

# AUSTRALIAN ROTAVIRUS SURVEILLANCE PROGRAM: ANNUAL REPORT, 2009/2010

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## Abstract

The Australian Rotavirus Surveillance Program together with 15 collaborating laboratories Australia-wide conducts a laboratory based rotavirus surveillance program. This report describes the genotypes of rotavirus strains responsible for the hospitalisation of children with acute gastroenteritis during the period 1 July 2009 to 30 June 2010, the 3rd year of surveillance following introduction of rotavirus vaccines into the National Immunisation Program. Seven hundred and seventy-eight faecal samples were referred to the centre for G and P genotype analysis using hemi-nested multiplex reverse transcription-polymerase chain reaction. Of the 422 confirmed as rotavirus positive, genotype G1P[8] was the dominant type nationally, representing 49.3%, followed by genotype G2P[4] (21.1%). Genotypes G3P[8], G4P[8] and G9P[8] each represented less than 3% of circulating strains nationally. The dominance of G1P[8] was in part associated with a large outbreak of severe gastroenteritis in the Northern Territory in 2010. The identification of uncommon rotavirus genotype combinations has increased since vaccine introduction, with G1P[4], G2P[8] and G9P[4] identified during this survey. Single strains of G1P[6] and G4P[6] were identified during this study period. This survey continues to highlight the fluctuations in rotavirus genotypes, and results from this survey suggest there is limited genotype selection based on vaccine usage. However, the large G1P[8] outbreak of gastroenteritis in the Northern Territory may have resulted from vaccine pressure on wild-type strains. *Commun Dis Intell* 2010;34(4):427–434.

Keywords: Rotavirus, gastroenteritis, genotypes, disease surveillance

## Introduction

Rotaviruses are the most important cause of dehydration, hospitalisation and death due to severe gastroenteritis in young children worldwide<sup>1</sup> Two live oral rotavirus vaccines have been developed (Rotarix® [GlaxoSmithKline] and RotaTeq® [Merck]) in an effort to decrease the large disease burden. Both vaccines were shown to be safe and highly effective in prevention of severe diarrhoea and hospitalisation due to rotavirus infections during large phase III clinical and efficacy trials, each involving over 60,000 children worldwide.<sup>2,3</sup>

Rotavirus vaccines have been commercially available in Australia from 2006 and were introduced into the Australian National Immunisation Program (NIP) for all infants from 1 July 2007. Each state health department made independent decisions on which vaccine to use; Victoria, South Australia, and Queensland selected RotaTeq, while New South Wales, Western Australia (changed to RotaTeq from May 2009), the Northern Territory, Tasmania and the Australian Capital Territory selected Rotarix. Introduction of rotavirus vaccines to the NIP is aimed to decrease the large social and economic burden of rotavirus disease in Australia. In the pre-vaccine era diarrhoea accounted for up to 50% of childhood hospitalisations in Australia, which represents 10,000 children hospitalised each year.<sup>4</sup>

The Australian Rotavirus Surveillance Program has been reporting the changing annual pattern of dominant genotypes in the Australian population since 1999. Over this period, the results have highlighted the diversity of rotavirus strains capable of causing disease in children, and provided the baseline information of the changing pattern of circulating strains, prior to vaccine introduction.<sup>5</sup>

The introduction of vaccines into Australia will increase population immunity. This is likely to have an impact on circulating wild-type strains. However, exactly what will happen is difficult to predict as strain replacement and changes in the prevalence of common genotypes, as well as emergence of new or rare genotypes, are all possible. Thus continuing genotype surveillance should identify the effects that each vaccine program has on circulating wild-type strains.

This report describes the surveillance and genotype characterisation of rotavirus strains causing severe gastroenteritis in young children 5 years of age or younger in Australia for the period 1 July 2009 to 30 June 2010.

## 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 National Rotavirus Reference Centre (NRRC) Melbourne, together with relevant age and sex details. Viral

RNA was extracted from each specimen using an RNA extraction kit (Qiamp Viral mini extraction kit, Qiagen) according to the manufacturers instructions. Double stranded RNA was used to determine the G and P genotype of each specimen by hemi-nested multiplex reverse transcription-polymerase chain reaction (RT-PCR) assay, using G or P specific oligonucleotide primers.<sup>6,7</sup>

## Results

### Number of isolates

A total of 778 specimens were received for analysis from 15 collaborating centres in Victoria, Western Australia, the Northern Territory, New South Wales, Queensland, South Australia and Tasmania. Samples were not obtained from the Australian Capital Territory. Thus the sample collection is likely to be highly representative of all Australian children hospitalised with acute gastroenteritis. Four hundred and twenty-two specimens from Victoria (n=55), Western Australia (n=98), New South Wales (n=35), Queensland (n=78), South Australia (n=18), Tasmania (n=1) and the Northern Territory (n=137), were confirmed as rotavirus positive using a combination of in-house EIA and RT-PCR analysis. The remaining 356 specimens contained either insufficient specimen for genotyping (n=154), or the specimen was not confirmed to be positive for rotavirus (n=202), and were not analysed further.

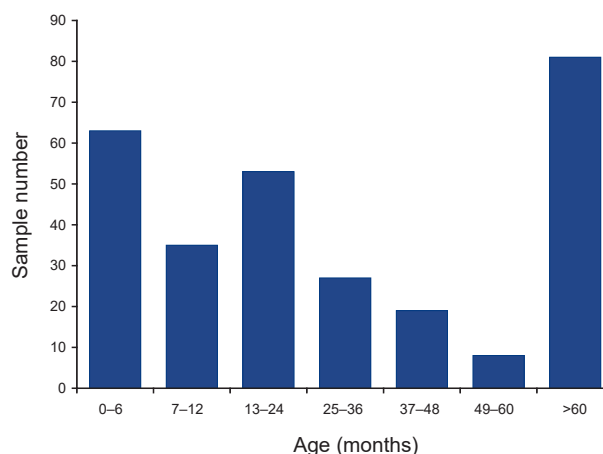
The Royal Children's Hospital in Melbourne was able to access approximately 55% of all faecal specimens identified as containing rotavirus antigen from children less than 5 years of age. However the collection rate at other centres was unknown.

### Age distribution

The overall age distribution of children with acute rotavirus gastroenteritis is depicted in Figure 1. During the reporting period, 22% of cases were from infants 0–6 months of age, 12% were from infants 7–12 months of age, and 19% from patients 13–24 months of age. Overall, 71% of samples were from children aged 5 years or less. Eighty-one samples were obtained from individuals more than 5 years of age; 23 were collected from children 5–10 years of age, 11 were from individuals 10–20 years of age, 31 were from individuals 21–80 years of age, and 16 were from individuals aged 80–100 years.

During the study period, slightly more specimens from male than female children (n=144 vs 141) were obtained for analysis.

**Figure 1: Distribution of rotavirus samples, Australia, 1 July 2009 to 30 June 2010, by age group**



### Genotype distribution

The rotavirus genotypes identified in Australia from 1 July 2009 to 30 June 2010 are shown in Table 1.

G1P[8] strains were the most common genotype identified, representing 49.3% of all specimens analysed, and was identified in all collaborating centres except Tasmania. It was the dominant type in the Northern Territory and Queensland, and 2nd most common strain in Western Australia, New South Wales and Victoria. G2P[4] strains were the 2nd most common type nationally, representing 21.1% of all specimens, and was the dominant type in South Australia and Western Australia. It represented 27% of samples in New South Wales, but less than 2% in Victoria, Queensland and the Northern Territory. G3P[8] or G3Pnt strains were the dominant type in Victoria representing 60% of strains. G3P[8] were identified in the three eastern states (Victoria, New South Wales and Queensland) and Western Australia, overall however, they represented only 6.6% of strains nationally. Five G9P[8] strains, one each from Sydney, Darwin, Melbourne and Perth, comprised 1.2% of samples analysed. A single G4P[8] strain was identified in Darwin, a single G4P[6] was identified in Tasmania, and a single G1P[6] was identified in Sydney.

Fourteen strains were found to possess uncommon genotype combinations of VP4 and VP7; 5 G1P[4] strains were identified in Queensland, Perth and Darwin and 4 G2P[8] strains were identified in Newcastle, Darwin and Melbourne. Three G9P[4] strains were identified in Sydney and Alice Springs. Single G8 strains were identified in Darwin and Perth. Eleven (2.6%) rotavirus samples contained multiple types.

Table 1: . Rotavirus G and P genotypes in Australia, 1 July 2009 to 30 June 2010

Centre	Type	G1P[8]		G2P[4]		G3P[8]		G4P[8]		G9P[8]		Mix*		Other†		Non-type	
		%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n
<b>New South Wales</b>																	
Sydney (POW)	7	28.6	2	28.6	2	28.6	2	-	0	14.2	1	-	0	-	0	-	0
Sydney (Westmead)	25	24.0	6	20.0	5	4.0	1	-	0	4.0	1	-	0	8.0	2	40.0	10
Newcastle	3	33.3	1	33.3	1	-	0	-	0	-	0	-	0	33.3	1	-	0
<b>Northern Territory</b>																	
Alice Springs	56	85.7	48	3.6	2	-	0	-	0	-	0	1.8	1	3.5	2	5.4	3
Darwin	33	72.7	24	3.0	1	-	0	-	0	-	0	-	0	3.0	1	21.3	7
Western Diagnostic (NT)	48	77.1	37	-	0	-	0	2.1	1	2.1	1	4.2	2	6.2	3	8.3	4
<b>Queensland</b>																	
	78	84.6	66	1.3	1	7.6	6	-	0	-	0	1.3	1	1.3	1	3.9	3
<b>South Australia</b>																	
Adelaide	18	5.5	1	83.5	15	-	0	-	0	-	0	5.5	1	-	0	5.5	1
<b>Tasmania</b>																	
Hobart	1	-	0	-	0	-	0	-	0	-	0	-	0	100.0	1	-	0
<b>Victoria</b>																	
Melbourne	55	14.6	8	1.8	1	29.0	16	-	0	1.8	1	3.6	2	3.6	2	45.6	25
<b>Western Australia</b>																	
PathWest WA	91	15.4	14	61.5	56	3.3	3	-	0	1.1	1	4.4	4	3.3	3	11.0	10
Perth	7	14.3	1	71.4	5	-	0	-	0	-	0	-	0	-	0	14.3	1
<b>Total</b>	<b>422</b>	<b>49.3</b>	<b>208</b>	<b>21.1</b>	<b>89</b>	<b>6.6</b>	<b>28</b>	<b>0.2</b>	<b>1</b>	<b>1.2</b>	<b>5</b>	<b>2.6</b>	<b>11</b>	<b>3.8</b>	<b>16</b>	<b>15.2</b>	<b>64</b>

\* Mix

Alice Springs G1P[4]/P[8]  
 Western Diagnostic (NT) 2xG1P[4]/P[8]  
 Queensland G1P[4]/P[8]  
 Melbourne G2P[4]/[8], G3/G2P[non-typeable]  
 PathWest WA G8P[4]/P[8], 2x G1/G2P[8], G1/G4P[8]  
 Adelaide G9/G2P[4]

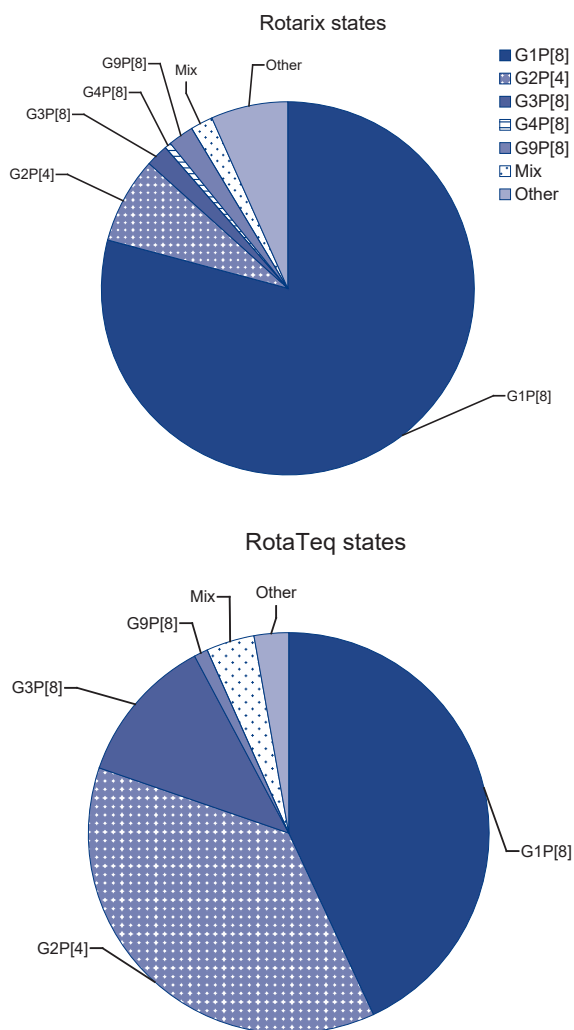
† Other

Westmead G1P[6], G9P[4]  
 Newcastle G2P[8]  
 Alice Springs 2x G9P[4]  
 Darwin G2P[8]  
 Western Diagnostic 3x G1P[4]  
 Queensland G1P[4]  
 PathWest WA G1P[4], G8P[4]/P[8], G8 P[non-typeable]  
 Melbourne 2x G2P[8]

In 15.2% of samples either a G- or P-Type, or both, could not be assigned (Table 2). These are likely to be samples with virus numbers below the detection 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. Future studies will include further characterisation of the genes encoding the outer capsid proteins of these strains.

The distribution of G and P genotypes in states using Rotarix (New South Wales, the Northern Territory and Tasmania) compared with distribution in states using RotaTeq (Victoria, Queensland, South Australia and Western Australia) is shown in Figure 2.

**Figure 2: Overall distribution of rotavirus G and P genotypes identified in Australian children based on vaccine usage for period 1 July 2009 to 30 June 2010**



Rotarix was used in New South Wales, Tasmania, and the Northern Territory.

RotaTeq was used in Victoria, South Australia, Western Australia and Queensland

The number of samples analysed was fairly consistent, with 149 strains from Rotarix sites and 209 from RotaTeq sites. However, 83% of samples in the Rotarix sites were from the Northern Territory, whereas strains from Victoria, Queensland and Western Australia represented 14%, 36% and 42% of the total number of samples analysed from states using RotaTeq.

Analysis of fully G and P typeable samples revealed that in Rotarix states G1P[8] strains dominate (79.2%), with G2P[4] strains comprising 7.4% of specimens. In RotaTeq states, G1P[8] was also dominant, identified in 43% of strains, G2P[4] comprised 37.3% of strains and G3P[8] represented 12% of strains. A slight increase in G3P[8] (12% vs 2%) and mixed strains (3.8% vs 2%) was observed in the states that introduced RotaTeq when compared with those using Rotarix. Conversely, a slight increase in uncommon strains was observed in Rotarix states versus RotaTeq states (6.7% vs 2.9%).

Faecal specimens were received from 24 children who developed rotavirus gastroenteritis after being vaccinated with RotaTeq. Vaccine virus was identified in seven of these cases by RT-PCR and sequence analysis. In addition, Rotarix vaccine was identified in a faecal specimen received from 1 child who developed rotavirus gastroenteritis after being vaccinated with Rotarix.

## Discussion

This report from the Australian Rotavirus Surveillance Program, covering the period 1 July 2009 to 31 June 2010, describes the annual epidemics and geographic distribution of rotavirus genotypes causing disease in Australian children. The surveillance program has showed that genotype G1P[8] re-emerged as the dominant genotype nationally, representing 49.3% of all strains. In part, this corresponded with a large outbreak of acute gastroenteritis in the Northern Territory during May to June 2010, as well as its emergence as the dominant type in Queensland and New South Wales. Genotype G2P[4] was the 2nd predominant type nationally, comprising 21.1% of all strains characterised. It was the dominant type in South Australia and Western Australia. Victoria was the only location where genotype G3 (G3P[8] or G3Pnt) was the dominant type, and continues to highlight its important role in acute gastroenteritis observed during past 2 surveys.<sup>8,9,10</sup> This survey highlights the ongoing fluctuations in the dominant genotypes, and reveals the return of G1P[8] as the dominant genotype nationally, similar to that observed in 2006–07 and 2007–08.

Similar to other reports,<sup>5,8,9</sup> multiple common genotypes (G1P[8], G2P[4], G3P[8], G4P[8])

Table 2: G and P genotype assignments in non-typeable specimens

Centre	Total	P non-typeable								G non-typeable			G & P non-typeable	
		G1	G2	G3	G4	G8	G9	G9	P[4]	P[6]	P[8]	NT		
New South Wales														
Sydney (POW)	0	-	-	-	-	-	-	-	-	-	-	-	-	-
Sydney (Westmead)	10	-	-	-	-	-	-	-	-	8	-	1	1	-
Newcastle	0	-	-	-	-	-	-	-	-	-	-	-	-	-
Northern Territory														
Alice Springs	3	-	1	-	-	-	-	-	-	-	1	-	-	-
Darwin	7	-	1	-	-	-	-	1	1	3	-	-	1	-
Western Diagnostic (NT)	4	4	-	-	-	-	-	-	-	-	-	-	-	-
Queensland														
	3	3	-	-	-	-	-	-	-	-	-	-	-	-
South Australia														
Adelaide	1	-	-	-	-	-	-	-	-	-	-	-	-	-
Tasmania														
Hobart	0	-	-	-	-	-	-	-	-	-	-	-	-	-
Victoria														
Melbourne	25	2	1	19	1	-	-	-	1	-	-	-	-	1
Western Australia														
PathWest WA	10	2	-	-	-	-	-	1	2	-	-	-	1	4
Perth	1	1	-	-	-	-	-	-	-	-	-	-	-	-
Total (%)	64	12 (18.8%)	3 (4.7%)	19 (29.7%)	1 (1.5%)	2 (3.1%)	6 (9.4%)	11 (17.2%)	1 (1.5%)	3 (4.7%)	6 (9.4%)			

and G9P[8]) continue to co-circulate within the Australian population causing significant disease with G1 and G2 identified in six states and territories, and G3 being identified in four states. In contrast, G4 and G9 strains each represented minor circulating strains.

The parallel usage of both vaccines in Australia provides a unique opportunity to compare the effect of each vaccine on the circulating wildtype strains. During the first 2 years post-vaccine introduction, differences have been observed in genotype distribution when vaccine usage was compared.<sup>8,9,10</sup> As previously reported the emergence of G2P[4] strains were more commonly identified in locations using Rotarix vaccine, while G3P[8] strains were more common in locations using RotaTeq.<sup>10,11,12</sup> In the current survey, the two locations where G2P[4] strains were dominant both used RotaTeq vaccine in their vaccine program. The lack of association with Rotarix vaccine seen during this survey period was supported by the emergence of G2P[4] reported in vaccinated populations in Nicaragua (RotaTeq) and to a lesser extent in non-vaccinated populations in Europe.<sup>13,14</sup> The association of G3P[8] and RotaTeq observed previously in our study, was not confirmed here. G3P[8] was only observed in one of the three states using RotaTeq vaccine, in the other states either G1P[8] or G2P[4] were the dominant strain. Therefore for both Rotarix and RotaTeq no clear association of vaccine driven genotype selection was observed during the 3rd year post-vaccine introduction.

The 2009–10 reporting period was also characterised by a large outbreak of acute diarrhoea in the Northern Territory between May and June 2010, caused by a G1P[8] virus. Unlike the recent G9P[8] and G2P[4] outbreaks in the Northern Territory, the G1P[8] represents an identical genotype as the Rotarix vaccine. It is unknown whether this strain emerged due to a lack of protection by the vaccine or by natural variation, since not all the subjects from whom samples were obtained were eligible for vaccination and the vaccination status of vaccine-eligible infants is unknown. However, the emergence of G1P[8] maybe an example of vaccine pressure, such that it resulted from genetic drift such that a divergent G1P[8] lineage was selected.

The rapid emergence and global spread of G9 and G12 strains in less than a decade illustrates the potential with which rotavirus can evolve.<sup>15</sup> Thus uncommon rotavirus types continue to be of worldwide interest because of the possible impact they could have on future rotavirus vaccine programs. This year several uncommon VP7/VP4 genotype combinations were again identified; including G1P[4], G2P[8], and G9P[4], several of which have existed in Australia for 2–3 years, albeit in low num-

bers. Similar to past surveys<sup>8,9</sup> G8 strains continue to be identified circulating in low levels in Australian children. The identification of uncommon G and P genotype combinations has increased in Australia since vaccine introduction, and may suggest that the wild-type strains are under an increased state of flux. Vaccine pressure may cause more mixing of strains with greater selection pressure occurring to identify 'fitter' strains, or natural variants that will replicate in a setting with greater immune pressure.

In Australia, where rotavirus mortality was rare prior to vaccine introduction, the decision to implement infant rotavirus vaccination was based upon the morbidity caused by rotavirus and the predicted cost-effectiveness of vaccination.<sup>16</sup> Recently the impact of rotavirus vaccination has shown not only reductions in rotavirus positive tests and hospital encounters, but also reductions in non-rotavirus coded episodes of gastroenteritis.<sup>17,18</sup> Importantly reductions in childhood gastroenteritis have been observed at all hospital levels. This impact has also been reported in others setting including the United States of America and Belgium.<sup>19,20</sup> These early data provide reassurance that vaccination has impacted directly and possibly indirectly upon gastroenteritis morbidity. However, in the Northern Territory vaccine effectiveness was not as high as seen elsewhere in Australia, where protection was evident for young infants with severe disease, but not for all cases resulting in hospitalisation.<sup>21</sup> It is possible that a waning of vaccine-induced immunity or increasing immunity from natural infection might account for the apparent decline in vaccine effectiveness amongst older infants in this setting.

The previous report (2008/09),<sup>9</sup> which represented the 2nd year of vaccine usage, showed a change in age distribution of children admitted to hospital. This observed increase in infants in the 0–6 month age group was also observed in the current survey where the highest proportion of children admitted to hospital was in the 0–6 month age group. Thus since the 2007/08 survey, the proportion of hospitalisation in the 0–6 month age group has increased from 14% to 27% to 30.7%. Interestingly, 71% of the infants in this age group were less than 3 months of age, an age group too young to receive complete vaccination. The proportion of children in the 7–12 month age group remained similar since the vaccine was introduced, but importantly lower than pre-vaccine rates.

This survey has further highlighted the continued fluctuations in rotavirus genotypes across Australia, and tends to support the notion that there is limited genotype selection based on vaccine usage. However, the rapidly changing genotype patterns do illustrate a more dynamic wild-type population. The recent report that estimated that a single novel rotavirus

strain could emerge and spread worldwide in less than a decade re-enforces the need for thorough and continued rotavirus surveillance. Thus the ongoing evolution of the wild-type strains circulating in Australia, under constant vaccine pressure will require close monitoring to identify any changes that may impact on vaccine effectiveness.

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