

# Report of the Australian Rotavirus Surveillance Program 2003–2004

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## Abstract

The National Rotavirus Reference Centre together with collaborating laboratories Australia-wide has conducted rotavirus surveillance since June 1999. This report describes the serotypes of rotavirus strains responsible for the hospitalisation of children with acute gastroenteritis during the period 1 July 2003 to 30 June 2004. We examined 688 faecal samples using monoclonal antibody immunoassays, reverse transcription-polymerase chain reaction and polyacrylamide gel analysis. This revealed that serotype G1 has re-emerged as the major serotype nationally, representing 40 per cent of all strains, followed by serotype G3 (25.7%) serotype G2 (17.1%) and serotype G9 (11.7%). However, there is substantial geographic variation in the prevalence of rotavirus serotypes. These findings have implications for vaccine development strategies which have targeted prevention of disease due to serotypes G1-G4. *Commun Dis Intell* 2004;28:481–485.

*Keywords:* rotavirus, surveillance

## Introduction

Group A rotaviruses are the single most important cause of severe gastroenteritis in young children worldwide. An estimated 400,000–500,000 children die annually of severe diarrhoea, however few deaths occur in developed countries.<sup>1</sup> Rotavirus induced disease accounts for between 25–50 per cent of all hospitalisations for diarrhoea, with 10,000 Australian children hospitalised each year.<sup>2</sup> There is wide acceptance of the need for a vaccine to prevent rotavirus disease in children under five years of age throughout the world, with several vaccines under development. National rotavirus surveillance provides an understanding of the epidemiology of rotavirus in Australia, an important component for success in vaccine development and implementation.

The previous rotavirus surveillance report from the National Rotavirus Surveillance Program, covering the period July 2002–June 2003, highlighted the potential importance of uncommon serotypes such as serotype G9. Serotype G9 was first described in Australia in 1997<sup>3</sup> and since then has steadily increased in prevalence to become the dominant serotype nationally during the 2002/2003 period, representing 74.7 per cent of samples.<sup>4</sup> This was the first time since surveillance began in 1993, that serotype G1 was not the dominant type in Australia.

The surveillance and characterisation of rotavirus strains causing annual epidemics of severe diarrhoea in young children in Australia continues to be undertaken by the National Rotavirus Reference Centre in Melbourne, together with seven collaborating centres. In this report we describe the results of the Australian Rotavirus Surveillance program for the period 1 July 2003 to 30 June 2004, and identify the geographic distribution of the predominant rotavirus serotypes.

## Methods

Rotavirus detection was undertaken by enzyme immunoassay (EIA) or latex agglutination in collaborating laboratories. Rotavirus positive specimens were collected, stored frozen and forwarded to Melbourne, together with relevant age and sex details. Specimens were then tested using an in-house monoclonal antibody (MAb) based serotyping EIA. The EIA employed a panel of MAbs specific for the major glycoprotein VP7 of the outer capsid of the five major group A human rotavirus serotypes (G1, G2, G3, G4 and G9).<sup>5</sup> Strains which could not be assigned a serotype were genotyped by reverse transcription/polymerase chain reaction (RT/PCR) using serotype specific oligonucleotide primers.<sup>6</sup> Polyacrylamide gel electrophoresis (PAGE) was used to classify rotavirus strains genetically into electropherotypes and to confirm the sharing of the same electropherotype between collaborating centres.

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## Results

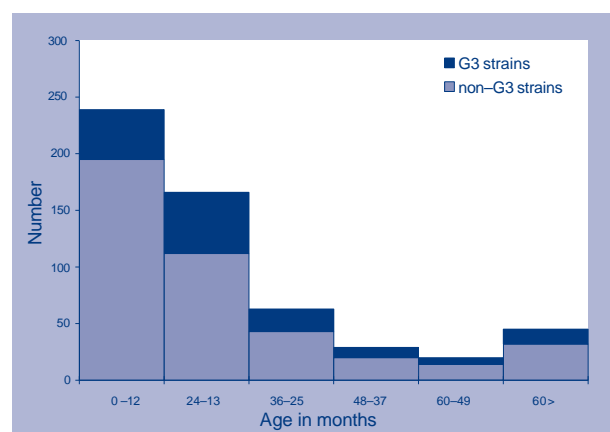
### Number of isolates

Six hundred and eighty-eight specimens were received for analysis from Melbourne and the collaborating centres in Western Australia, Northern Territory and New South Wales. During the sampling period, no samples were collected from South Australia, Queensland or Tasmania. Collections in those states resumed in the financial year beginning July 2004. A total of 608 specimens were confirmed as rotavirus positive using our in-house EIA assay. Specimens containing insufficient specimen for testing ( $n=29$ ) or specimens that were not confirmed to be positive for rotavirus ( $n=51$ ) were not analysed further.

### Age distribution

The overall age distribution of the children with acute gastroenteritis was typical of rotavirus infection (see Figure 1). In the reporting period, 42.5 per cent of cases were from infants 12 months of age or less, 29.5 per cent were from patients 13–24 months of age, and 11.2 per cent were from patients 25–36 months of age. Overall, 83.3 per cent of samples were from children three years or less, and 92 per cent were from children five years or less. When the age distribution was broken down according to serotypes, patients aged more than 12 months were significantly more likely to have a serotype G3 infection (69.9%) than infants aged less than 12 months of age (30.1% Chi-square = 11.87,  $P<0.001$ , Figure 1).

**Figure 1. Age distribution versus infecting serotype**



Slightly more male children than female were admitted to hospital during the year, (male to female ratio 1.3:1).

### Serotype distribution

The rotavirus serotypes identified in Australia from July 1, 2003 to June 30, 2004 are shown in the Table. Serotype G1 was the most common type identified, representing 40 per cent of all specimens. It was the dominant strain in only two centres (Melbourne and Sydney), and was identified in six of the seven centres. Serotype G3 was the second most common serotype nationally and represented 25.7 per cent of specimens over all. It was identified in four centres and was the dominant type in Perth and Western Australian Path Centre. The two Western Australian centres represent different geographic locations, one urban (Perth) and one remote, north western Western Australia (WA Path Centre). Serotype G2 was identified in three centres and represented 17.1 per cent of all specimens. It was the dominant type in Alice Springs. Serotype G9 was the third most common serotype and represented 11.7 per cent of all specimens. It was identified in all seven centres, but, was the dominant type in only two centres (Darwin and Gove). A single serotype G4 isolate was identified in Melbourne.

During the reporting period, 1.3 per cent of the rotavirus samples analysed contained multiple serotypes, and in 4.1 per cent of the samples a serotype was not identified. The latter could be samples with virus numbers below the detection limits of our assays. Alternatively, these could represent unusual serotypes not identified using standard methods. For example, we identified five specimens from Alice Springs which exhibited a super short RNA electropherotype but were not typeable. Future studies will include further characterisation of the genes encoding the outer capsid proteins of these strains.

### Discussion

National rotavirus surveillance from 1 July 2003 to 30 June 2004 was highlighted by the finding that serotypes G1, G2, G3 and G9 were each the dominant type in at least one of the collaborating centres.

Serotype G1 was the dominant serotype nationally comprising 40 per cent of all strains. This replaced serotype G9, which was the dominant strain nationally for the previous two years.<sup>4</sup> Serotype G9 persisted as the dominant strain in Darwin, but not elsewhere in Australia. Serotype G1, G2 and G3 were the common types in other centres. Serotype G1 was identified in six centres and was the dominant type in Melbourne and Sydney and the second most common serotype in two other centres Perth and WA Path centre. The re-emergence of serotype G1 as the dominant strain reinforces the importance of this serotype. G1 was the dominant serotype in

**Table. Rotavirus G serotypes in Australia, 1 July 2003 to 30 June 2004**

Centre	Total number	Serotype percentage (number)						
		G1	G2	G3	G4	G9	Mixed serotypes	No result <sup>‡</sup>
Melbourne	131	86.2 (113)	0	2.3 (3)	0.8 (1)	3.8 (5)	0.8 (1)	6.1 (8)
Sydney	40	75 (30)	0	0	0	20 (8)	2.5 (1)	2.5 (1)
Perth*	148	31.1 (46)	4 (6)	56.1 (83)	0	5.4 (8)	2 (3)	1.4 (2)
WA PathCentre*	137	32.1 (44)	6.6 (9)	49.7 (68)	0	5.8 (8)	0.7 (1)	5.1 (7)
Alice Springs	115	4.4 (5)	77.4 (89)	0	0	10.4 (12)	1.7 (2)	6.1 (7)
Darwin	33	15.1 (5)	0	6.1 (2)	0	78.8 (26)	0	0
Gove	4	0	0	0	0	100 (4)	0	0
	608 <sup>†</sup>	40 (243)	17.1 (104)	25.7 (156)	0.2 (1)	11.7 (71)	1.3 (8)	4.1 (25)

\* The two Western Australian centres represent different geographic area, one urban (Perth) and one remote Western Australia (WA Path Centre).

† An additional 80 specimens were omitted from analysis due to insufficient sample or specimen was not confirmed to be rotavirus positive.

‡ No result - unable to be serotyped with monoclonal antibodies or genotyped by RT/PCR.

surveys conducted in Australia from 1993 to 1996<sup>7</sup>, and during the 1999/2000 and 2000/2001 surveys.<sup>8,9</sup> These findings are supported by epidemiological studies conducted throughout the world which have continued to identify serotype G1 as the dominant serotype.<sup>10-14</sup>

The decline in prevalence of serotype G9 has been as dramatic as its emergence. Serotype G9 was first identified during Australia-wide surveillance in 1997,<sup>3</sup> and became the second most prevalent serotype nationally during the 1999/2000 and 2000/2001 surveys, representing 10 per cent and 18.1 per cent respectively of specimens collected in those years.<sup>8,9</sup> G9 became the dominant strain nationally in 2001/2002, comprising 40 per cent of the strains<sup>15</sup> and 2002/2003 comprising 74.7 per cent.<sup>4</sup> However, during the current survey, G9 while present in each centre, represented only 11.7 per cent of all strains. The decline in the prevalence of G9 strains around Australia in 2003/2004 was associated with an increase in the prevalence of G1 and G3 strains.

The increase in prevalence of serotype G3 in Australia has been dramatic. During the four previous surveys conducted Australia-wide, (1999/2000, 2000/2001, 2001/2002 and 2002/2003) serotype G3 represented less than two per cent of all strains. However during this survey, the prevalence of serotype G3 has increased to 25.7 per cent and was the dominant strain in West Australia. The high prevalence of G3 in Australia is remarkable when compared with the low prevalence rates of this serotype reported in other countries.<sup>10-14</sup> Interestingly, the emergence of serotype G3 has also been recently identified in two regions (Qinhuangdao and Zhengzhou) in a recent

study from China,<sup>16</sup> and represented 45 and 80 per cent of isolates from each region. Whether these G3 strains move eastward from Western Australia to Sydney and Melbourne, and have an Australia-wide impact similar to serotype G9, will be followed with interest during the next rotavirus season. The increase in prevalence of serotype G3 appears to have been associated with changes in the age distribution of children infected with rotavirus, when compared to non-G3 strains. While the majority of children (87%) infected with rotavirus were under three years of age, the G3 strains infected children aged 13–24 months more frequently than children aged 12 months or less ( $p < 0.001$ ). In contrast, over 50 per cent of the children infected with the other rotavirus serotypes were under 12 months of age. This data suggests that pre-existing antibodies may not protect against subsequent severe re-infection with the serotype G3 strain.

An outbreak of severe gastroenteritis again swept through Alice Springs in Central Australia causing a major impact on health care facilities in January 2004. Serotype G2 was responsible for this year's outbreak. The previous G2 outbreak in Alice Springs in 1993, was shown to be due to an unusual G2 strain derived by reassortment between subgroup I and subgroup II human strains.<sup>17</sup> The 2004 strain possessed the standard short pattern electropherotype and subgroup I antigenicity. Serotype G2 strains have previously been responsible for intermittent epidemics in several of the other centers during the past 12 years, including in Perth in 1993, Melbourne in 1994, and Sydney in 2001.<sup>7,9</sup>

These results together with those of previous years highlight the continuing change in the prevalence and emergence of new rotavirus serotypes. Multi-centre surveillance of rotavirus is important to continue to monitor strains in Australia. These results contribute to worldwide knowledge of rotavirus epidemiology and essential to inform the development of new rotavirus vaccines.

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#### **New South Wales**

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