Australian Rotavirus Surveillance Program, 2010/2011

AUSTRALIAN ROTAVIRUS SURVEILLANCE PROGRAM
ANNUAL REPORT, 2010/11
Carl D Kirkwood, Susie Roczo, Karen Boniface, Ruth F Bishop, Graeme L Barnes, Australian Rotavirus Surveillance Group

Abstract
The Australian Rotavirus Surveillance Program together with 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 1 July 2010 to 30 June 2011. This report represents the fourth year of surveillance following introduction of rotavirus vaccines into the National Immunisation Program. One thousand one hundred and twenty-seven faecal samples were referred to the centre for G and P genotype analysis using hemi-nested multiplex reverse transcription-polymerase chain reaction. Eight hundred and sixteen samples were confirmed as rotavirus positive. Of these, 551 were collected from children under 5 years of age, while 265 were from older children and adults. Genotype analysis revealed that a change in the dominant type occurred in this reporting period, such that genotype G2P[4] was the dominant type nationally, representing 51% of samples, followed by genotype G1P[8] (26.1%). Genotypes G3P[8] represented 11% of samples while G4P[8] re-emerged as an important genotype, and was identified in 6% of samples. Uncommon rotavirus G and P combinations continue to be identified, with G2P[8] and G9P[4] identified during this survey. Differences in genotype distribution based on vaccine usage continue to be evident in Australian states. This survey continues to highlight the fluctuations in rotavirus genotypes, with an annual change in dominant genotypes suggesting a more dynamic wild-type population. Commun Dis Intell 2011;35(4):281–287.

Keywords: Rotavirus, gastroenteritis, genotypes, disease surveillance

Introduction
Rotaviruses are a major cause of severe diarrhoea in young children worldwide.1 The development of two live oral rotavirus vaccines Rotarix® (GlaxoSmithKline) and RotaTeq® (Merck) was undertaken in an effort to decrease the substantial disease burden. Extensive clinical trials have shown both vaccines to be safe and highly effective in the prevention of severe diarrhoea and hospitalisation due to rotavirus infections.2,3 In Australia, rotavirus vaccines were introduced into the National Immunisation Program (NIP) for all infants from 1 July 2007, with all state health departments making independent decisions on which vaccine to use. RotaTeq is administered in Victoria, South Australia, Western Australia (since May 2009) and Queensland, while Rotarix is in use in New South Wales, the Northern Territory, Tasmania and the Australian Capital Territory. In the pre-vaccine era, rotavirus infection accounted for up to 10,000 childhood hospitalisations for diarrhoea each year.4 The introduction of rotavirus vaccines into the NIP has shown an early impact on the large disease burden of rotavirus, with significant declines in hospitalisation and emergency room visits reported since vaccine introduction.5 Postmarketing surveillance for intussusception following rotavirus vaccination has revealed no overall increase, although there is some evidence of a slight elevated risk after the first dose of both vaccines.6

The National Rotavirus Surveillance Program has been reporting the changing annual pattern of dominant genotypes in the Australian population since 1999. Over this period, results have highlighted the diversity of rotavirus strains capable of causing disease in children, and provided the baseline information of the pattern of circulating strains prior to vaccine introduction.7 The introduction of rotavirus vaccines into Australia will increase the population immunity to rotavirus, this in turn is likely to impact on the epidemiology of circulating wild-type strains. Changes in the prevalence of common genotypes, as well as emergence of new or rare genotypes are all possible. Therefore, investigation of circulating rotavirus genotypes will provide insight into whether vaccine introduction has impacted on virus epidemiology, and provide findings that can validate prior assumptions concerning the consequences of vaccination programs.

This report describes the genotype characterisation of rotavirus strains causing severe gastroenteritis in children in Australia for the period 1 July 2010 to 30 June 2011.

Methods
Rotavirus positive specimens detected by enzyme immunoassay (EIA) or latex agglutination in collaborating laboratories across Australia were col-
Viral ribonucleic acid (RNA) was extracted from each specimen using an RNA extraction kit (Qiamp Viral mini extraction kit, Qiagen) according to the manufacturers instructions. The G and P genotype of each specimen was determined by hemi-nested multiplex reverse transcription/polymerase chain reaction (RT-PCR) assays. The first round RT-PCR was 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 reaction was conducted using specific oligonucleotide primers. A G and P genotype was assigned for each sample based on agarose gel analysis of second round PCR products.

Results

Number of isolates

A total of 1,127 specimens were received for analysis from 16 collaborating centres across Australia; located in Victoria, Western Australia, the Northern Territory, New South Wales, Queensland, South Australia and Tasmania.

Eight hundred and sixteen samples were confirmed as rotavirus positive by EIA (ProspecT, OXOID) or RT-PCR analysis. Of these, 551 were from children under 5 years of age and 265 samples were from older children and adults. The remaining 311 specimens contained either insufficient specimen for genotyping (n = 42), or the specimen was not confirmed to be positive for rotavirus (n = 269), and were not analysed further.

Age distribution

In the current survey period 551 specimens were from children aged 5 or less. In this cohort, 20.2% of cases were from infants 0–6 months of age, 10.2% were from infants 7–12 months of age, 20.7% from infants 13–24 months of age, 15.3% from infants 25–36 months of age, 18% from children 37–48 months of age and 15.5% from children 49–60 months of age. Sixty-six samples were obtained from children 5–10 years of age, 24 were from individuals aged 10–20 years, 72 were from individuals aged 21–80 years, and 103 were from individuals aged 80–100 years.

Genotype distribution

The rotavirus genotypes identified in Australian children 5 years of age or younger, from 1 July 2010 to 30 June 2011 are shown in the Table.

G2P[4] strains were the most common genotype identified, representing 51% of all specimens analysed, and was identified in all states and territories. It was the dominant type in five locations, New South Wales, Western Australia, South Australia, Queensland and Victoria, representing between 36% and 71% of strains in these locations. In the Northern Territory G2P[4] represented 4.2% of samples.

G1P[8] strains were the second most common type nationally, representing 26.1% of all specimens, and was the dominant type only in the Northern Territory, representing 86.1% of strains. It was the second most common strain in another four locations, Queensland, Western Australia, South Australia and Victoria.

G3P[8] strains were identified in all locations, representing 11.1% of strains nationally. It was the second most common strain in New South Wales (15.7%), and was the third most common strain in Queensland, Western Australia, South Australia and Victoria.

G4P[8] strains represented the fourth most common type Australia-wide, being identified in four locations (Western Australia, Queensland, New South Wales and Victoria). In New South Wales and Western Australia it represented 12.3% and 8.4% of strains respectively.

Three G9P[8] strains were identified, one each in New South Wales, the Northern Territory and South Australia. A G3P[6] strain was identified during this study period in Western Australia.

Eleven samples were found to possess uncommon genotype combinations of VP4 and VP7; five G2P[8] strains were identified in New South Wales (n = 2), the Northern Territory (n = 2), and Western Australia. Three G9P[4] strains were identified in Victoria (n = 2) and Queensland, while additional P[4] strains identified associated with G4, G8 or G9 VP7 proteins in New South Wales, Tasmania Queensland and Victoria. Two G8P[9] strains were identified in the Northern Territory and Western Australia. A single G2P[5] strain which resembles the genotype of a component of the RotaTeq vaccine was identified in Western Australia.

Thirteen samples containing multiple G and/or P genotypes were identified. While, in less than 1% of samples either a G- or P-Type could not be assigned. 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.
Table: Rotavirus G and P genotype distribution in Australian children ≤ 5 years, 1 July 2010 to 30 June 2011

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<td>279</td>
<td>11</td>
<td>58</td>
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Other genotypes identified:

- G2P[5] PathWest
- G2P[8] Westmead/ Newcastle/ Alice Springs (x2)/ PathWest
- G3P[6] PathWest
- G8P[9] Darwin/ PathWest
- G9P[4] Qld health/ Royal Children’s Hospital (x2)
Over 300 rotavirus samples were collected from older children and adults. The majority of these that were confirmed as rotavirus and genotyped were collected from South Australia and Western Australia (168/199). Genotype analysis of the rotavirus samples from older individuals (> 10 years of age) showed a similar distribution to that observed in young children, with G2P[4] being the dominant genotype.

The Figure details the distribution of rotavirus G and P genotypes in states using Rotarix (New South Wales, the Northern Territory and Tasmania) compared with the distribution in states using RotaTeq (Victoria, Queensland, South Australia and Western Australia). Analysis of fully G and P typeable samples revealed that in RotaTeq states, G2P[4] was the dominant genotype, identified in 59.8% of strains, while G1P[8] comprised 18.4% of strains. In Rotarix states, G1P[8] strains were dominant (46.6%), while G2P[4] strains comprised 29.8% of specimens. G3P[8], G4P[8] and G9P[8] strains were all identified at similar rates in both settings. Rare or uncommon strains appeared to occur at slightly higher rate in Rotarix studies (4.3%) when compared with RotaTeq states at 1.8%.

The number of samples analysed differed significantly between Rotarix and RotaTeq sites, with 386 samples analysed from RotaTeq locations and 161 from Rotarix locations. This in part is due to the large number of samples obtained during this reporting period from Western Australia. In a subset analysis where Western Australia genotypes were removed, the genotype distributions in the remaining samples did not differ significantly than those obtained using the complete dataset.

Faecal specimens were received from 19 children who developed rotavirus gastroenteritis after being vaccinated with either RotaTeq or Rotarix. RotaTeq vaccine virus was identified in two of these cases by RT-PCR and sequence analysis.

**Discussion**

The Australian Rotavirus Surveillance Program report for 1 July 2010 to 30 June 2011 describes the annual cases and geographic distribution of rotavirus genotypes causing disease in Australian children. The surveillance program identified that genotype G2P[4] emerged as the dominant genotype nationally, representing 49.8% of all strains. This genotype was the dominant type in five of the seven states or territories where samples were collected. Genotype G1P[8] was the second predominant type nationally, comprising 26.3% of all strains, however it was the dominant type only in the Northern Territory and Queensland. Genotype G3P[8] represented the third most common genotype, representing more than 10% of strains nationally. The emergence of G4P[8] as an important cause of disease in this period is the first time during the past 5 years that it has been an important genotype. Previously, G4 strains have represented less than 1% of the circulating strains. This report highlights the continual fluctuations in genotypes, and reveals that G2P[4] re-emerged as the dominant genotype, an occurrence observed previously during 2008–09 season.

The fluctuations in rotavirus genotypes appear more pronounced than in the pre-vaccine period. In the 4 years since vaccine introduction, a different genotype has emerged as the dominant type each
year. In contrast, in the 11 years pre-vaccine introduction, G1P[8] was the dominant type in eight of the 11 rotavirus seasons,\textsuperscript{7,11–13}

Australia continues to provide a unique opportunity to compare the effect of each vaccine on the circulating wild-type strains. During the first 2 years post-vaccine introduction, differences have been observed in genotype distribution depending on the vaccine used.\textsuperscript{13} 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.\textsuperscript{13} In the third season post vaccine, G2P[4] strains were dominant in RotaTeq locations and G1P[8] in Rotarix locations.\textsuperscript{13} During this reporting period G2P[4] remained dominant in RotaTeq locations, as well as two of the three states using Rotarix: New South Wales and Tasmania. In the remaining Rotarix state, G1P[8] remained the dominant type. Thus differences were evident in genotype distribution, however it is unclear whether this is a selection process specific for each type of vaccine or a generic effect.

The worldwide interest in uncommon rotavirus genotypes continues because of the possible impact they could have on rotavirus vaccine programs. Several uncommon VP7/VP4 genotype combinations were again identified; including G1P[4], G2P[8], and G9P[4]. These continue to persist in low numbers at similar levels, as reported in two previous surveillance reports. Since vaccine introduction, the prevalence of these uncommon types has increased. However, it is not clear whether this is due to vaccine introduction exerting an increase selective pressure or simply natural variation is unclear at the moment.

This report details a significant increase in rotavirus positive samples in adults. This is considered to be a real increase demonstrated by an increasing proportion of rotavirus positive samples among adults compared with previous years and not an artefact of increased overall sample numbers. The increase of severe diarrhoea in adults in South Australia and Western Australia may reflect the first evidence that changes in antigenic profile of commonly circulating genotypes is occurring. The changes in recently circulating strains may allow them to evade immune protection generated by exposure to older historical strains. Ongoing analysis of the outbreaks are underway, however, the emergence of rotavirus in these settings may be due to waning of existing immunity, and/or changes in antigenic makeup of the current wild-type strains. Recent reports from the United States of America (USA) have also detailed several rotavirus diarrhoeal outbreaks in the elderly in nursing homes.\textsuperscript{15} Rotavirus has previously been shown to cause 16% of diarrhoeal outbreaks in elderly populations,\textsuperscript{16} but whether the rates have increased in past years is unclear. Further study is required to understand the role rotavirus has in the elderly population in settings such as nursing homes.

Surveillance of genotype distribution post vaccine introduction has been investigated in several other countries including the USA and Belgium.\textsuperscript{17,18} In Belgium, where Rotarix is mainly in use, a significant increase in G2P[4] has been observed for the first 2 years of vaccine use,\textsuperscript{17} while in the USA where RotaTeq is predominantly used, G3P[8] has predominated for several years post vaccine introduction.\textsuperscript{18,19} Further evaluation of genotype distribution in multiple countries is required to understand whether vaccine driven selection is indeed present.

This survey has further highlighted the continued fluctuations in rotavirus genotypes across Australia. However, the rapidly changing genotype patterns do illustrate a more dynamic wild-type population thus suggesting that vaccine pressure may be speeding up the selection process. This is supported by the observation of an increase in cases in older children and adults during the current survey period. Therefore the ongoing evolution of the wild-type strains circulating in Australia will require close monitoring to identify any changes that may emerge and impact on vaccine effectiveness.

**Acknowledgements**

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Dr Kirkwood is supported by a CDA Fellowship, National Health and Medical Research Council.

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.

The laboratories contributing samples were:

Princess Margaret Hospital for Children, Subiaco, Western Australia

Division of Microbiology, PathWest LM, The Queen Elizabeth Medical Centre, Nedlands, Western Australia

The Microbiology Department, Royal Darwin Hospital, Casuarina, Northern Territory

The Department of Microbiology, Western Diagnostic Pathology, Northern Territory and Western Australia
The Microbiology Department, Alice Springs Hospital, Alice Springs, Northern Territory

The Virology Division, Prince of Wales Hospital, New South Wales

The Microbiology Department, The Children’s Hospital at Westmead, New South Wales

The Microbiology Department, John Hunter Hospital, Newcastle, New South Wales

Forensic and Scientific Services, Queensland Health, Herston, Queensland

Pathology Queensland, Herston, Queensland

The Queensland Paediatric Infectious Diseases Laboratory, Royal Children’s Hospital, Brisbane, Queensland

The Queensland Health laboratories in Townsville, Cairns and Gold Coast, Queensland

The Virus Laboratory Institute of Medical and Veterinary Science, Adelaide, South Australia

Royal Hobart Hospital and the Communicable Disease Prevention Unit, Department of Health and Human Services, Hobart, Tasmania

The Virology Department, Royal Children’s Hospital, Parkville, Victoria

The Department of Microbiology, Melbourne Pathology, Victoria

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Dr A Kesson, Ms I Tam and members of the Microbiology Department, The Children’s Hospital at Westmead

Dr R Givney and members of the Microbiology Department, John Hunter Hospital, Newcastle

Northern Territory

Dr P Southwell, Ms J Hennessy and members of the Microbiology Department, Royal Darwin Hospital, Casuarina

Dr M Leung, Ms E Langford and members of the Department of Microbiology, Western Diagnostic Pathology, Northern Territory and Western Australia

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

Ms H Cook, Centres for Disease Control, Darwin

Queensland

Dr M Lyon, Mr M Finger, Forensic and Scientific Services, Queensland Health, Herston

Dr M Nissen and department members, Pathology Queensland, Herston

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Mr R Enbom, Ms G Gilmore, Ms P Derrington and members of the Queensland Health laboratories in Townsville, Cairns and Gold Coast

South Australia

Professor G Higgins, Ms L Payne and members of the Virus Laboratory Institute of Medical and Veterinary Science, Adelaide

Tasmania

Mr D Coleman, Mr D Jones and members of the Communicable Disease Prevention Unit, Department of Health and Human Services, Hobart

Victoria

Dr R Alexander and members of the Virology Department, Royal Children’s Hospital, Parkville

Ms L Prendergast and members of the Department of Microbiology, Melbourne Pathology

Dr J Buttery, Mrs D Kotsanas and members of the Department of Microbiology, Monash Medical Centre, Clayton

Western Australia

Dr K Lindsay and members of the Virology Department, Princess Margaret Hospital for Children, Subiaco

Dr D Smith, Dr G Harnett, Ms N Cooper, Ms E Reyes and members of Division of Microbiology, PathWest LM, The Queen Elizabeth Medical Centre, Nedlands
Australian Rotavirus Surveillance Program, 2010/2011

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References