Annual report

Australian Rotavirus Surveillance Program: Annual Report, 2016

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

Abstract

This report from the Australian Rotavirus Surveillance Program (ARSP) 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 2016. During this period, 949 faecal specimens were referred for rotavirus G and P genotype analysis, of which 230 were confirmed as positive for wildtype rotavirus, and 184 were identified as rotavirus vaccine-like. Genotype analysis of the 230 samples from both children and adults revealed that G2P[4] was the dominant genotype in this reporting period nationally, identified in 29% of samples, followed by equine-like G3P[8] and G12P[8] (19% and 15% respectively). Genotype distribution remained distinct between States using RotaTeq® and Rotarix® vaccines. In RotaTeq® States, G12P[8] strains were more common, while G2P[4] and equine-like G3P[8] genotypes were more common in Rotarix® States and Territories. This report highlights the continued dominance of G12P[8] strains in RotaTeq® States and co-dominance of G2P[4] and equine-like G3P[8] in States and Territories using Rotarix®.

Keywords: rotavirus, gastroenteritis, genotypes, disease surveillance, Australia, vaccine, RotaTeq®, Rotarix®

Introduction

Rotaviruses, from the Reoviridae family, 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] The segmented nature of rotavirus has been attributed as one of the major processes by which the virus can evolve, since it allows for reassortment both within and between human and animal strains, leading to the occurrence of unusual and novel rotavirus strains.[2] Rotaviruses are the most common cause of severe diarrhoea in young children worldwide, estimated to have caused 215,000 deaths in 2013 worldwide.[3] The latest figures are significantly lower than previous estimates of 611,000 deaths per annum[4], primarily due to the introduction of rotavirus vaccines, such as Rotarix® [GlaxoSmithKline] and RotaTeq® [Merck]. These two live attenuated oral rotavirus vaccines have been shown to be safe and highly effective in the prevention of severe diarrhoea due to rotavirus infection[5,6], 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.[7] Since 1 July 2007, rotavirus vaccines have been included in the Australian National Immunisation Program (NIP), with excellent uptake in subsequent years across the nation. 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.[8]

Before the introduction of rotavirus vaccines in Australia, rotavirus had accounted for ~10,000 childhood hospitalisations for diarrhoea each year.[9] A significant impact on acute gastroenteritis disease burden has been observed since vaccine introduction, with studies showing a

78% decline across Australia in both rotavirus coded and non-rotavirus coded hospitalisations in children under 5 years of age.[8,10,11]

The ARSP has characterised and reported the G- and P- genotypes of rotavirus strains causing severe disease in Australian children since 1999. Surveillance data generated by the ARSP has shown that strain diversity, as well as temporal and geographic changes occur each year.[12] Ongoing characterisation of circulating rotavirus genotypes will provide insight into whether vaccine introduction has impacted on virus epidemiology, altered circulating strains, or caused vaccine escape strains, which could 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 2016.

Methods

Rotavirus positive specimens detected by quantitative Reverse Transcription Polymerase Chain Reaction (RT-PCR), enzyme immunoassay (EIA), or latex agglutination in collaborating laboratories across Australia were collected, stored frozen and forwarded to the Australian National Rotavirus Reference Centre Melbourne, together with relevant age and gender details. The laboratories contributing samples for 2016 were:

- Microbiology Department, Canberra Hospital, 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, Central Coast, Gosford, New South Wales.
- Douglas Hanly Moir Pathology, New South Wales.
- Royal North Shore Hospital, St. Leonards, 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, Townsville, Queensland.
- Microbiology and Infectious diseases laboratory, SA Pathology, Adelaide, South Australia.
- Molecular Medicine, Pathology Services, Royal Hobart Hospital, Hobart, Tasmania.
- The Serology Department, Royal Children's Hospital, Parkville, Victoria.
- QEII Microbiology Department, PathWest Laboratory Medicine WA, Perth, Nedlands, Western Australia.

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, 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].[13-17] 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 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).[18]

Samples sent or identified as vaccine-like were confirmed for vaccine by amplifying a portion of the inner capsid VP6 gene, using human Rot3/Rot5 primers and Superscript III One-Step RT-PCR System with Platinum Taq DNA Polymerase (Invitrogen), as previously described.[19,20]

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 949 faecal specimens were collected during the period 1 January to 31 December 2016 for analysis from 18 collaborating centres across Australia, located in the Australian Capital Territory (ACT), New South Wales (NSW), Northern Territory (NT) Queensland (Qld), South Australia (SA), Tasmania (Tas), Victoria (Vic) and Western Australia (WA).

In 2016, 414 rotavirus positive samples from patients clinically diagnosed with acute gastroenteritis were identified. For analysis, these samples were divided based on whether a sample had no vaccine component identified (described herein as "wildtype rotavirus") or had a vaccine component identified based on VP6 or VP7 sequence analysis ("vaccine-like"). A total of 230 samples were confirmed as wildtype rotavirus positive by EIA (ProSpecT, OXOID) or RT-PCR analysis. Of these, 107 were collected from children under 5 years of age, and 123 were from older children and adults. An additional 535 specimens contained either insufficient specimen for genotyping (n=10), were duplicates of samples already analysed (n=43) or the specimen was not confirmed to be positive for rotavirus (n=482) and were thus not analysed further.

In addition, 184 samples were identified as rotavirus vaccine-like by VP6 and/or VP7 sequencing. The majority of these samples (n=162) were received from South Australia, where a duplex rapid real time qRT-PCR assay that could differentiate between wildtype and RotaTeq® NSP3 was used.[21] Of these, 101 were sent from hospital settings, while the remaining 61 were from general practices across South Australia. These samples were already identified as positive by the collaborator's rotavirus and RotaTeq®-specific qRT-PCR assays, and were consequently included in this surveillance report. Other RotaTeq® samples were identified from Qld Regional (n=1), Townsville Pathology, Qld (n=1), Royal Children's Hospital, Vic (n=3), and PathWest, WA (n=6). Rotarix® vaccine was identified in 11 cases by VP7 sequence. These samples were provided by Westmead, NSW (n=8), John Hunter Hospital, NSW (n=1) and Royal Hobart Hospital, Tas (n=1).

Wildtype rotavirus specimens:

Age distribution for wildtype rotavirus infections

From 1 January to 31 December 2016, 46.5% of rotavirus positive samples were obtained from children under 5 years of age (Table 1). A total of 14.3% of wildtype rotavirus positive samples were from children 13-24 months of age, and 35.2% of samples were from individuals older than 20 years of age.

In the samples from children under 5 years of age, almost a third of all samples (30.8%) were identified in children 13-24 months old, while the next most common age group was 7-12 months where 19.6% of cases were found.

Wildtype rotavirus genotype distribution

Genotype analysis was performed on the 230 confirmed rotavirus positive cases from children and adults (Table 2). G2P[4] was the most common genotype identified nationally, representing 29% of all specimens analysed. This genotype was identified as the dominant genotype in NSW and WA, representing 41% and 33% of strains respectively. G2P[4] was also the only strain detected in ACT, however only 3 rotavirus positive samples were received from ACT for this reporting period.

A previously described equine-like G3P[8] strain[22,23] was the second most common genotype found in Australia, representing 19% of all strains nationally (Table 2). The majority of these equine-like G3P[8] samples were found in NSW, representing 33% of all strains identified within the State. G12P[8] strains were the third most common genotype identified nationally, representing 15% of all specimens. G12P[8] strains were dominant in Qld, SA and Vic, representing 37%, 29% and 38% of each state total, respectively. Other common genotypes identified nationally in 2016 included G9P[8] (10%), G1P[8] (7%), and G3P[8] (6%).

Twenty-nine (13% of rotavirus positive) specimens did not fall into a common genotype category (Table 3). Whilst two samples were of mixed genotype (G1/G3P[8] and equine-like G3 P[4]/P[8]), the remaining 27 samples represented 12 uncommon rotavirus strains. Six of these strains included unusual combinations, such as G1P[6], G2P[8], G3P[4], G9P[4], G9P[6], and G12P[6]. The remaining 6 were represented by strains that contained an animal VP7 and/or VP4 component. Feline/canine-like G3P[3] were identified in 2 samples from the NT, while bovine-like strains such as G6P[14] (n=4), G8P[8] (n=3), G8P[14] (n=2), G10P[5] (n=1), and G10P[14] (n=1) were identified in multiple States across Australia.

A G- or P- genotype could not be assigned to 3 samples (Table 2). Two of these were G-non typeable samples from SA (G-non typeable P[4]) and Tas (G-non typeable P[8]). The third sample was an equine-like G3 P[non typeable] from Qld. The partially non typeable samples could be due to either low viral load, mutations in the primer annealing regions, or inhibitors within

Table 1: Age distribution of rotavirus wildtype gastroenteritis cases

Age (months)	Age (years)	n=	% of total	% under 5 years
0-6		13	5.7	12.1
7-12	≤1	21	9.1	19.6
13-24	1-2	33	14.3	30.8
25-36	2-3	20	8.7	18.7
37-48	3-4	12	5.2	11.2
49-60	4-5	8	3.5	7.5
Subtotal		107	46.5	-
61-120	5-10	23	10.0	
121-240	10-20	19	8.3	
241-960	20-80	58	25.2	
961+	>80	23	10.0	
Total		230	-	

Table 2: Rotavirus G and P genotype distribution in infants, children and adults, 1 January to 31 December 2016

Centre	Type total	G1P[8]		G2P[4]		G3P[8]		G3P[8]*		G9P[8]		G12P[8]		Other†		Non-type‡		Neg
		%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	n
Australian Capital Territory																		
ACT	3	-	0	100	3	-	0	-	0	-	0	-	0	-	0	-	0	0
New South Wales																		
Prince of Wales Hospital	23	-	0	74	17	-	0	22	5	4	1	-	0	-	0	-	0	0
Westmead	19	11	2	42	8	-	0	16	3	16	3	-	0	16	3	-	0	22
Westmead - CIDM	14	-	0	29	4	7	1	7	1	36	5	7	1	14	2	-	0	6
John Hunter	15	-	0	13	2	-	0	80	12	7	1	-	0	-	0	-	0	5
Other §	11	-	0	27	3	9	1	55	6	-	0	-	0	-	0	9	1	4
NSW subtotal:	82	2	2	41	34	2	2	33	27	12	10	1	1	6	5	1	1	37
Northern Territory																		
Alice Springs	1	-	0	100	1	-	0	-	0	-	0	-	0	-	0	-	0	4
Royal Darwin Hospital	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	1
Other §	2	-	0	-	0	-	0	-	0	-	0	-	0	100	2	-	0	2
Northern Territory subtotal:	3	-	0	33	1	-	0	-	0	-	0	-	0	67	2	-	0	7
Queensland																		
Pathology Brisbane	3	33	1	-	0	-	0	33	1	-	0	33	1	-	0	-	0	4
Qld Regional	15	-	0	27	4	33	5	-	0	-	0	33	5	7	1	-	0	186
Pathology Townsville	1	-	0	-	0	-	0	-	0	-	0	100	1	-	0	-	0	3
Queensland subtotal:	19	5	1	21	4	26	5	5	1	-	0	37	7	5	1	-	0	193
South Australia																		
Adelaide	78	12	9	17	13	6	5	9	7	9	7	29	23	17	13	1	1	142
Tasmania																		
Hobart	4	-	0	-	0	-	0	50	2	25	1	-	0	-	0	25	1	2
Victoria																		
Royal Children's Hospital	8	-	0	-	0	-	0	13	1	13	1	38	3	38	3	-	0	26
Western Australia																		
PathWest	33	12	4	33	11	6	2	18	6	12	4	3	1	15	5	-	0	75
TOTAL	**230**	**7**	**16**	**29**	**66**	**6**	**14**	**19**	**44**	**10**	**23**	**15**	**35**	**13**	**29**	**1**	**3**	**482**

* Equine-like G3P[8]
† See Table 3
‡ A specimen where G and/or P genotype was not determined
§ NSW: Faecal specimens which were received from Pathology North, Central Coast Gosford, NSW; Douglas Hanly Moir Pathology, NSW; Royal North Shore Hospital, Sydney, NSW; Pathology Brisbane, Qld; Qld Regional, Qld; Adelaide, SA
§ NT: Faecal specimens which were received with SA/Pathwest WA samples

Table 3: Mixed and unusual G and P genotypes identified in infants, children and adults, 1 January to 31 December 2016

Genotype	RotaTeq®				Rotarix®		Total
	Qld	SA	Vic	WA	NSW	NT	
G1P[6]	-	1	-	-	-	-	1
G2P[8]	-	1	-	-	-	-	1
Feline/canine G3P[3]	-	-	-	-	-	2	2
G3P[4]	-	2	1	-	-	-	3
G6P[14]	-	2	1	-	1	-	4
G8P[8]	-	-	-	3	-	-	3
G8P[14]	-	1	-	-	1	-	2
G9P[4]	1	1	1	1	3	-	7
G9P[6]	-	1	-	-	-	-	1
G10P[5]	-	1	-	-	-	-	1
G10P[14]	-	1	-	-	-	-	1
G12P[6]	-	1	-	-	-	-	1
Mixed G1/G3P[8]	-	1	-		-	-	1
Equine G3 mixed P[4]/P[8]	-		-	1	-	-	1
Total:							**29**

the extracted RNA, which could have prevented the function of the enzymes used in the RT and/or PCR steps.

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

107 wildtype rotavirus samples in total were collected from children under 5 years of age (Table 4). Within this cohort, G2P[4] was the most common genotype identified, found in 28% of all samples. Equine-like G3P[8] was the second most common genotype (19%), and G12P[8] strains were the third most common genotype (17%). G9P[8] and G1P[8] strains represented minor genotypes of children in this cohort, identified in 8% and 7% of samples respectively (Table 4).

Table 4: Rotavirus G and P genotype distribution in infants and children under 5 years of age, 1 January to 31 December 2016

Centre	Type total	G1P[8]		G2P[4]		G3P[8]		G3P[8]*		G9P[8]		G12P[8]		Other†		Non-type‡		Neg
		%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	n
Australian Capital Territory																		
ACT	2	-	0	100	2	-	0	-	0	-	0	-	0	-	0	-	0	0
New South Wales																		
Prince of Wales Hospital	11	-	0	73	8	-	0	27	3	-	0	-	0	-	0	-	0	0
Westmead	15	7	1	33	5	-	0	20	3	20	3	-	0	20	3	-	0	22
Westmead - CIDM	12	-	0	33	4	8	1	8	1	42	5	-	0	8	1	-	0	4
John Hunter	8	-	0	-	0	-	0	100	8	-	0	-	0	-	0	-	0	3
Other §	4	-	0	25	1	25	1	50	2	-	0	-	0	-	0	-	0	1
NSW subtotal:	50	2	1	36	18	4	2	34	17	16	8	-	0	8	4	-	0	30
Northern Territory																		
Alice Springs	1	-	0	100	1	-	0	-	0	-	0	-	0	-	0	-	0	4
Royal Darwin Hospital	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	1
Other §	2	-	0	-	0	-	0	-	0	-	0	-	0	100	2	-	0	2
Northern Territory subtotal:	3	-	0	33	1	-	0	-	0	-	0	-	0	67	2	-	0	7
Queensland																		
Pathology Brisbane	1	100	1	-	0	-	0	-	0	-	0	-	0	-	0	-	0	0
Qld Regional	12	-	0	17	2	33	4	-	0	-	0	42	5	8	1	-	0	114
Pathology Townsville	1	-	0	-	0	-	0	-	0	-	0	100	1	-	0	-	0	3
Queensland subtotal:	14	7	1	14	2	29	4	-	0	-	0	43	6	7	1	-	0	117
South Australia																		
Adelaide	20	10	2	20	4	5	1	5	1	5	1	50	10	5	1	-	0	90
Tasmania																		
Hobart	2	-	0	-	0	-	0	50	1	-	0	-	0	-	0	50	1	2
Victoria																		
Royal Children's Hospital	4	-	0	-	0	-	0	25	1	-	0	25	1	50	2	-	0	12
Western Australia																		
PathWest	12	33	4	25	3	17	2	-	0	-	0	8	1	17	2	-	0	71
TOTAL	**107**	**7**	**8**	**28**	**30**	**8**	**9**	**19**	**20**	**8**	**9**	**17**	**18**	**11**	**12**	**1**	**1**	**329**

* Equine-like G3P[8]
† See Table 3
‡ A specimen where G and/or P genotype was not determined
§ NSW: Faecal specimens which were received from Pathology North, Central Coast Gosford, NSW; Douglas Hanly Moir Pathology, NSW; Royal North Shore Hospital, Sydney, NSW; Pathology Brisbane, Qld; Qld Regional, Qld; Adelaide, SA

§ NT: Faecal specimens which were received with SA/Pathwest WA samples

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

A total of 123 rotavirus samples were collected from children over the age of 5 years and adults (Table 5). This cohort was similar to the under 5 years of age group, in that G2P[4] was the main genotype identified (29%), followed by equine-like G3P[8] (20%) and G12P[8] (14%). G9P[8] was more prominent, found in 11% of all samples within this cohort.

Distribution of genotypes according to vaccine type in children less than 5 years of age

G- and P- genotypes of the 107 rotavirus positive samples were divided according to vaccine use (Figure). In states where RotaTeq® is in use, G12P[8] strains were the dominant genotype in children less than 5 years, identified in 36% of samples, compared to no observations in Rotarix® States. G2P[4] was the second most common genotype identified in RotaTeq® States (18%). Genotypes G1P[8] and G3P[8] were the third most common genotypes representing 14% of all samples individually. In locations using Rotarix®, G2P[4] strains were dominant, identified in 37% of strains, followed by equine-like G3P[8], identified in 32% of samples. By comparison, equine-like G3P[8] was only detected in 4% of all samples from States administering RotaTeq®.

Vaccine-like rotavirus specimens:

Age distribution for rotavirus vaccine cases

During the 2016 reporting period, 184 samples were identified as rotavirus vaccine by VP6 and/or VP7 sequencing (Table 6). Of these, 90.2% were from 0-6 month old patients, while 16% were from 7-12 month old patients. Two outlying samples were collected from older children in SA and Qld, aged 20 months and 40 months old respectively.

Genotype distribution of specimens containing rotavirus vaccine component

Of the 184 samples that had sequence confirmation of vaccine-like VP6 and/or VP7, 141 samples had been processed further for genotype analysis (Table 7). All samples identified with components of the Rotarix® vaccine (n=11) were genotyped as G1P[8], while samples containing RotaTeq® vaccine components (n=130) had more varied genotype combinations, due to the pentavalent nature of the vaccine. The most common combination identified by agarose gel electrophoresis was G1P[nt], identified in 48/130 samples, followed by G1P[8] (21/130). Single genotypes were identified in 17 samples, including G2, G4, and G6 with either a P[8] or P[non typeable]. Note, G6 samples had to be sequence confirmed, as primers for this bovine vaccine component are not included in the routine G-typing primer mix. G6 would present as G12 (~382bp band) in agarose gels if the G12 primer was included in the G-mix. Other combinations included various mixed G-types that contained two to four of all human virus components (G1, G2, G3 and G4) of the RotaTeq® vaccine, with either a P[8] or P[non typeable] type. Fully non typeable genotype results were attributed to 14 samples.

The majority of these P[nt] samples were most likely due to the bovine P[5] component of the RotaTeq® vaccine, for which a separate hemi-nested RT-PCR with specific bovine primers would have had to be used to identify the P[5] component. Due to time constraints, this was not performed for these samples.

Discussion

This 2016 ARSP report describes the distribution of rotavirus genotypes and geographic differences of rotavirus strains causing disease in Australia, for the period of 1 January to 31 December 2016. A reduction in confirmed rotavirus positive samples was observed during 2016, where only 230 samples were confirmed as positive with a wildtype rotavirus strain, and another 184 with a vaccine-like rotavirus strain. Of the 230 wildtype rotavirus specimens, 29% were

Table 5: Rotavirus G and P genotype distribution in children over 5 years of age and adults, 1 January to 31 December 2016

Centre	Type total	G1P[8]		G2P[4]		G3P[8]		G3P[8]*		G9P[8]		G12P[8]		Other†		Non-type‡		Neg
		%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	n
Australian Capital Territory																		
ACT	1	-	0	100	1	-	0	-	0	-	0	-	0	-	0	-	0	0
New South Wales																		
Prince of Wales Hospital	12	-	0	75	9	-	0	17	2	8	1	-	0	-	0	-	0	0
Westmead	4	25	1	75	3	-	0	-	0	-	0	-	0	-	0	-	0	0
Westmead - CIDM	2	-	0	-	0	-	0	-	0	-	0	50	1	50	1	-	0	2
John Hunter	7	-	0	29	2	-	0	57	4	14	1	-	0	-	0	-	0	2
Other§	7	-	0	29	2	-	0	57	4	-	0	-	0	-	0	14	1	3
NSW subtotal:	32	3	1	50	16	0	0	31	10	6	2	3	1	3	1	3	1	7
Queensland																		
Pathology Brisbane	2	-	0	-	0	-	0	50	1	-	0	50	1	-	0	-	0	4
Qld Regional	3	-	0	67	2	33	1	-	0	-	0	-	0	-	0	-	0	72
Queensland subtotal:	5	-	0	40	2	20	1	20	1	-	0	20	1	-	0	-	0	76
South Australia																		
Adelaide	58	12	7	16	9	7	4	10	6	10	6	22	13	21	12	2	1	52
Tasmania																		
Hobart	2	-	0	-	0	-	0	50	1	50	1	-	0	-	0	-	0	0
Victoria																		
Royal Children's Hospital	4	-	0	-	0	-	0	-	0	25	1	50	2	-	1	-	0	14
Western Australia																		
PathWest	21	-	0	38	8	-	0	29	6	19	4	-	0	14	3	-	0	4
TOTAL	123	7	8	29	36	4	5	20	24	11	14	14	17	14	17	2	2	153

* Equine-like G3P[8]
† See Table 3
‡ A specimen where G and/or P genotype was not determined
§ NSW: Faecal specimens which were received from Douglas Hanly Moir Pathology, NSW & Pathology Brisbane, Qld

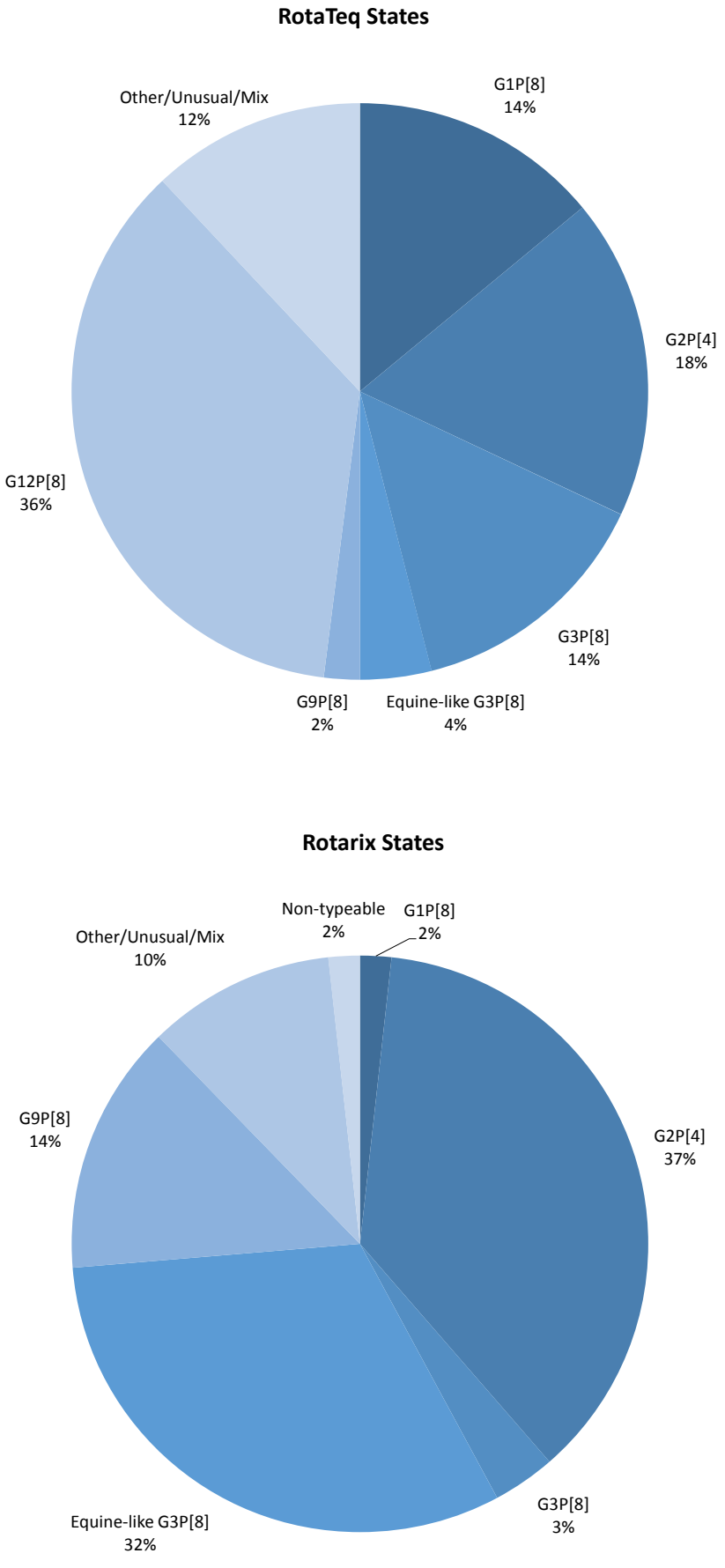

Figure: Overall distribution of wildtype rotavirus G- and P- genotypes identified in Australian children under 5 years of age, based on vaccine usage, Australia, 1 January to 31 December 2016

Table 6: Age distribution of gastroenteritis cases, where a rotavirus vaccine component was identified:

Age (months)	Age (years)	n=	% of total
0-6		166	90.2
7-12	≤1	16	8.7
13-24	1-2	1	0.5
37-48	3-4	1	0.5
Total		184	-

genotyped as G2P[4], 19% as equine-like G3P[8], and 15% as G12P[8], ending the four-year dominance of G12P[8] nationally.[23] However, distinct differences in genotype distribution based on vaccine usage continued to occur. As previously reported, G12P[8] was more common in States administering RotaTeq® compared to Rotarix®, whereas G2P[4] and equine-like G3P[8] was more prominent in locations using Rotarix®.[23] This ongoing distinction in circulating rotavirus genotypes between vaccine groups has not been reported previously in other countries that have had both vaccines added to their NIP, most likely due to the lack of geographical segregation of vaccine use. Only one vaccine-related trend has been described in the literature, where G2P[4] rotavirus gastroenteritis was associated with countries that use the Rotarix® vaccine, including Austria, Australia, Belgium, and countries within Latin America.[24-29] However, there still is a lack of evidence that could distinguish whether these observations are due to a temporal coincidence, since G2P[4] was found indiscriminately in both vaccinated and unvaccinated countries, or vaccine-induced immunological pressure, since the Rotarix® G1P[8] vaccine is less efficient against the heterotypic G2P[4] strain.[29,30]

Of the 184 samples identified as vaccine-strain, 166 were primarily from infants aged 0-6 months (90.2%), where subclinical shedding of rotavirus vaccine is expected.[31] A change in diagnostic methods at collaborating laboratories from less-sensitive ELISA or latex agglutination assays (conventional), to highly sensitive real time qRT-PCR, is the most likely cause for such an increase in rotavirus vaccine positive stool specimens, since conventional methods generally would not detect low level vaccine shedding.[32] This would also help explain the unusually high amount of negative or unconfirmed rotavirus cases reported for this year (n=482/949). It is plausible that the acute gastroenteritis for these patients was instead caused by another agent, however the ARSP does not have access to patient comorbidity records to comment further. On the other hand, two patients aged 20 months and 40 months were found to have vaccine-like rotavirus strain present in their stool specimens. It is possible that the vaccine strain was present in these two patients after horizontal transmission from a recently vaccinated sibling, a vaccine/wildtype reassortment strain infection, or the patient was immunocompromised and therefore unable to clear the vaccine efficiently.[19,33] Full genome characterisation of these two strains would help elucidate the cause of vaccine components being present in these older patients.

Despite a marked drop in overall rotavirus positive samples from 1,031 in 2015, to 230 in 2016, the number of specimens in which an unusual genotype was identified was similar to the number detected in 2015.[23] Furthermore, the genotypes identified within both surveillance periods included strains such as feline/canine G3 (P[3] or P[9]), G8 (P[4], P[8], or P[14]), G10P[14] and G12 (P[4] or P[6]).[23] These observations are unusual, in that a particular uncommon strain may occur sporadically within a year as a single case or a sporadic localised outbreak, rather than persisting across multiple years in different States across Australia. An example of this is the feline/canine-like G3P[3], which was detected in Australian States: NT (n=4), SA (n=1), and WA (n=1) in 2015, and in the NT (n=2) in 2016. This strain (with either P[3] or P[9]) has also been reported in multiple countries such as South Korea, China, Brazil, and the USA, but only as 1-3 cases in total across multiple years.[34-38] Similarly, bovine-human strains such as G6P[14] and G8P[14], were considered uncommon in Australia; identified in 6 individual cases over an 11-year surveillance period when combined (1997-2007).[12] In 2016 alone, 4 G6P[14] and 2

Table 7: Rotavirus G and P genotypes identified in rotavirus vaccine-like cases:

		P[8]	P[nt]
Rotarix®	G1 (Rix)	11	-
	G1 (Teq)	21	48
	G2	1	3
	G4	3	7
RotaTeq®*	G6 (VP7 seq)	3	-
	Mixed G1/G2/ G3/G4	7	3
***130/173 genotyped**	Mixed G1/G2/G4	1	-
	Mixed G1/G3	1	2
	Mixed G1/G3/G4	2	1
	Mixed G1/G4	3	8
	G-non typeable	2	14

G8P[14] strains have been identified, together with other uncommon human-animal reassortant rotavirus strains, such as G10P[14].

Animal rotavirus strains are considered to be attenuated to humans, however, multiple reassortment events between human and animal strains can lead to chimeric viruses that have more human segments, increasing their ability to infect and replicate within a human host.[39,40] This improved adaptation to the human host, together with a lack of pre-existing population immunity against such new strains, creates a niche for novel human-animal reassortant rotaviruses that have the potential to spread globally and persist within the human population, as seen with G9 and G12 strains.[41,42] These two strains were considered to be the product of multiple reassortment events between human and swine rotavirus.[40] The factors behind this increase in animal-human reassortant strains need to be elucidated, as these novel strains have the potential to become epidemiologically important. The recent global emergence of an equine-human reassortment G3P[8] strain, predominantly in vaccinated countries, raised the question of whether the increase in zoonotic strains was due to immunological pressures from the vaccine itself. Indeed, the prolonged differences in genotype diversity between vaccine groups described here suggests that the vaccines may be inducing selective pressures that favour certain genotypes. However, the introduction of vaccines cannot be the sole reason for the observed increase in zoonotic strain prevalence, as such events occurred prior to vaccine introduction, as shown with G9 and G12 strains.[40] Nevertheless, continued surveillance is vital for understanding how vaccines can affect rotavirus evolution and genotype diversity. Furthermore, continued epidemiological surveillance will gain insight into how these changes in rotavirus diversity can alter vaccine effectiveness in children.

Despite the continuous changes in circulating rotavirus genotypes, the introduction of both RotaTeq® and Rotarix® to the Australian NIP has substantially impacted on the rotavirus burden in Australia. It is estimated that for the 6 years post vaccine implementation, ~77,000 hospitalisations and ~3 deaths were prevented; 90% of which were for children under 5 years of age.[11] Such an impact was less noticeable in other healthcare outcomes, suggesting that there has been a shift in the severity of symptoms from severe to less critical outcomes.[11] This report supports the idea that vaccine implementation to the Australian NIP has been effective on reducing the burden caused by rotavirus infections, as shown by the decrease in rotavirus positive samples received for 2016. This decrease in sample number appears to be a true depiction of rotavirus epidemiology in Australia, when compared to available State-specific notifiable disease reports. For 2016, Western Australia reported an annual crude rate of 6.6/100,000 rotavirus infections per population, compared to an average of 16.9/100,000 over the preceding four years.[43]

In this 2016 annual report, an overall reduction in rotavirus positive samples was described. G2P[4], equine-like G3P[8], and G12P[8] continue to cause significant disease in Australia, however G12P[8] impacted more in RotaTeq® States, while equine-like G3P[8] and G2P[4] were associated with States administering Rotarix®. The continued dominance of G12 in RotaTeq® states only, and the increase in occurrence of novel strains such as the various animal-like G3, G8, G10, P[3], P[5] and P[14] strains, demonstrate a highly evolving and hard to predict trend in circulating genotypes since vaccine introduction to the Australian NIP. The contin-

ued variations in the wildtype strain population will remain a challenge to vaccine effectiveness and will require continued monitoring.

Corresponding Author

Mrs Susie Roczo-Farkas

Enteric Virus Group, Level 5, Murdoch Childrens Research Institute, Royal Children's Hospital, Flemington Road , Parkville, Victoria, 3052.
Ph: 03 8341 6383
Email: susie.roczofarkas@mcri.edu.au

Authors details

Mrs Susie Roczo-Farkas, Research Assistant, MCRI

Dr Carl D Kirkwood, Senior Program Officer, Bill and Melinda Gates Foundation

Prof Julie E Bines, Group Leader, Enteric Virus Group and Rotavirus Group, MCRI and the Australian Rotavirus Surveillance Group

Enteric Virus Group, Murdoch Childrens Research Institute, Royal Children's Hospital, Flemington Road, Parkville, Victoria, 3052.

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The Australian Rotavirus Surveillance Group includes:

Australian National Rotavirus Reference Centre

Mrs Susie Roczo-Farkas; Coordinator, Research Assistant, Enteric Virus Group, MCRI

Associate Prof Carl Kirkwood; Director (prior to August 2015), Enteric Virus Group, MCRI

Prof Julie Bines; Director (after August 2015), Enteric Virus Group, MCRI

Australian Capital Territory

Ms S Bradbury, Ms E Malinsky and members of members of the Microbiology Department, Canberra Hospital

New South Wales

Prof W. Rawlinson, Prof. M. Lahra, Mr J Merif and members of the Virology Division, SEALS, Prince of Wales Hospital

Dr A. Kesson, Ms I Tam and members of the Virology Department, The Children's Hospital at Westmead

Dr V Sintchenko, T Olna, Centre for Infectious Diseases and Microbiology, Westmead Hospital

Dr R. Givney, S Pearce, K Delves and members of the Microbiology Department, John Hunter Hospital, Newcastle

Mr D Spence and members of the Microbiology Department, Pathology North Central Coast, Gosford, New South Wales

Dr M Wehrhahn and members of the Douglas Hanly Moir Pathology, New South Wales

Ms R Timmins and members of the Department of Microbiology, Royal North Shore Hospital, St. Leonards, New South Wales

Mr T McNeill, Northern Sydney Public Health, Hornsby, New South Wales

Northern Territory

Dr R Baird, Ms J Hennessy, Ms P Smith and members of the Microbiology Department, Royal Darwin Hospital, Tennant Creek Hospital, Gove District Hospital and Katherine District Hospital

Mr J McLeod and members of the Microbiology Department, Alice Springs Hospital, Alice Springs

Ms H Cook, Centre for Disease Control, Darwin

Queensland

Mr F Moore, Ms J McMahon, Forensic and Scientific Services, Queensland Health, Herston

Dr G Nimmo, Dr C Bletchly, Ms S Ye and department members, Microbiology division, Pathology Queensland Central laboratory, Herston

Dr S Lambert and members of the Queensland Paediatric Infectious Diseases laboratory, Royal Children's Hospital, Brisbane

Ms G Gilmore and members of the Queensland Health laboratory, Townsville

South Australia

Prof G Higgins, Ms S Schepetiuk and members of the Microbiology and Infectious diseases laboratory SA Pathology, Adelaide.

Tasmania

Dr Jan Williamson and members of Molecular Medicine, Pathology Services, Royal Hobart Hospital, Hobart, Tasmania.

Victoria

Miss P Adamopolous and members of the Serology Department, Royal Children's Hospital, Parkville.

Western Australia

Prof Smith, Dr A Levy, Mrs J Lang and members of QEII Microbiology Department, PathWest Laboratory Medicine WA, Perth

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