vaccine introduction. *Commun Dis Intell* 2014;38(4):E334–E342.

Keywords: rotavirus, gastroenteritis, genotypes, disease surveillance

## Introduction

Rotaviruses are triple layered dsRNA viruses that belong to the *Reoviridae* family. They contain 11 gene segments that encode the 6 structural proteins found in the virion and 6 non-structural proteins produced inside cells during viral replication.<sup>1</sup>

Rotaviruses are the most common cause of severe diarrhoea in young children worldwide.<sup>2</sup> Vaccines have been developed to reduce the significant morbidity and mortality associated with infection. Two live attenuated oral rotavirus vaccines; Rotarix® [GlaxoSmithKline] and RotaTeq® [Merck], have been shown to be safe and highly effective in the prevention of severe diarrhoea due to rotavirus infection.<sup>3,4</sup> Both vaccines were included into the funded National Immunisation Program of Australia from 1 July 2007. 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. Since 2006, rotavirus vaccines have been licensed in over 125 countries and included in the national vaccination schedules of 59 predominantly high and middle-income countries worldwide.<sup>5</sup>

Historically in Australia, rotavirus infection accounted for up to 10,000 childhood hospitalisations for diarrhoea each year.<sup>6</sup> The introduction of rotavirus vaccines has seen a significant impact on the disease burden, with state based studies in New South Wales, Queensland, South Australia and Victoria showing a substantial decline in both rotavirus coded and non-rotavirus coded hospitalisations and emergency room visits since vaccine introduction.<sup>7–10</sup>

The annual circulation patterns of rotavirus genotypes causing disease in Australian children has been documented by the Australian Rotavirus Surveillance Program since 1997. The strain diversity and temporal and geographic changes observed each year provides the baseline information vital to assist vaccine introduction and ongoing evaluation.<sup>11</sup> Vaccine introduction has increased the population immunity to wild type rotavirus strains, which is likely to impact on the epidemiology of circulating strains. Therefore, characterisation of circulating rotavirus genotypes 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 the vaccination programs.

In this report we describe the genotype of rotavirus strains causing severe gastroenteritis in Australia for the period 1 January to 31 December, 2013.

## 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 laboratories in Townsville, Cairns and Gold Coast, Queensland
- Microbiology and Infectious Diseases Laboratory, SA Pathology, Adelaide, South Australia
- The Serology Department, Royal Children's Hospital, Parkville, Victoria
- Princess Margaret Hospital for Children, Subiaco, Western Australia
- Division of Microbiology, PathWest Laboratory Medical WA, The Queen Elizabeth II Medical Centre, Nedlands, Western Australia

Viral RNA was extracted from 10%–20% faecal extracts of each specimen using the QIAamp Viral RNA mini extraction kit (Qiagen) according to the manufacturer's instructions. The rotavirus G and P genotype were determined for each sample by the 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].<sup>12–16</sup> Two new G3 primers were included in the G typing assay: EQ3 fwd-ctgcatacgcctaattctacacaagg, and EQ3 rev-gatcgtacaagtagccgtagtaac. 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 (Thermo Fisher, Aus), as per the manufacturer's instructions to confirm the presence of rotavirus antigen.

## Results

### Number of isolates

During the period 1 January to 31 December 2013, a total of 1,035 faecal specimens were collected for analysis from 18 collaborating centres across Australia; located in Victoria, Western Australia, the Northern Territory, New South Wales, Queensland, South Australia, and the Australian Capital Territory.

Of these, 828 were confirmed as rotavirus positive by EIA (ProSpecT, OXOID) or RT-PCR analysis. Of these, 503 samples were collected from children under 5 years of age, and 325 samples were from older children and adults. An additional 207 specimens contained either insufficient specimen for genotyping ( $n=3$ ), or the specimen was not confirmed to be positive for rotavirus ( $n=204$ ) and these were not analysed further.

### Age distribution

During the reporting period, 61% of samples were obtained from children under 5 years of age (Table 1). Overall, 17.9% of isolates were from infants 0–6 months of age, 9.9% were from infants 7–12 months of age, 16.1% were from children 13–24 months of age, and 9.4% were from children 25–36 months of age. A total of 14.4% of samples were from children 5 years and 1 month to 10 years of age, and 21.3% of samples were from individuals older than 21 years of age, which included 5.6% from adults over the age of 80 years.

### Genotype distribution

All of the 828 confirmed rotavirus samples collected from children and adults from 7 locations in New South Wales, the Northern Territory, Queensland, Western Australia, South Australia,

**Table 1: Age distribution of gastroenteritis cases**

Age range (months)	n	% of total
0–6	148	17.9
7–12	82	9.9
13–24	133	16.1
25–36	78	9.4
37–48	31	3.7
49–60	30	3.6
61–120	119	14.4
121–240	28	3.4
241–960	130	15.7
961+	46	5.6
Unknown	3	0.4
Total	828	na

Victoria and the Australian Capital Territory, underwent genotype analysis (Table 2). G12P[8] strains were the most common genotype identified nationally, representing 33% of all specimens analysed. This genotype was identified as the dominant type in 3 states, Queensland, Victoria and South Australia, representing 42%, 68% and 61% of strains respectively. Genotype G12P[8] strains were only identified in one other location, Western Australia, during this survey period and represented 9% of strains.

G3P[8] strains were the 2nd most common genotype identified nationally, representing 31% of all specimens. This genotype was identified in all 7 states and territories, and was the dominant type in the Northern Territory and Western Australia, where it represented 93% and 44% respectively.

G2P[4] strains were the 3rd most common genotype nationally, representing 14% of all specimens. It was identified in 5 states and was the dominant type in New South Wales, representing 59% of strains.

In this survey period, G1P[8] was the 4th most common genotype representing only 10% of strains analysed; however it was identified in all states and territories.

Genotypes G4P[8] and G9P[8] each represented less than 2% of the total specimens typed. Several rare or uncommon genotype combinations were identified, including 3 G12P[6] strains in Western Australia, 2 G9P[9] strains in South Australia, and single G3P[14], G2P[8] and G6P[8] strains in Queensland, Western Australia and South Australia. Of 10 samples

that contained multiple G and/or P genotypes, 7 were identified as being vaccine component strains by sequence analysis. A total of 32 samples contained a non-typeable G– and/or P genotype. The non-typeable samples are likely to be samples that contain low virus amounts, 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.

Twenty-nine faecal specimens collected through routine surveillance were identified that contained a component of the RotaTeq vaccine; these were from Western Australia and South Australia. In addition, faecal specimens were received from 15 children who developed gastroenteritis after being vaccinated. A RotaTeq vaccine component was identified in 5 samples, while a G9P[8] strain was identified in a single sample. The RotaTeq vaccine virus components were identified by RT-PCR and sequence analysis.

#### **Analysis of genotypes identified in samples from children less than 5 years of age**

A total of 503 rotavirus samples were collected from children under 5 years of age. Genotype G3P[8] strains were the most commonly identified; found in 39.2% of samples, and G12P[8] strains were the 2nd most common genotype; identified in 31% of samples. G1P[8] was the 3rd most common genotype; identified in 11.3% of samples. G2P[4], G4P[8] and G9P[8] all represented minor genotypes in children in this study, and were identified in 4.6%, 2.4% and 1.6% of samples respectively (Table 3).

Analysis of G and P genotyping results revealed that in states where RotaTeq is in use, G12P[8] was the dominant genotype in children less than 5 years of age, identified in 42.5% of samples, while G3P[8] was the 2nd most common, identified in 22.3% of strains (Figure). G1P[8] was the 3rd most common genotype representing 13.6% of samples. In states where Rotarix is used, G3P[8] strains were dominant, identified in 84.6%, while genotype G1P[8] and G2P[4] were identified in 5.1% of strains.

A degree of consistency in genotype distribution within each vaccine type was observed, for example, in 3 of the 4 RotaTeq states (Queensland, Victoria and South Australia) G12P[8] was the dominant genotype. However, in states using Rotarix (New South Wales, Northern Territory and Australian Capital Territory), G3P[8] was dominant only in the Northern Territory. The small number of samples (n=138) from locations using Rotarix may have influenced these comparisons.

Table 2: Rotavirus G and P genotype distribution in Australian infants, children and adults, 1 January to 31 December 2013

Centre	Total	G1P[8]	G2P[4]	G3P[8]	G4P[8]	G9P[8]	G9P[9]	G12P[8]	G12P[6]	G6P[8]	G3P[14]	G2P[8]	Mix*	Non-typ†	Vaccine	Neg	Insuff	
		%	%	%	%	%	%	%	%	%	%	%	%	%	%	n	n	
<b>Australian Capital Territory</b>																		
ACT	3	33	1	0	0	0	0	0	0	0	0	0	0	33	1	0	0	0
<b>New South Wales</b>																		
Sydney (POW)	33	15	5	52	17	24	8	0	0	0	0	0	0	9	3	0	0	7
Sydney (Westmead)	16	6	1	75	12	0	0	6	1	0	0	0	0	13	2	0	0	1
Newcastle (JH)	2	50	1	50	1	0	0	0	0	0	0	0	0	0	0	0	0	1
<b>Northern Territory</b>																		
Alice Springs	53	0	0	0	0	94	50	0	0	0	0	0	2	4	2	0	0	5
Darwin	68	3	2	0	0	96	65	0	0	0	0	0	1	0	0	0	0	7
Other‡	4	25	1	0	0	75	3	0	0	0	0	0	0	0	0	0	0	1
<b>Queensland</b>																		
Pathology (Brisbane)	53	17	9	19	10	13	7	0	0	0	0	2	1	2	1	0	0	13
Qld regional	25	24	6	16	4	12	3	0	0	8	2	0	0	4	1	0	0	46
Pathology (Townsville)	9	33	3	0	0	33	3	0	0	0	0	0	0	0	0	0	0	15
Pathology (Gold Coast)	4	50	2	0	0	0	0	25	1	0	0	0	0	0	0	0	0	12
<b>South Australia</b>																		
Adelaide	231	6	14	10	22	6	14	3	8	0	1	2	61	140	0	0	0	8
<b>Victoria</b>																		
RCH	87	10	9	13	11	10	9	1	1	1	0	0	64	56	0	0	0	19
Monash	23	13	3	0	0	4	1	0	0	0	0	0	83	19	0	0	0	0
<b>Western Australia</b>																		
PathWest WA	202	12	25	17	35	44	88	1	2	5	11	0	9	18	1	3	0	15
Perth (P Marg)	15	20	3	0	0	47	7	0	0	7	1	0	0	0	0	0	0	34
Total	828	10	85	14	112	31	259	1	12	2	15	0	2	33	273	0	3	204

207 specimens were omitted from analysis due to insufficient sample or because the specimen was not confirmed to be rotavirus positive

**Non-typables:**

ACT: 1x NT/NT

Perth: 8x G-non typeable P[8]

SA: 1x NT/NT (EIA +ve), 1x G2 P[non typeable], 7x G1 P[non typeable], 6x G4P[non typeable], 4x G12 P[non typeable], 1x G-non typeable P[4], 6x G-non typeable P[8], 1x G-non typeable P[9]?

A.S. (NT): 2x G-non typeable P[8]

POW G3 P[non typeable], G-non typeable P[8], NT/NT

Westmead: 1x G2 P[non typeable], 1x G3 P[non typeable]

Table 3: Rotavirus G and P genotype distribution in Australian children ≤ 5 years, 1 January to 31 December 2013

Centre	Total	G1P[8]		G2P[4]		G3P[8]		G4P[8]		G9P[8]		G12P[8]		G12P[6]		G6P[8]		G3P[14]		G2P[8]		Mix*		Non-type†		Vaccine		Neg		Insuff						
		%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n					
<b>Australian Capital Territory</b>																																				
ACT	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	0	0	0	0	0	0	0	0			
<b>New South Wales</b>																																				
Sydney (POW)	13	15	2	38	5	31	4	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		
Sydney Westmead	4	25	1	50	2	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	0	0	0	
Newcastle (JH)	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	0	0	0	0	0	0	0	0	0	0	
<b>Northern Territory</b>																																				
Alice Springs	52	0	0	0	0	94	49	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1	4	2	-	-	5	0	0	0	0	0		
Darwin	62	3	2	0	0	95	59	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0	0	-	-	5	0	0	0	0	0	0		
Other*	3	0	0	0	0	100	3	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		
<b>Queensland</b>																																				
Pathology (Brisbane)	24	25	6	8	2	21	5	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	0	0
Qld regional	10	20	2	20	2	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	0	0	0	0
Pathology Townsville	5	60	3	0	0	20	1	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	0	0
Pathology (Gold Coast)	2	0	0	0	0	0	0	50	1	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
<b>South Australia</b>																																				
Adelaide	123	8	10	5	6	2	2	7	8	0	0	61	75	0	0	1	1	0	0	0	0	0	0	0	0	6	7	11	14	3	0	0	0	0		
<b>Victoria</b>																																				
RCH	56	11	6	5	3	13	7	2	1	2	1	68	38	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Monash	15	13	2	0	0	0	0	0	0	0	0	87	13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<b>Western Australia</b>																																				
PathWest WA	119	15	18	3	3	52	62	2	2	5	6	9	11	0	0	0	0	0	0	0	0	0	0	0	0	2	2	12	14	6	2	0	0	0	0	
Perth (P Marg)	13	23	3	0	0	38	5	0	0	8	1	8	1	0	0	0	0	0	0	0	0	0	0	0	0	8	1	8	1	30	0	0	0	0	0	
Total	503	11.3	57	4.6	23	39.2	197	2.4	12	1.6	8	31	156	0	0	0.2	1	0	0	0	0.2	1	0.6	3	3.2	16	5.8	29	-	-	-	-	-	-		

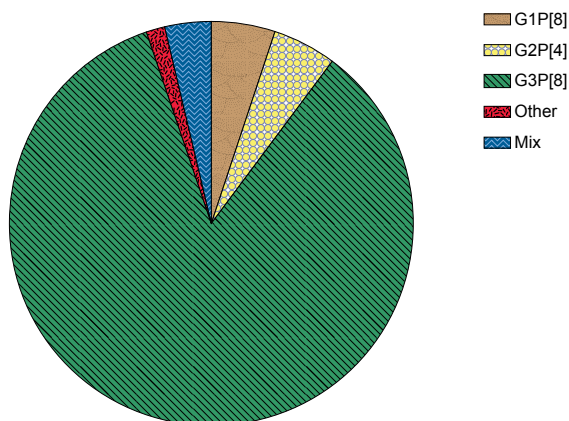


## Discussion

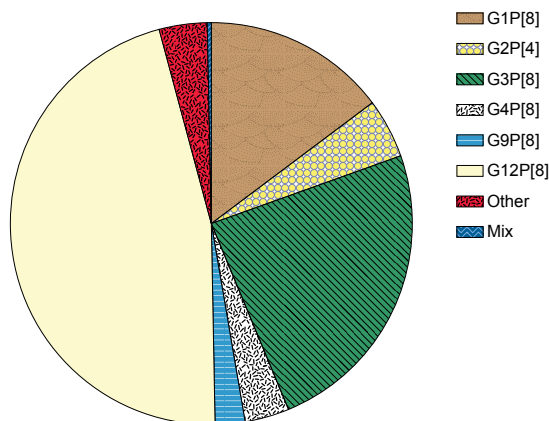
The Australian Rotavirus Surveillance Program report for the period 1 January to 31 December 2013 describes the annual distribution of rotavirus genotypes and geographic differences in genotypes causing disease in Australia. In 2013, the surveillance program identified that genotype G12P[8] emerged as the dominant genotype nationally, representing 33% of all strains, being the dominant genotype in 3 states; Queensland, Victoria and South Australia. Genotype G3P[8] was the 2nd most common genotype nationally, comprising 28% of all strains, but was the dominant genotype in 2 locations, Western Australia and the Northern Territory. Genotype G2P[4] represented the 3rd most common genotype, representing more than 14% of strains nationally, and was the dominant genotype in New South Wales.

**Figure: Overall distribution of rotavirus G and P genotypes identified in Australian children based on vaccine usage for period 1 January to 31 December, 2013**

### Rotarix states



### RotaTeq states



Since 2007–2008, genotypes G1P[8] and G2P[4] have alternated as the dominant genotype causing disease across Australia.<sup>17–20</sup> The identification of G12P[8] as the dominant genotype this year is the first time since vaccine introduction that neither of these genotypes were dominant. In 2012, the emergence of G12P[8] strains represented more than 20% of strains, being identified as the 3rd most common genotype, and were observed circulating in Western Australia, the Northern Territory, Queensland and South Australia.<sup>20</sup> This report found that G12P[8] did not spread across the country, rather it continued to emerge in two of the locations (Queensland and South Australia) and cause a greater proportion of disease in those settings. Victoria was the only new location where G12P[8] strains emerged in 2013. Prior to 2012, G12P[8] strains only represented a sporadic and rare cause of disease in Australia. In other countries, G12P[8] generally continues to represent an uncommon cause of disease.<sup>21</sup> However, similar to Australia, a few countries have seen the emergence of G12 in recent years. In West Africa, G12 strains represented more than 80% of strains in 2011–2012,<sup>22</sup> while in the Basque Country of Spain, G12P[8] was the predominant genotype, causing 65% of rotavirus gastroenteritis.<sup>23</sup> This Spanish outbreak was characterised by a broad geographical distribution (rural and urban) and affected both infants and children.<sup>23</sup> The sudden emergence and predominance of G12P[8] rotaviruses in several locations suggest that they may soon become a major human rotavirus genotype. Importantly, in an efficacy trial of Rotarix conducted in South Africa and Malawi, vaccination was shown to provide comparable protection against a range of circulating genotypes including G12 strains.<sup>24</sup> The presence of the P[8] VP4 protein in the G12 strains suggests that both rotavirus vaccines are likely to be effective against the emergence of G12P[8] strains.

This report saw the emergence of G3P[8] strains as the 2nd most common genotype across Australia. In previous years, G3P[8] strains have been observed as generally the 3rd most common type, representing 4%–11% of strains in any given year, and only on 2 occasions was it the most common type in one location; Melbourne 2006–07 and 2009–10.<sup>17–20</sup> The identification of G3P[8] as the dominant type in 2 locations within the same year is unique. In part, its emergence may be due to its unusual G3 VP7 protein, which on preliminary sequence analysis is genetically more similar to equine G3 strains than other human strains (unpublished observations, C Kirkwood). Further sequence analysis of the whole genome of this genotype is required to determine whether other genes are unique.

In the previous surveillance report in 2012, a single genotype G3P[14] rotavirus strain was identified in a 12-year-old child presenting to the Emergency Department of the Royal Children's Hospital, Melbourne, with gastroenteritis. Full genome sequence analysis revealed that the strain contained the novel genome constellation G3-P[14]-I2-R3-C3-M3-A9-N2-T6-E2-H3.<sup>25</sup> The genome was genetically divergent from previously characterised lapine viruses and the genes were distantly related to a range of human bovine-like strains and animal strains of bovine, bat and canine/feline characteristics.<sup>25</sup> This highlights that novel strains are capable of causing disease in Australian children, and an interest in uncommon rotavirus genotypes continues because of the possible impact they could have on rotavirus vaccination programs.

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. In the current survey, G12P[8] strains were the most common in locations using RotaTeq vaccine, however, none were observed in locations using Rotarix vaccine. In contrast, G3P[8] represented the most common type in Rotarix locations, and was second most common in RotaTeq locations. Differences in genotype distribution based on vaccine usage have been observed each year since vaccine introduction.<sup>26</sup> During the post vaccine years 1, 2 and 5, G2P[4] strains were more common in states and territories using Rotarix, and during year 4, in states using RotaTeq. G1P[8] strains were more common in the other 4 years in locations using Rotarix. G3P[8] were more common in RotaTeq states in years 2008—09, and 2009–10, after which they occurred at similar rates in years 4 and 5.<sup>17–20</sup> Thus consistent differences in genotype distribution linked to a particular vaccine may be starting to emerge.

This survey of rotavirus strains causing disease between 1 January and 31 December 2013 highlights the emergence of G12P[8] rotavirus as the dominant genotype in Australia. The emergence of G12P[8] and to a lesser extent G3P[8] illustrates a unique change to the genotype patterns in Australia, further highlighting the continual changes in the wild type virus population and suggest a more dynamic virus population is present in the current post vaccine era than observed in the pre-vaccine era.

Whether the introduction of vaccine is exerting an increase due to immune pressure or whether the increase is simply due to natural variation is still unclear, but the identification of G12 and unusual G3 strains strengthens the need to continue rotavirus surveillance in both humans and animals.

Therefore, continued surveillance of the wild type strains circulating in Australia is required to monitor any changes that may emerge and impact vaccine effectiveness.

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