



**Australian Government**  
**Department of Health**

# COMMUNICABLE DISEASES INTELLIGENCE

2021      Volume 45  
<https://doi.org/10.33321/cdi.2021.45.6>

## **Australian Rotavirus Surveillance Program: Annual Report, 2018**

Susie Roczo-Farkas, Julie E Bines, and the Australian Rotavirus Surveillance Group

# Communicable Diseases Intelligence

ISSN: 2209-6051 Online

This journal is indexed by Index Medicus and Medline.

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# Australian Rotavirus Surveillance Program: Annual Report, 2018

Susie Roczo-Farkas, Julie E Bines, and the Australian Rotavirus Surveillance Group

## Abstract

This report, from the Australian Rotavirus Surveillance Program and collaborating laboratories Australia-wide, describes the rotavirus genotypes identified in children and adults with acute gastroenteritis during the period 1 January to 31 December 2018. During this period, 690 faecal specimens were referred for rotavirus G- and P- genotype analysis, including 607 samples that were confirmed as rotavirus positive. Of these, 457/607 were wild-type rotavirus strains and 150/607 were identified as rotavirus vaccine-like. Genotype analysis of the 457 wild-type rotavirus samples from both children and adults demonstrated that G3P[8] was the dominant genotype nationally, identified in 52% of samples, followed by G2P[4] (17%). The Australian National Immunisation Program, which previously included both RotaTeq and Rotarix vaccines, changed to Rotarix exclusively on 1 July 2017. Continuous surveillance is needed to identify if the change in vaccination schedule could affect rotavirus genotype distribution and diversity in Australia.

Keywords: rotavirus; gastroenteritis; genotype; surveillance; Australia; vaccine; RotaTeq; Rotarix; G3; P[8]; P[25]; G3P[8]; mis-priming; mis-typing; G9; primer slippage

## Introduction

Rotaviruses, of the *Reoviridae* family, are triple-layered capsid, double-stranded RNA viruses that contain a segmented genome, consisting of 11 gene segments that encode six structural proteins and six non-structural proteins.<sup>1</sup> A major process by which rotaviruses can evolve is attributed to the segmented genome, as this allows for reassortment both within and between human and animal strains, leading to the emergence of novel rotavirus strains.<sup>2</sup> A binary classification system is commonly used by surveillance programs to describe rotavirus genotypes based on the two outer capsid proteins, VP7 (G for glycoprotein) and VP4 (P for protease-sensitive).<sup>3</sup> Globally, there are five common genotype combinations identified in humans: G1P[8], G2P[4], G3P[8], G4P[8], and G9P[8]; however, G12P[8] has also increased in prevalence across multiple continents.<sup>4</sup> In addition, whole genome classification is used to assign genotypes to each gene: Gx-P[x]-Ix-Rx-

Cx-Mx-Ax-Nx-Tx-Ex-Hx, which are notations for the VP7-VP4-VP6-VP1-VP2-VP3-NSP1-NSP2-NSP3-NSP4-NSP5/6 encoding genes, respectively.<sup>5</sup> The majority of human rotavirus genomes fall under two genotype constellations: Wa-like (genogroup 1: G1/3/4/9/12-P[8]-I1-R1-C1-M1-A1-N1-T1-E1-H1); and DS-1-like (genogroup 2: G2-P[4]-I2-R2-C2-M2-A2-N2-T2-E2-H2).<sup>3,5</sup> A third genogroup, AU-1-like, is also detected in humans, however less frequently (genogroup 3: G3-P[9]-I3-R3-C3-M3-A3-N3-T3-E3-H3).<sup>3,5</sup>

Rotaviruses are the most common cause of severe diarrhoea in young children worldwide, and are estimated to have caused 215,000 deaths globally in 2013.<sup>6</sup> To reduce this burden, the rotavirus vaccines Rotarix<sup>®</sup> [GlaxoSmithKline] and RotaTeq<sup>®</sup> [Merck] have been introduced in the immunisation programs of 100 countries, with a further 11 countries planning to introduce the vaccines in 2019.<sup>7</sup> Both vaccines were included in the Australian

National Immunisation Program (NIP) on 1 July 2007, leading to a significant reduction in both rotavirus-coded and non-rotavirus-coded hospitalisations of children  $\leq 5$  years of age with acute gastroenteritis.<sup>8-10</sup> Over the first six years after implementation of the rotavirus immunisation program,  $\sim 77,000$  hospitalisations were prevented, 90% of which were in children  $\leq 5$  years, with indications of herd protection occurring in older age groups.<sup>10</sup> RotaTeq was administered in Queensland, South Australia, and Victoria, whereas Rotarix was administered in the Australian Capital Territory, New South Wales, the Northern Territory, and Tasmania. Western Australia initially administered Rotarix and changed to RotaTeq in May 2009.<sup>8</sup> On 1 July 2017, all states and territories in Australia changed to Rotarix.<sup>11</sup>

The Australian Rotavirus Surveillance Program (ARSP) has characterised and reported rotavirus genotypes causing severe disease in Australian children  $\leq 5$  years of age since 1999. Surveillance data generated by the ARSP has shown that changes in genotype diversity, as well as temporal and geographic changes, occur each year.<sup>12</sup> Furthermore, differences in genotype dominance were observed when comparing jurisdictions by vaccine use, suggesting that the RotaTeq and Rotarix vaccines exert different immunological pressures.<sup>12</sup> Ongoing characterisation of circulating rotavirus genotypes will provide insight into whether changes in vaccine immunisation programs could impact virus epidemiology, could alter circulating strains, or could cause vaccine escape strains. This could, in turn, have ongoing consequences for the success of current and future vaccination programs.

This report describes the G- and P- genotype distribution of rotavirus strains causing severe gastroenteritis in Australia, for the period 1 January to 31 December 2018. This is the first report of the ARSP that describes a full 12 months after exclusive use of Rotarix vaccine within the NIP.

## Methods

Rotavirus-positive faecal specimens (detected by quantitative Reverse Transcription Polymerase Chain Reaction (RT-qPCR), enzyme immunoassay (EIA), or latex agglutination in collaborating laboratories across Australia) were collected, stored frozen, and forwarded to the Australian Rotavirus Reference Centre (NRRC) Melbourne, together with metadata including date of collection (DOC); date of birth (DOB); gender; postcode; and the collaborating laboratory rotavirus RT-qPCR Ct values. These specimens were received from the following 13 collaborating centres across Australia, located in New South Wales (NSW), Queensland (Qld), South Australia (SA), Tasmania (Tas.), Victoria (Vic.), and Western Australia (WA) (n = number of specimens received from the indicated state):

- Microbiology Department, SEALS-Randwick, Prince of Wales Hospital, NSW (n = 15).
- Virology Department, The Children's Hospital at Westmead, NSW (n = 20).
- Microbiology Department, John Hunter Hospital, Newcastle, NSW (n = 12).
- Microbiology Department, Central Coast, Gosford, NSW (n = 4).
- Douglas Hanly Moir Pathology, NSW (n = 32).
- Queensland Paediatric Infectious Diseases laboratory, Royal Children's Hospital, Brisbane, Qld (n = 145).
- Queensland Health laboratory, Townsville, Qld (n = 2).
- Microbiology and Infectious diseases laboratory, SA Pathology, Adelaide, SA (n = 136).
- Molecular Medicine, Pathology Services, Royal Hobart Hospital, Hobart, Tas. (n = 1).

- Department of Microbiology, Monash Medical Centre, Clayton, Vic. (n = 56).
- Molecular Infectious Department, Australian Clinical Labs, Clayton, Vic. (n = 20).
- Serology Department, Royal Children's Hospital, Parkville, Vic. (n = 71).
- QEII Microbiology Department, PathWest Laboratory Medicine, Nedlands, WA (n = 176).

No samples were sent directly from the Northern Territory (NT) or the Australian Capital Territory (ACT) for 2018. Samples from the Northern Territory were referred to interstate collaborators that routinely perform rotavirus diagnostic tests: Microbiology and Infectious diseases laboratory, SA Pathology, Adelaide, SA (n = 5), and QEII Microbiology Department, PathWest Laboratory Medicine, Nedlands, WA (n = 2). These samples were subsequently forwarded to the NRRC together with the other rotavirus positives listed above.

Upon receipt, samples were allocated a unique laboratory code and entered into the NRRC sample tracking database (Excel and REDCap). Samples were then stored at -80 °C until analysed. The presence of rotavirus antigen was confirmed using the ProSpecT™ Rotavirus Test, a commercial rotavirus EIA assay (Thermo Fisher), as per the manufacturer's instructions. Samples confirmed as rotavirus positive underwent genotyping analysis, whereas unconfirmed samples (EIA negative) were not processed further (Figure 1).

Viral RNA was extracted from 10–20% 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 RT-PCR assay. The first-round RT-PCR reactions were performed using the Qiagen One Step RT-PCR kit (Qiagen), in conjunction with 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 G1, G2, G3, G4, G8, and G9, or P types P[4], P[6], P[8], P[9], P[10], and P[11].<sup>13–16</sup> The G- and P- genotype of each sample was assigned using agarose gel electrophoresis and analysis of second-round PCR products. Samples for which no amplicon was present in the second round, or which had inconclusive results, were designated as G- or P- non-typeable.

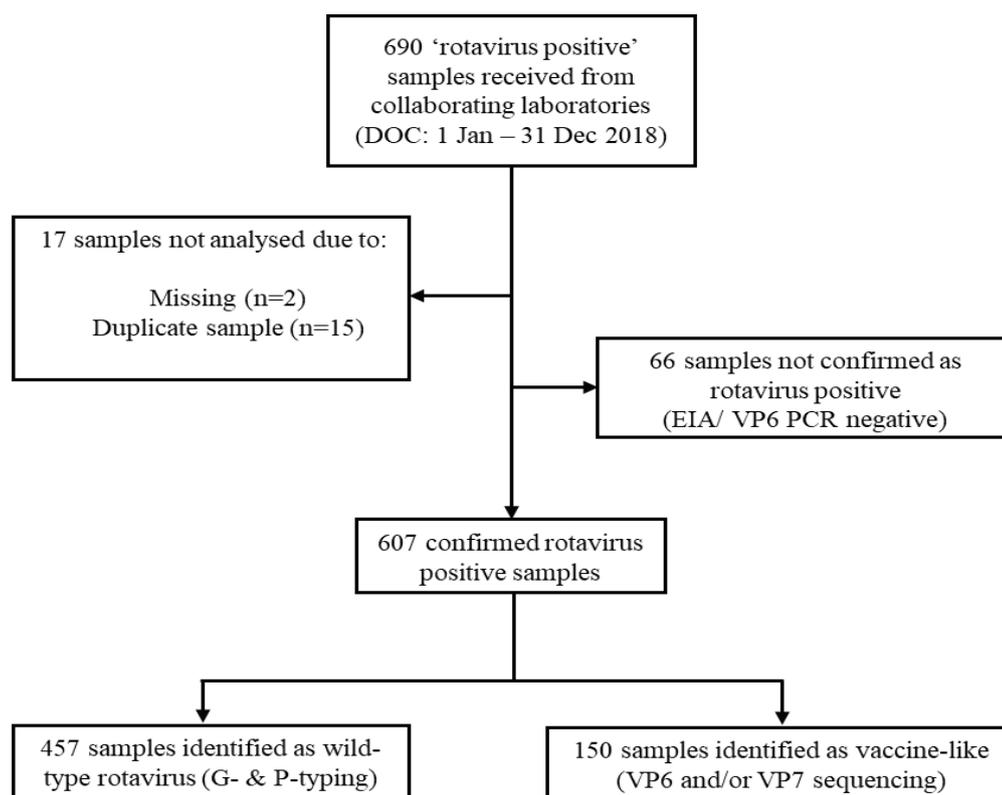
The VP7 and VP4 nucleotide sequence from PCR non-typeable samples was determined by Sanger sequencing, as the primers used in the current second-round G-typing protocol could not assign a genotype to equine-like G3, G12, and unusual or uncommon rotavirus strains. Suspect vaccine excretion cases were also sequenced. Selection criteria for vaccine suspect include age under 8 months, and genotyped as G1P[8] (Rotarix) or any G1/G3/G4 type with P[nt] (RotaTeq). First round VP7 or VP4 amplicons were purified for sequencing using the Wizard SV Gel for PCR Clean-Up System (Promega), according to the manufacturer's protocol. Purified DNA, together with oligonucleotide primers (VP7F/R or VP4F/R), was sent to the Australian Genome Research Facility, Melbourne, and sequenced using an ABI PRISM BigDye Terminator Cycle Sequencing Reaction Kit (Applied Biosystems) in an Applied Biosystems 3730xl DNA Analyzer (Applied Biosystems). Electropherograms were visually analysed and edited using Sequencher v.4.10.1. Genotype assignment was determined using BLAST<sup>i</sup> and RotaC v2.0.<sup>17,ii</sup> Comparison of VP7 sequences to primer sequences was performed using MEGA version 6.<sup>18</sup>

Samples sent or identified as vaccine-like were confirmed as exhibiting genes of vaccine origin by amplifying a portion of the inner capsid VP6 gene, using human Rot3/Rot5 primers and

i <http://blast.ncbi.nlm.nih.gov/Blast.cgi>.

ii <http://rotac.regatools.be>.

Figure 1: Stool sample flowchart



Superscript III One-Step RT-PCR System with Platinum Taq DNA Polymerase (Invitrogen), as previously described.<sup>19,20</sup>

## Results

### Number of specimens

A total of 690 rotavirus-positive faecal specimens were sent to the NRRC during the period 1 January to 31 December 2018, for genotyping analysis (Figure 1). A subset of samples were not analysed due to samples either not received ( $n = 2$ ), duplicate ( $n = 15$ ), or unable to be confirmed as rotavirus positive by EIA ( $n = 66$ ).

In 2018, 607 rotavirus-positive samples were identified from patients clinically diagnosed with acute gastroenteritis. For analysis, these samples were classified based on whether a sample had no vaccine component identified

(described herein as ‘wild-type rotavirus’) or had a vaccine component identified based on VP6 or VP7 sequence analysis (‘vaccine-like’). A total of 457 samples were confirmed as wild-type rotavirus positive by EIA (ProSpecT, OXOID) and RT-PCR analysis. Of these, 207 were collected from children  $\leq 5$  years of age, and 250 were from older children and adults. In addition, 150 samples were identified as rotavirus vaccine-like by VP6 and/or VP7 sequencing.

### Rotavirus-positive samples identified by month, compared to national notification rates

Rotavirus-positive samples were analysed by date of collection [month], to determine the peak season represented (Figure 2). The majority of wild-type specimens were collected during June–October, peaking in September ( $n = 82/457$ ), which coincides with the winter-spring

period in the southern hemisphere. The least number of specimens were positive during the March–May (autumn) season. This trend has also been observed when reviewing the National Notifiable Diseases Surveillance System (NNDSS) national notification rates, where notification rates were lowest in April 2018 (0.7 per 100,000 population), but peaked between June and September (1.1–1.3 per 100,000 population).<sup>21</sup> Notification rates were higher than average in January 2018; however, this is likely attributed to the completion of multiple outbreaks that occurred in 2017.<sup>22</sup> Vaccine-like rotavirus strains were progressively detected each month (range: 7–19 samples identified each month).

## Wild-type rotavirus specimens

### Age distribution for wild-type rotavirus infections

From 1 January to 31 December 2018, 45.3% (n = 207/457) of rotavirus-positive samples were obtained from children ≤ 5 years of age (Table 1). Of the subset of children in the ≤ 5 years of age category, 35.3% of cases (n = 73/207) were identified in children 13–24 months old, while the next most common age group was 7–12 months which accounted for 20.8% of cases (n = 43/207). In addition, 40.5% of all samples (185/457) were from individuals older than 20 years of age.

## Wild-type rotavirus genotype distribution

Genotype analysis was performed on the 457 confirmed rotavirus-positive cases from children and adults (Table 2). G3P[8] was the most common genotype identified nationally, representing 52% of all specimens analysed. This genotype was identified as the dominant genotype in the Northern Territory, Queensland, South Australia, Victoria, and Western Australia, representing 100%, 41%, 59%, 60%, and 66% of strains respectively.

G2P[4] was the second most common genotype found in Australia, representing 17% of all strains nationally (Table 2). The largest number were from New South Wales, representing 25% of all strains identified within the state. G2P[4] was also prevalent in Queensland (26%), South Australia (14%), Victoria (11%), and Western Australia (12%). Other common genotypes identified nationally in 2018 included G9P[8] (10%), equine-like G3P[8] (5%), and G8P[8] (4%).

Forty (9% of rotavirus positive) specimens were identified as ‘other’, listed in Table 3. G9P[4] was detected in Queensland (n = 3), South Australia (n = 1), Victoria (n = 1), Western Australia (n = 2), and New South Wales (n = 9). Two samples were of mixed genotype (G3/G9P[8], and G9P[4]/P[8]). The remaining 22 samples represented 12 uncommon rotavirus genotypes.

**Table 1: Age distribution of wild-type rotavirus gastroenteritis cases**

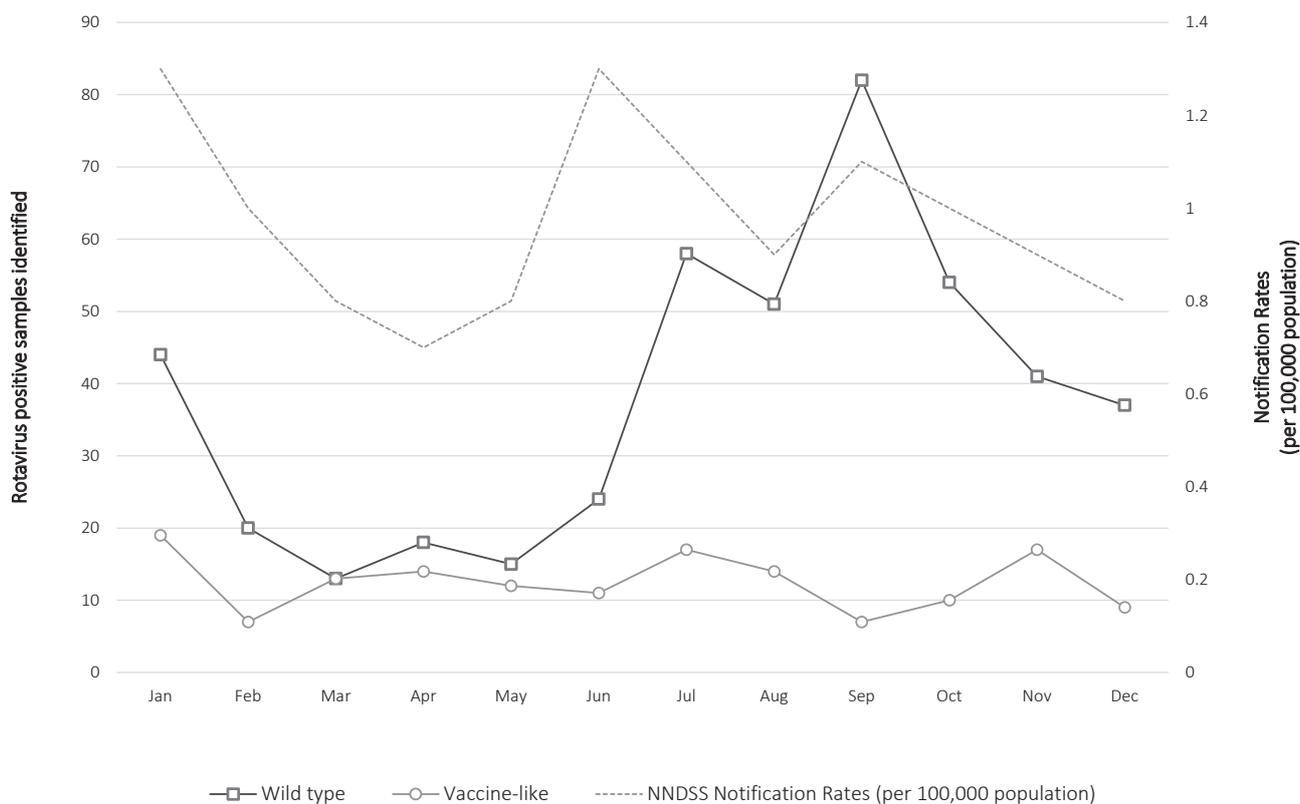
Age (months)	Age (years)	Number of cases	Percentage of total	Percentage under 5 years
0–6	≤1	28	6.1	13.5
7–12		43	9.4	20.8
13–24	1–≤2	73	16.0	35.3
25–36	2–≤3	32	7.0	15.5
37–48	3–≤4	19	4.2	9.2
49–60	4–≤5	12	2.6	5.8
<b>Subtotal</b>		<b>207</b>	<b>45.3</b>	<b>100</b>
61–120	5–≤10	37	8.1	
121–240	10–≤20	28	6.1	
241–960	20–≤80	155	33.9	
961+	>80	30	6.6	
<b>Total</b>		<b>457</b>	<b>100</b>	

**Table 2: Rotavirus G and P genotype distribution in infants, children and adults, 1 January to 31 December 2018**

Jurisdiction	Type total <sup>a</sup>		G1P[8]		G2P[4]		G3P[8]		G8P[8]		G9P[8]		G12P[8]		Non-type <sup>c</sup>		Other <sup>d</sup>		
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	
Australian Capital Territory	ND	–	0	–	0	–	0	–	0	–	0	–	0	–	0	–	0	–	
New South Wales	57	3.5	14	24.6	8	14.0	4	7.0	1	1.8	6	10.5	0	–	5	8.8	17	29.8	
Northern Territory	6	–	0	–	6	100.0	0	–	0	–	0	–	0	–	0	–	0	–	
Queensland	100	1.0	26	26.0	41	41.0	9	9.0	4	4.0	12	12.0	0	–	0	–	7	7.0	
South Australia	83	1.2	12	14.5	49	59.0	3	3.6	0	–	11	13.3	0	–	1	1.2	6	7.2	
Tasmania	ND	–	0	–	0	–	0	–	0	–	0	–	0	–	0	–	0	–	
Victoria	75	1.3	8	10.7	45	60.0	1	1.3	6	8.0	6	8.0	1	1.3	4	5.3	3	4.0	
Western Australia	136	0.7	16	11.8	90	66.2	4	2.9	6	4.4	9	6.6	0	–	3	2.2	7	5.1	
<b>Total</b>	<b>457</b>	<b>6</b>	<b>1.3</b>	<b>76</b>	<b>16.6</b>	<b>239</b>	<b>52.3</b>	<b>21</b>	<b>4.6</b>	<b>17</b>	<b>3.7</b>	<b>44</b>	<b>9.6</b>	<b>1</b>	<b>0.2</b>	<b>13</b>	<b>2.8</b>	<b>40</b>	<b>8.8</b>

a ND: no data.  
b Equine-like G3P[8].  
c A specimen where G- and/or P- genotype was not determined.  
d See Table 3.

**Figure 2: Number of analysed wild-type and vaccine-like specimens compared to NNDSS rotavirus notification rates per 100,000 population,<sup>a</sup> Australia, 1 January to 31 December, 2018**



<sup>a</sup> NNDSS – National Notifiable Diseases Surveillance System notification rates for rotavirus.<sup>21</sup> Note: Victoria did not report notification rates in 2018.

Four of these samples included unusual genotype combinations, such as G1P[4], G1P[6], G2P[8], and G12P[6]. The remaining 18 samples exhibited an animal (i.e. bovine, equine, bat, or wild boar) VP7 and/or VP4 gene: G3P[6] (n = 1), G3P[9] (n = 1), G4P[6] (n = 1), G9P[9] (n = 1), G6P[14] (n = 4), G10P[14] (n = 1), and G10P[25] (n = 1).

### Genotypes identified in samples from children ≤ 5 years of age

A total of 207 wild-type rotavirus samples in total were collected from children ≤ 5 years of age (Table 4). Within this subset, G3P[8] was the most common genotype identified, found in 65% of all samples, followed by G2P[4] as the second most common genotype (11%). G1P[8], equine-like G3P[8],<sup>23</sup> G8P[8], G9P[8], and G12P[8], represented minor genotypes, identified in 2–5% of all samples (Table 4).

### Genotypes identified in samples from individuals > 5 years of age

A total of 250 rotavirus-positive samples were collected from children > 5 years of age and adults (Table 5). Similar to the ≤ 5 years of age cohort, G3P[8] was the dominant genotype identified (42%), followed by G2P[4] (22%) and G8P[8] (11%).

### Vaccine-like rotavirus specimens:

#### Age distribution for rotavirus vaccine cases

During the 2018 reporting period, 150 samples were identified as rotavirus vaccine-like by VP6 and/or VP7 sequencing, of which 96% (n = 144/150) were from the 0–6 months of age group. Vaccine-like rotavirus genes were also detected in patients aged 11 months and 1, 9, 36, 56, and 85 years old.

**Table 3: Mixed and unusual G and P genotypes identified in infants, children and adults, 1 January to 31 December 2018**

Genotype	Qld	SA	Vic.	WA	NSW	Total
G1P[4]	–	–	–	–	1	1
G1P[6]	–	–	–	–	1	1
G2P[8]	–	–	–	2	–	2
G3P[9]	–	–	–	1	–	1
G4P[6]	–	–	1	–	–	1
G9P[4]	3	1	1	2	9	16
G9P[9]	–	–	–	1	–	1
G12P[6]	–	–	1	–	1	2
Equine-like G3P[4]	–	–	–	1	–	1
G6P[14]	–	3	–	–	1	4
G8P[14]	2	2	–	–	2	6
G10P[14]	–	–	–	–	1	1
G10P[25]	1	–	–	–	–	1
Mixed G3/G9 P[8]	–	–	–	–	1	1
Mixed G9 P[4]/P[8]	1	–	–	–	–	1
<b>Total</b>	<b>7</b>	<b>6</b>	<b>3</b>	<b>7</b>	<b>17</b>	<b>40</b>

### Genotype distribution of specimens containing rotavirus vaccine component

The 150 samples that had sequence confirmation of vaccine-like VP6 and/or VP7 were processed further for genotype analysis. All samples identified as Rotarix (n = 149) were genotyped as G1P[8], while a single RotaTeq-like sample was typed as G1P[non-typeable]. The P [non-typeable] result for this sample was likely due to the bovine P[5] component of RotaTeq; however, the separate hemi-nested RT-PCR required to genotype P[5] was not performed, as it is not routinely used for rotavirus surveillance.

### Incorrect genotype assignment of G3 specimens as G9 by gel electrophoresis:

Mis-priming events were detected frequently in samples that were sequenced as various strains of G3, but were genotyped by gel electrophoresis as mixed G3/G9 or G9. An investigation of the primer binding region for the G9 primer used in the genotyping assay revealed that the G9 primer shared highly homologous regions

with some Australian G3 strains (Figure 3). The sample WAPC3112 was genotyped as G9 by gel electrophoresis, however shared 99% sequence identity with a G3 strain identified in Russia (RVA/Human-wt/RUS/Omsk/O211/2007/G3P[9]). This particular sample was homologous to the G9 primer for 6 bases at the 3' end, signifying that the G9 primer was able to easily bind to the template DNA. Other samples that were genotyped as mixed G3/G9 but were revealed as G3 by sequencing (not mixed infection) also showed high levels of homology with the G9 primer, however the primer-binding region was less conserved than for WAPC3112. The mis-typed specimens also had 'TTG' that was one base out from the 'TTG' of the 5' end of the G9 primer. This shift would have been sufficient for primer slippage to occur, which was observed in these samples as smeared G3 bands on the electrophoresis gel. Equine-like G3 samples identified in 2018 had no G9 band present in the electrophoresis gel, which could be attributed to the 'CGGC' at the 3' end of the G9 primer binding region (Figure 3).

**Table 4: Rotavirus G and P genotype distribution in infants and children ≤ 5 years of age, 1 January to 31 December 2018**

Jurisdiction	Type total <sup>a</sup>	G1P[8]		G2P[4]		G3P[8]		G3P[8] <sup>b</sup>		G8P[8]		G9P[8]		G12P[8]		Non-type <sup>c</sup>		Other <sup>d</sup>	
		n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Australian Capital Territory	ND	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-
New South Wales	26	2	7.7	7	26.9	3	11.5	2	7.7	0	-	1	3.8	0	-	2	7.7	9	34.6
Northern Territory	6	0	-	0	-	6	100.0	0	-	0	-	0	-	0	-	0	-	0	-
Queensland	47	1	2.1	8	17.0	26	55.3	3	6.4	1	2.1	6	12.8	0	-	0	-	2	4.3
South Australia	26	0	-	2	7.7	19	73.1	1	3.8	0	-	1	3.8	0	-	1	3.8	2	7.7
Tasmania	ND	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-
Victoria	34	0	-	2	5.9	22	64.7	0	-	3	8.8	3	8.8	1	2.9	2	5.9	1	2.9
Western Australia	68	1	1.5	3	4.4	59	86.8	0	-	2	2.9	0	-	0	-	2	2.9	1	1.5
<b>Total</b>	<b>207</b>	<b>4</b>	<b>1.9</b>	<b>22</b>	<b>10.6</b>	<b>135</b>	<b>65.2</b>	<b>6</b>	<b>2.9</b>	<b>6</b>	<b>2.9</b>	<b>11</b>	<b>5.3</b>	<b>1</b>	<b>0.5</b>	<b>7</b>	<b>3.4</b>	<b>15</b>	<b>7.2</b>

a ND: no data.

b Equine-like G3P[8].

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

d See Table 3.

**Figure 3: Comparison of 2018 G3 strain sequence to G9 primer sequence**

G9 primer	5'	C	T	G	A	T	G	A	C	T	A	C	T	A	A	A	T	A	C	3'	Genotype result (gel)	Sequence analysis - top BLAST hit (final result)
WAPC3112	T	.	G	A	.	C	.	A	.	G	.	C	.	.	.	.	.	.	.	.	G9	G3 (99% identity to "RVA/Human-wt/RUS/Omsk/0211/2007/G3P[9]")
SA3057	T	.	G	A	G	C	.	.	.	A	.	C	.	.	.	.	C	.	.	.	G3/G9	G3 (99.88% identity to "RVA/Human-wt/IND/hb14-18/IVRI/G3P8")
SA3080	T	.	G	A	G	C	.	.	.	A	.	C	.	.	.	.	C	.	.	.	G3/G9	G3 (99.88% identity to "RVA/Human-wt/IND/hb14-18/IVRI/G3P8")
Q2092	T	.	G	A	G	C	.	.	.	A	.	C	.	.	.	.	C	.	.	.	G3/G9	G3 (99.75% identity to "RVA/Human-wt/IND/hb14-18/IVRI/G3P8")
POW507	T	.	G	A	.	C	.	.	.	A	.	C	.	.	.	.	C	.	.	.	G3/G9	G3 (99.5% identity to "RVA/Human-wt/ETH/BD265/2016/G3P[8]")
NSW186	.	.	A	.	.	.	.	T	.	.	.	C	G	G	C	.	.	.	.	.	non typeable	Equine-like G3 (100% identity to "RVA/Human-wt/BRA/IAL-R608/2015/G3P[8]")
NSW193	.	.	A	.	.	.	.	T	.	.	.	C	G	G	C	.	.	.	.	.	non typeable	Equine-like G3 (100% identity to "RVA/Human-wt/BRA/IAL-R608/2015/G3P[8]")
Q2119	.	.	A	.	.	.	.	T	.	.	.	C	G	G	C	.	.	.	.	.	non typeable	Equine-like G3 (100% identity to "RVA/Human-wt/BRA/IAL-R608/2015/G3P[8]")
WAPC3085	.	.	A	.	.	.	.	T	.	.	.	C	G	G	C	.	.	.	.	.	non typeable	Equine-like G3 (100% identity to "RVA/Human-wt/BRA/IAL-R608/2015/G3P[8]")
NSW202	.	.	A	.	.	.	.	T	.	.	.	C	G	G	C	.	.	.	.	.	non typeable	Equine-like G3 (99.87% identity to "RVA/Human-wt/BRA/IAL-R608/2015/G3P[8]")
Q2120	.	.	A	.	.	.	.	T	.	.	.	C	G	G	C	.	.	.	.	.	non typeable	Equine-like G3 (99.87% identity to "RVA/Human-wt/BRA/IAL-R608/2015/G3P[8]")
Q2140	.	.	A	.	.	.	.	T	.	.	.	C	G	G	C	.	.	.	.	.	non typeable	Equine-like G3 (99.72% identity to "RVA/Human-wt/BRA/IAL-R608/2015/G3P[8]")

Table 5: Rotavirus G and P genotype distribution in children over 5 years of age and adults, 1 January to 31 December 2018

Jurisdiction	Type total <sup>a</sup>	G1P[8]		G2P[4]		G3P[8]		G3P[8] <sup>b</sup>		G8P[8]		G9P[8]		G12P[8]		Non-type <sup>c</sup>		Other <sup>d</sup>	
		n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Australian Capital Territory	ND	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-
New South Wales	31	0	-	7	22.6	5	16.1	2	6.5	1	3.2	5	16.1	0	-	3	9.7	8	25.8
Northern Territory	0	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-
Queensland	53	0	-	18	34.0	15	28.3	6	11.3	3	5.7	6	11.3	0	-	0	-	5	9.4
South Australia	57	1	1.8	10	17.5	30	52.6	2	3.5	0	-	10	17.5	0	-	0	-	4	7.0
Tasmania	ND	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-
Victoria	41	1	2.4	6	14.6	23	56.1	1	2.4	3	7.3	3	7.3	0	-	2	4.9	2	4.9
Western Australia	68	0	-	13	19.1	31	45.6	4	5.9	4	5.9	9	13.2	0	-	1	1.5	6	8.8
<b>Total</b>	<b>250</b>	<b>2</b>	<b>0.8</b>	<b>54</b>	<b>21.6</b>	<b>104</b>	<b>41.6</b>	<b>15</b>	<b>6.0</b>	<b>11</b>	<b>4.4</b>	<b>33</b>	<b>13.2</b>	<b>0</b>	<b>0.0</b>	<b>6</b>	<b>2.4</b>	<b>25</b>	<b>10.0</b>

a ND: no data.  
b Equine-like G3P[8].  
c A specimen where G- and/or P- genotype was not determined.  
d See Table 3.

## Discussion

The 2018 Australian Rotavirus Surveillance Program (ARSP) report describes the distribution of rotavirus genotypes causing gastroenteritis in Australia, for the period of 1 January to 31 December 2018. This is the first year where Rotarix was used exclusively in the Australian NIP, after Queensland, South Australia, Victoria, and Western Australia changed from RotaTeq to Rotarix on 1 July 2017.<sup>11,22</sup> The introduction of rotavirus vaccines to immunisation programs has been reported to change rotavirus epidemiological patterns from annual to biennial peaks, a trend that has previously been observed through the ARSP.<sup>24,25</sup> Indeed, compared to 2017, the 2018 reporting period overall had low incidence of national rotavirus notifications for Australia, which was reflected in the low number of rotavirus positives received for this reporting period.<sup>21,22</sup> This was further supported by the observation that the peak of wild-type rotavirus specimens received by the ARSP reflected the rotavirus notification seasonal trend identified by the NNDSS.<sup>21</sup> Although rotavirus notifications peaked in June, the largest number of wild-type rotavirus specimens were received in September, demonstrating that some collection-bias has occurred as collaborators were encouraged to actively collect samples when the rotavirus season commenced.

During this reporting period, it was observed that children aged 13–24 months and adults 20–80 years of age were the main age groups affected by rotavirus infection. This shift in age towards an older population has previously been observed by the ARSP and by global surveillance.<sup>22,26–30</sup> Waning immunity (both vaccine and naturally-acquired) and child-to-adult transmission are potential mechanisms that could have led to an increase of rotavirus incidents in the older population. Therefore, the question was raised whether current licensed rotavirus vaccinations should be considered for other age groups such as the elderly, in order to further reduce rotavirus disease burden.<sup>27</sup>

Vaccine-like genes were detected consistently throughout the calendar year, and did not reflect the rotavirus seasonal peak. Importantly, rotavirus vaccine-like strains were not only detected in the expected cohort of recently-vaccinated children (0–8 months of age), but also in 6 individuals that ranged in ages from 11 months to 85 years of age. These observations are only based on a single gene (VP7); however, reassortment between wild-type and vaccine strains has been previously observed, and could be transmitted in the community if the vaccine-derived G1P[8] strain is fit for replication in the human host.<sup>31,32</sup> Sequence confirmation of vaccine-like samples (i.e. G1P[8], and G1/G3/G4 with P[8] or P[nt] if RotaTeq is in use) is of relevance, otherwise the incidence of wild-type infections involving common genotypes such as G1P[8], G3P[8], or G4P[8] could be over-represented.

Since vaccine introduction in the Australian NIP, the G1P[8] strain has drastically decreased in prevalence from 53.4% in the pre-vaccine era to 26.2% in the vaccine era.<sup>12</sup> An unexpected observation was the dominance of G3P[8] in five out of six states and territories. Since vaccine introduction to the NIP, G3P[8] has circulated at very low levels in Australia, ranging from 1.8% to 10% of all genotypes identified (2012 and 2008 calendar years respectively), excepting 2011, where it was the second-most-dominant strain nationally (36.1%).<sup>12</sup> Prior to vaccine introduction, G3 was infrequently detected, with peaks during 2003 (20.1%), 2004 (36.1%), and 2006 (28.5%).<sup>12</sup> These figures exclude the DS-1-like equine-like G3P[8] variant identified from 2012 onwards, which became a dominant strain in Australia during 2013–2017, primarily in states and territories using Rotarix.<sup>12,23</sup> The replacement of Wa-like G3P[8] strains by DS-1-like equine-like G3P[8] was also observed in Brazil, where Rotarix vaccine is in use.<sup>33</sup> These observations, predominantly in countries using the Rotarix vaccine, raised the question of whether vaccines could induce selective pressures that favour zoonotic strains.<sup>33</sup> Nonetheless, the emergence of unusual reassortant G3 strains has also been reported in countries without rotavirus vaccine; thus, further investigation is required to elucidate how novel/

unusual strains can emerge and spread between countries with and without rotavirus vaccination programs.<sup>34,35</sup>

It is unclear how or why G3P[8] emerged as the dominant genotype in Australia; however, a combination of various factors such as selective immune pressures (either from vaccination or natural infection); accumulation of point mutations; and genetic reassortment may have contributed to the fitness and sustainability of existing and novel rotavirus strains. Other countries that recently have reported an upsurge of G3P[8] strains include Bangladesh and India, in districts that did not have a rotavirus vaccine immunisation program available at the time.<sup>34,35</sup> G3P[8] re-emerged in 2016 (17%) after 11 years in Dhaka, Bangladesh, while G1P[8] dominance was replaced by G3P[8] (44.6%) in the Kolkata region of Eastern India during the 2015–2016 surveillance period.<sup>34,35</sup> Furthermore, the Eastern India surveillance identified multiple strains of G3 co-circulating, including Wa-like G3P[8] and DS-1-like G3P[4], that had mutations in major neutralising epitope regions of VP7.<sup>34</sup> The VP7 sequences generated during the course of their surveillance had > 99.75% nucleotide similarity to some of the G3 specimens analysed in this 2018 report, suggesting that these new G3's are able to rapidly disseminate and cause disease, even to countries with high vaccine uptake.

Although both globally-licensed vaccines have been shown to provide broad heterotypic protection against common genotypes that have Wa-like or DS-1-like genogroup constellations (i.e. G1P[8], G2P[4], G3P[8], G4P[8], and G9P[8]), an increase in inter-genogroup reassortant strains, unusual genotypes, and zoonotic strains, including equine-like G3P[8] and G12P[8], has created uncertainty over how vaccines will perform against these novel or unusual rotavirus strains.<sup>33,36</sup> Despite a low number of specimens received for 2018, 40/457 specimens were not genotyped as one of the common genotypes listed in Table 2. Instead, 13 unusual or rare genotype combinations were detected that appear to be potential inter-genogroup reassortants such as G1, G3, G9 with P[4], G2P[8],

or zoonotic in nature, including bovine-like G8, G10, P[6], P[14]; bat-like G3; and feline-like G3, P[3], and P[9]. A single G10P[25] strain has also been identified, presenting a genotype combination not described previously in the literature. P[25] has been reported in Nepal, Bangladesh, India, and South Korea, in association with a G11 genotype, and in Taiwan in association with a G3 genotype.<sup>37–43</sup> Phylogenetic analysis of these P[25] strains reveal human-porcine reassortant events had occurred; however, the host species origin could not be deduced.<sup>40</sup> Further characterisation of the unusual strains detected during this surveillance period would be useful to identify new genotype constellations that could potentially evade protection inferred by natural or vaccine-acquired immunity.

Whole genome characterisation is required to identify unusual genotype constellations; however, this method is not routinely used due to the increased resources required. Although VP7 and VP4 genotyping identifies the genotypes that are circulating, it does not distinguish between pre-existing genotypes and new reassorted variants that may evade vaccine-induced immunity. Moreover, the binary system alone does not make allowances for the comparison of local strains in relation to global strains identified within a given period. A subset of the G3 genotypes identified in Australia in 2018 are similar to the Indian 2015 reassortant strains. These samples were recognised due to smearing of the G3 band on the electrophoresis gel, in conjunction with a non-specific band at ~180 base pairs (corresponding to G9). Sequence confirmation was undertaken to confirm these mixed G3/G9 specimens, however only G3 was identified (Figure 3). A comparison of the G9 primer and these specimens revealed that the gene sequence had induced primer slippage due to the 'TTG' at the 5' end, while the remainder of the primer had high homology towards the 3' end of the G9 primer (Figure 3). Sub-optimal annealing temperatures of the multiplex PCR, as well as the conserved region at the 3' end of the primer, caused some samples to be mis-typed as G9. Of interest, the equine-like G3 samples collected in 2018 had no mis-typing issues, which could be attributed to the 'CGGC' at the 3' end.

Previously, there have been problems with some of the equine-like G3 mis-typing as G9 upon gel electrophoresis. Aside from G3/G9 mis-typing, other common mis-priming issues identified by the ARSP include G8 and RotaTeq bovine G6 presenting as G1 and/or G12, when the G12 primer was included in the mix.<sup>44</sup> Porcine-like G4 also genotyped as either G12, G3, or G9. These results highlight the importance of confirming electrophoresis results by a secondary method such as sequencing.

In this 2018 annual report, we document the change in circulating rotavirus genotypes during the first full year of surveillance following the change of all states and territories to Rotarix within the NIP. A reduction of rotavirus-positive specimens was identified when compared to 2017, where multiple outbreaks caused by genotype G2P[4], equine-like G3P[8], and G8P[8] strains occurred.<sup>22</sup> G3P[8] was the dominant genotype across 5 out of 6 states, while G2P[4] was the second most prominent genotype identified. Previously we have observed differences in circulating rotavirus strains dependent on the specific vaccine administered in states in Australia, but we would anticipate it may take up to 5 to 10 years to detect an alteration in circulating strains in response to a change in vaccine administered in the NIP. Observing the impact of this change in vaccine in the presence of high vaccine coverage and a robust surveillance system has the potential to inform vaccine-related decisions in Australia and globally.

## Acknowledgements

The Rotavirus Surveillance Program is supported by grants from the Australian Government Department of Health, and GlaxoSmithKlineBiologicalsSA[Study#116120]. The Murdoch Children's Research Institute (MCRI) is supported by the Victorian Government's Operational Infrastructure Support program. GlaxoSmithKline Biologicals SA was provided the opportunity to review a preliminary version of this manuscript for factual accuracy but the authors are solely responsible for final content and interpretation. The

authors received no financial support or other form of compensation related to the development of the manuscript.

We thank H Tran for providing technical assistance, and Dr C M Donato for reviewing the manuscript and consultation for primer binding analysis.

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.

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