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 the period 1 July 2008 to 30 June 2009, the second year of surveillance following introduction of rotavirus vaccine into the National Immunisation Program. Five hundred and ninety-two faecal samples from across Australia were examined for G and P genotype using hemi-nested multiplex reverse transcription-polymerase chain reaction assays. Of the 445 confirmed as rotavirus positive, genotype G2P[4] was the dominant type nationally, representing 50.3%, followed by genotype G1P[8] (22.5%). Genotypes G3P[8], G4P[8] and G9P[8] each represented less than 5% of circulating strains nationally. Uncommon rotavirus genotype combinations, including G1P[4] (n = 6), G4P[4] (n = 2) and single strains of G1P[6] and G3P[6] were identified during this study period. The national dominance of G2P[4] was associated with a large outbreak of severe gastroenteritis in Alice Springs in early 2009. This is the first report to describe G2P[4] as the dominant genotype nationally. Whether vaccine pressure has resulted in emergence of this genotype is not yet known.

In Australia, each state health department made independent decisions on which vaccine to use; Victoria, South Australia, and Queensland selected RotaTeq, while New South Wales, Western Australia (changed to RotaTeq from May 2009), the Northern Territory, Tasmania and the Australian Capital Territory selected Rotarix.

The national rotavirus surveillance program has been reporting the changing annual pattern of dominant serotypes in the Australian population since 1999. Over this period our results have highlighted the diversity of rotavirus strains capable of causing disease in children, and providing the baseline information of the changing pattern of circulating strains, prior to vaccine introduction.

The impact of these 2 widely used vaccines on the natural pattern of circulating rotavirus strains is unknown and difficult to predict, given the different components of each vaccine. Continuing genotype surveillance should identify the effects that each vaccine program has on circulating strains – in particular, whether changes occur in genotype incidence and whether increased proportions of rare or uncommon types result.

In this report we describe the surveillance and characterisation of rotavirus strains causing the annual epidemics of severe diarrhoea in young children 5 years of age or younger in Australia for the period 1 July 2008 to 30 June 2009, the second year in which rotavirus vaccine has been included as part of the National Immunisation Program.

Methods

Rotavirus positive specimens detected by enzyme immunoassay (EIA) or latex agglutination in collaborating laboratories were collected, stored frozen and forwarded to the National Rotavirus Reference Centre (NRRC) Melbourne, together with relevant age and sex details. Viral RNA was extracted from each specimen using a RNA extraction kit (Qiamp Viral mini extraction kit, Qiagen) according to the manufacturer's instructions. Double stranded RNA was used to determine the G and P genotype of each specimen by
using a hemi-nested multiplex reverse transcription/polymerase chain reaction (RT-PCR) assay, using G or P specific oligonucleotide primers.9,10

**Results**

**Number of isolates**

A total of 592 specimens were received for analysis from Melbourne, Victoria, and the collaborating centres in Western Australia, the Northern Territory, New South Wales, Queensland and Tasmania (Table 1). Samples were not obtained from South Australia or the Australian Capital Territory. Four hundred and forty-five specimens were confirmed as rotavirus positive using a combination of our in-house EIA and RT-PCR. The remaining 147 specimens contained either insufficient specimen for testing, or the specimens were not confirmed to be positive for rotavirus so were not analysed further.

**Age distribution**

The overall age distribution of children with acute rotavirus gastroenteritis is depicted in Figure 1. In the reporting period, 31% of cases were from infants 0–12 months of age (19% from those less than 6 months of age, 12% from those 7–12 months of age), and 26% from patients 13–24 months of age. Overall, 81% of samples were from children aged 5 years or less.

During the study period, slightly more specimens from male than female children (n = 179 vs 172) were obtained for analysis.

**Genotype distribution**

The rotavirus genotypes identified in Australia from 1 July 2008 to 30 June 2009 are shown in Table 1. G2P[4] and G1P[8] strains were the most common, representing 72.8% of all specimens nationally. Of all strains analysed 50.3% were G2P[4] and were identified in all collaborating centres except Hobart, but were the dominant type only in the Northern Territory and Western Australia. In the Northern Territory, the G2P[4] strain was responsible for a large outbreak of severe acute gastroenteritis between February and May 2009. This outbreak accounted for 74.6% of the G2P[4] samples submitted nationally. G1P[8] strains were the second most common type nationally, representing 22.5% of specimens. G1P[8] strains were identified in all states and was the dominant type in Sydney, Brisbane, Melbourne and Hobart.

G3P[8] strains were identified in Sydney and Melbourne, where they were the second most dominant type identified in these locations (26.3% and 29.6% respectively). In the Northern Territory, Brisbane and Perth, G3P[8] represented less than 2% of samples in each location. Overall, G3P[8] represented only 4.2% of strains nationally. Five G9P[8] strains, two each from Sydney and Melbourne, and one from Perth, comprised 1.1% of samples analysed. Four G4 strains were identified, two genotyped as G4P[8] in Melbourne and two as G4P[4] in Alice Springs and Brisbane.

A total of 12 (2.8%) uncommon strains were identified. A single G8 strain was identified in Darwin. One Brisbane strain was found to be VP7 G1 and VP6 Subgroup I. Ten were found to possess uncommon combinations of VP4 and VP7 genes, with 6 G1P[4] strains identified in Western Australia, and single G1P[6] and G3P[6] strains found in Brisbane and Melbourne, respectively, in addition to the 2 G4P[4] strains mentioned above. Seven (1.6%) rotavirus samples contained multiple types.

In 16.4% of samples either a G– or P-Type, or both, could not be assigned (Table 2). These samples may contain virus numbers below the detection limits of our typing assays or have inhibitors within extracted RNA that prevent the function of the enzymes used in RT and/or PCR steps.

The distribution of G & P genotypes between states using Rotarix (New South Wales, the Northern Territory, Western Australia and Tasmania) compared with RotaTeq states (Victoria, Queensland) appears to differ, as shown in Figure 2.
Table 1: Rotavirus G and P genotypes, Australia, 1 July 2008 to 30 June 2009

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td><strong>New South Wales</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sydney (POW)</td>
<td>0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Sydney (Westmead)</td>
<td>19</td>
<td>42.1</td>
<td>8.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>10.5</td>
<td>2.6</td>
<td>0.0</td>
</tr>
<tr>
<td><strong>Northern Territory</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alice Springs</td>
<td>106</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.9</td>
<td>1.8</td>
<td>0.0</td>
<td>21.8</td>
</tr>
<tr>
<td>Darwin</td>
<td>57</td>
<td>1.8</td>
<td>1.8</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>1.8</td>
<td>1.8</td>
<td>0.0</td>
<td>8.8</td>
</tr>
<tr>
<td>Western Diagnostic</td>
<td>60</td>
<td>28.3</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>1.7</td>
<td>1.7</td>
<td>0.0</td>
<td>3.3</td>
</tr>
<tr>
<td><strong>Queensland</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brisbane</td>
<td>54</td>
<td>59.3</td>
<td>32.1</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>3.7</td>
<td>1.8</td>
<td>5.5</td>
<td>24.0</td>
</tr>
<tr>
<td><strong>Victoria</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Melbourne</td>
<td>27</td>
<td>33.3</td>
<td>9.6</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>3.7</td>
<td>11.2</td>
</tr>
<tr>
<td><strong>Western Australia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PathWest</td>
<td>78</td>
<td>20.5</td>
<td>16.2</td>
<td>3.8</td>
<td>3.0</td>
<td>53.8</td>
<td>42.0</td>
<td>2.6</td>
<td>1.3</td>
<td>1.3</td>
<td>16.7</td>
</tr>
<tr>
<td>Perth</td>
<td>39</td>
<td>36.0</td>
<td>14.1</td>
<td>7.6</td>
<td>3.0</td>
<td>18.0</td>
<td>7.0</td>
<td>0.0</td>
<td>5.0</td>
<td>0.0</td>
<td>30.8</td>
</tr>
<tr>
<td><strong>Tasmania</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hobart</td>
<td>5</td>
<td>60.0</td>
<td>3.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>40.0</td>
</tr>
<tr>
<td>Total</td>
<td>445</td>
<td>22.5</td>
<td>100.0</td>
<td>1.3</td>
<td>6.0</td>
<td>50.3</td>
<td>224.0</td>
<td>4.2</td>
<td>1.1</td>
<td>1.6</td>
<td>16.4</td>
</tr>
</tbody>
</table>

* An additional 147 specimens were omitted from analysis due to insufficient sample or because the specimen was not confirmed to be rotavirus positive.

**Mix**
- Alice Springs: G9 P[4]/[9]
- Darwin: G1/2 P[4]
- Brisbane: G1/3/9 P[8], G1/3 P non-typeable
- PathWest: G1 P[4]/[8]
- Perth: G1/2 P[8], G1/2 P[4]

**Other**
- Alice Springs: G4P[4]
- Western Diagnostic: G8 P non-typeable
- Brisbane: G1P[6], G4P[4], G1 + subgroup I
- Melbourne: G3P[6]
Analysis of fully G and P typeable samples revealed that in Rotarix states G2P[4] strains dominate (59%), whereas in RotaTeq states G1P[8] strains dominate (51%) and G2P[4] strains compose only 5% of fully typed specimens. A slight increase in G3P[8] (11% vs 3%), G4P[8] (2% vs 0%) and G9P[8] (2% vs 1%) strains was observed in the states which introduced RotaTeq when compared with those using Rotarix. The majority of all samples analysed however, (364 of the 445 samples nationally) came from Rotarix states, with almost two-thirds originating from the high disease environment in the Northern Territory, and this discrepancy in sample number has to be considered when interpreting the data.

Vaccine associated diarrhoea

Faecal specimens were received from 25 children who developed rotavirus diarrhoea after being vaccinated with RotaTeq in Victoria and Queensland. Vaccine virus was identified in five of these cases by RT-PCR and sequence analysis of the VP6 gene, while wild-type rotavirus was identified in 16 samples.

RotaTeq induced diarrhoea was confirmed in a child with severe combined immune deficiency. Serial stool samples were collected (n = 14) post immunisation, and RT-PCR and sequence analysis identified RotaTeq vaccine strain in all samples.11

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

<table>
<thead>
<tr>
<th>Centre</th>
<th>Total</th>
<th>P non-typeable</th>
<th>G1</th>
<th>G2</th>
<th>G4</th>
<th>G9</th>
<th>G non-typeable</th>
<th>G &amp; P non-typeable</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>New South Wales</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sydney (POW)</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sydney (Westmead)</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Northern Territory</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alice Springs</td>
<td>23</td>
<td></td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Darwin</td>
<td>5</td>
<td></td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Western Diagnostic</td>
<td>2</td>
<td></td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Queensland</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brisbane</td>
<td>13</td>
<td></td>
<td>12</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Victoria</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Melbourne</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Western Australia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PathWest</td>
<td>13</td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perth</td>
<td>12</td>
<td></td>
<td>4</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tasmania</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hobart</td>
<td>2</td>
<td></td>
<td></td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total (%)</td>
<td>73</td>
<td></td>
<td>20</td>
<td>9</td>
<td>1</td>
<td>2</td>
<td>33</td>
<td>3</td>
</tr>
</tbody>
</table>

Figure 2: Overall distribution of rotavirus G and P genotypes identified in Australian children based on vaccine usage for the period 1 July 2008 to 30 June 2009
Discussion

In this report covering the period 1 July 2008 to 30 June 2009, we describe the annual epidemics and geographic distribution of rotavirus genotypes causing disease in Australian children during the second year after the introduction of national rotavirus vaccination. The rotavirus surveillance program highlighted the emergence of genotype G2P[4] as the dominant genotype, nationally representing 50.2% of all strains. This large number corresponded with a large outbreak of acute gastroenteritis in Alice Springs during February to May 2009 and emergence as dominant type in Western Australia. Genotype G1P[8] was the second predominant type nationally, comprising 22.5% of all strains characterised. It was the dominant type along the eastern seaboard, in particular in Melbourne, Sydney and Brisbane. This survey continues to highlight the ongoing fluctuations in the dominant genotypes, and represented the second time in the past 5 years where G1P[8] was not the dominant genotype nationally. As the overall numbers of rotavirus gastroenteritis decrease in the post-vaccine era, the contribution of outbreaks to the analysis of circulating strains by the NRRC is likely to be increased.

Similar to other reports, multiple common genotypes (G1P[8], G2P[4], G3P[8], G4P[8] and G9P[8]) continue to co-circulate within the Australian population causing significant disease with G1, G2 and G3 being identified in at least 5 states and territories. Unlike the first year of vaccine introduction where G1, G2 and G3 were all identified in more than 10% of specimens, the proportion of G3, G4 and G9 strains were much less, each representing less than 5% of total isolates.

The prevalence of G2P[4] strains has increased during the past 2 surveys. During the 2005/06 period G2P[4] was identified in only 4 sites and represented less than 5% of the total number of strains nationally, whereas during the 2007/08 survey G2P[4] strains had become the second most common type nationally, being identified in 9 sites, and represented the dominant type in the Northern Territory. G2P[4] have previously been responsible for 2 large outbreaks in the Northern Territory, both prior to vaccine introduction in 1997 and 2004. This outbreak represents the first emergence of G2P[4] since vaccine has been introduced. The increased detection of G2P[4] strains has been restricted to 2 of 3 states using the monovalent vaccine, but this trend is of uncertain significance and will require ongoing investigation. The emergence of G2P[4] has also been reported in vaccinated populations in Brazil (Rotarix) and Nicaragua (RotaTeq), but also to a lesser extent in non-vaccinated populations in Latin America. In this setting, the proportion of rotavirus cases has significantly reduced since vaccine introduction, however G2P4 strains have virtually replaced all other strains as the cause of diarrhoea 1–2 years after vaccine introduction.

In a comparison of rotavirus types identified, based on vaccine usage in the various states, differences in the prevalence rates of various genotypes were identified. G2P[4] strains were more prevalent in states using Rotarix, whereas G1P[8] and G3P[8] strains were more prevalent in states using RotaTeq. Not all the subjects from whom samples were obtained were eligible for vaccination, and the vaccination status of vaccine-eligible infants is unknown. Consequently, it is difficult to ascertain whether these differences are due to a lack of protection by either vaccine or by natural variation. G2P[4] strains have previously caused large outbreaks, but then their prevalence has generally declined in the following year. It will be important to determine whether G2P[4] continues to remain a common genotype causing disease in the Northern Territory and Western Australia. If G2P[4] strains decrease, then their emergence during the current study period is more likely due to the natural fluctuations in rotavirus genotypes, than to vaccine pressure.

Uncommon rotavirus types continue to be of worldwide interest because of the possible impact they could have on future rotavirus vaccine programs. This year 2 unusual VP7/VP4 genotype combinations were identified; G1P[4] and G4P[4]. This follows the identification of a G2P8 strain in the 2007/08 survey. A single G8 strain was identified in Darwin, continuing the ongoing observation of G8 strains in Perth, Brisbane and Darwin in the past 2 surveys. Reports of uncommon strains continue to highlight their low level existence in Australian children.

In the second year of rotavirus vaccine usage we have observed a change in the age distribution of children admitted to hospital when compared with the previous 12 month period. Changes in 2 age groups were identified. An increase was observed in the 0–6 month group (23% vs 14%), while a decrease in the 7–12 month age group was observed (14% vs 24%). No differences in rates of hospital admissions were identified in children aged 1–2 and 2–3 years. The increase in the 0–6 month infants age group may be due to the presence of G2P[4] strains in Alice Springs and Western Australia where over 40% of infants in these locations were 0–6 months of age.

The second year of vaccine implementation has seen the emergence of G2P[4] as the dominant genotype. Interestingly, this was restricted to states using Rotarix, however, the differences in genotype distribution were potentially magnified by the large G2P[4] outbreak that occurred in Alice Springs.
These changes could therefore be the result of continual fluctuations in rotavirus genotypes, and the unpredictable nature of changes in the prevalence of rotavirus strains across Australia, rather than to vaccine pressure. Understanding the fluctuations in rotavirus genotypes, using multicentre national surveillance, is needed to evaluate vaccine efficacy in the long term.

Acknowledgements

The Australian Rotavirus Surveillance Program is supported by grants from the Australian Government Department of Health and Ageing, GlaxoSmithKline and CSL.

Dr Kirkwood is supported by an RD Wright Fellowship, National Health Medical and 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 Australian rotavirus surveillance group includes:

New South Wales

Prof W Rawlinson, Dr C McIver and members of the Virology Division, Prince of Wales Hospital

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

Northern Territory

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

Dr M Leung, Ms A Reed and members of the Department of Microbiology, Western Diagnostic Pathology, NT & WA

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

Ms Heather Cook, Centers for Disease Control Darwin

Queensland

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

Dr M Nissen and department members, Pathology Queensland, Herston

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

Tasmania

Mr D Coleman 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

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 and members of Division of Microbiology, PathWest LM, The Queen Elizabeth Medical Centre, Nedlands

Author details

Dr Carl D Kirkwood, Senior Research Fellow
Ms Karen Boniface, Research Assistant
Professor Ruth F Bishop AO, Senior Principal Research Fellow
Professor Graeme L Barnes, Senior Principal Research Fellow

National Rotavirus Reference Centre, Murdoch Childrens Research Institute, Royal Children's Hospital, Parkville, Victoria

Corresponding author: Dr Carl Kirkwood, Enteric Virus Research Group, Room P104B, 1st Floor Gantry, Murdoch Childrens Research Institute, Royal Children's Hospital, Flemington Road, PARKVILLE VIC 3052, Telephone: +61 3 8341 6439. Facsimile: +61 3 8341 6449. Email: carl.kirkwood@mcri.edu.au

References


