Australian Rotavirus Surveillance Program: Annual report, 2006–07

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Abstract

The National Rotavirus Reference Centre, together with collaborating laboratories Australia-wide, conducts a laboratory based rotavirus surveillance program. This report describes the serotypes of rotavirus strains responsible for the hospitalisation of children with acute gastroenteritis during the period 1 July 2006 to 30 June 2007. One thousand and two faecal samples from across Australia were examined using a combined approach of monoclonal antibody immunoassays, reverse transcription-polymerase chain reaction and polyacrylamide gel analysis. Serotype G1 was the dominant serotype nationally, representing 36.7% of all strains, followed by serotype G9 (31.1%), and serotype G3 (23.3%). Serotype G2 represented less than 5% of strains, while no serotype G4 strains were identified. All G1, G3 and G9 strains assayed for P genotype contained the P[8] genotype, bar one G1 strain, which possessed a P[6]. Uncommon rotavirus genotypes, G8 (n=1) and G12 (n=2) were identified in children with acute gastroenteritis during this study period. *Commun Dis Intell* 2007;31:375–379.

Keywords: annual reports, rotavirus, disease surveillance, epidemiology

Introduction

Rotaviruses are the single most important cause of dehydration, hospitalisation and death due to severe gastroenteritis in young children worldwide. An estimated 600,000 children die annually of severe diarrhoea, however few of these deaths occur in developed countries.[1] Rotavirus induced disease accounts for up to 50% of childhood hospitalisations for diarrhoea in Australia. This represents 10,000 children hospitalised each year,[2] costing an estimated $26 million in direct costs.

In an effort to decrease the huge social and economic burden of rotavirus disease in Australia, two new rotavirus vaccines (Rotarix® [GlaxoSmithKline] and Rotateq® [Merck]) have been licensed and included in the National Immunisation Program free of charge to all young infants from 1 July 2007. Both vaccines were demonstrated to be safe and highly effective in prevention of severe diarrhoea and hospitalisation due to rotavirus infections during large-scale phase III clinical and efficacy trials, each involving over 60,000 children worldwide.[3,4]

Since 1999 the Australian Rotavirus Surveillance Program, has reported the changing annual pattern of dominant serotypes together with the multiple types identified in the Australian population each year. Results highlight the diversity of rotavirus strains capable of causing disease in children. Of particular importance was the emergence in 1999 and dominance in 2002 of serotype G9 strains nationally,[5] as well as the recent re-emergence of serotypes G3 and G4 as major causes of acute gastroenteritis in Australian children.[5,6]

Surveillance of rotavirus serotypes will provide important data to inform rotavirus vaccine programs. The impact of these two 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 serotype surveillance should identify the effects that each vaccine program has on circulating strains, in particular, whether changes occur in serotype incidence and whether increased proportions of rare or uncommon types result.

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 collaborating laboratories across Australia. In this report we describe the surveillance results for the period 1 July 2006 to 30 June 2007, and identify the geographic distribution of the predominant rotavirus serotypes causing disease in Australian children.

Methods

Rotavirus positive specimens detected by enzyme immunoassay (EIA) or latex agglutination in collaborating laboratories were collected, stored frozen and forwarded to Melbourne, together with relevant age and sex of the patient. Specimens were then serotyped 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).[7] Strains that could not be assigned a G serotype were genotyped by using a hemi-nested multiplex reverse transcription/polymerase chain reaction (RT-PCR), using G specific oligonucleotide primers.[8] P genotypes were determined by using a hemi-nested multiplex RT-PCR assay.[9]

Polyacrylamide gel electrophoresis (PAGE) was used to classify rotavirus strains genetically into electropherotypes, and to examine the extent of sharing of the same electropherotype between collaborating centres.

Results

Number of isolates

A total of 1,002 specimens were received for analysis from Melbourne and the collaborating centres in Western Australia, the Northern Territory, and New South Wales (Table). Eight hundred and twenty-seven specimens were confirmed as rotavirus positive using our in-house EIA assay. Specimens containing insufficient specimen for testing (n=27), or specimens that were not confirmed to be positive for rotavirus (n=148) were not analysed further.

Age distribution

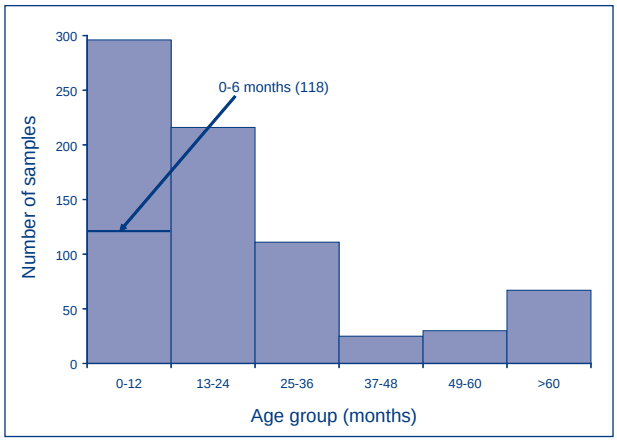
The overall age distribution of children with acute rotavirus gastroenteritis is depicted in the Figure. In the reporting period, 15.8% of cases were from infants aged 0–6 months, 23.9% of cases were from infants aged 7–12 months, 30% were from patients aged 13–24 months, and 14.9% were from patients aged 25–36 months. Overall, 84.6% of samples were from children aged 3 years or less, and 92% were from children aged 5 years or less.

During the study period, slightly more specimens from male than female children (n=446 vs. 309) were analysed.

Serotype distribution

The rotavirus serotypes identified in Australia from 1 July 2006 to 30 June 2007 are shown in the Table. Serotype G1 was the most common, representing 36.7% of all specimens, and was identified in all centres. It was the dominant strain in Sydney and Perth, and was the second most common type in Melbourne. Serotype G9 was the second most common serotype nationally, and represented 31.1% of specimens. It was identified in six of the eight collaborating centres but was the dominant type only in the Northern Territory, where it was responsible for a large outbreak of acute gastroenteritis between March and May 2007. Strains belonging to serotype G3 were the third most common type identified Australia-wide during this study period, and represented 23.3% of specimens. It was found in seven of the nine centres, and was dominant in Melbourne.

Cases of rotavirus, Australia, 1 July 2006 to 30 June 2007, by age group



Rotavirus G serotypes in Australia, 1 July 2006 to 30 June 2007

Centre	Total	Serotype													
		G1		G2		G3		G4		G9		mix		NR	
		%	n	%	n	%	n	%	n	%	n	%	n	%	n
Melbourne	180	29.4	53	13.9	25	39.4	71	0.0		14.4	26	0.0		2.8	5*
Sydney (POW)	32	75.0	24	0.0		0.0		0.0		21.9	7	0.0		3.1	1*
Sydney (Westmead)	25	32.0	8	28.0	7	8.0	2	0.0		24	6	0.0		8.0	2
Alice Springs	105	2.8	3	0.0		10.5	11	0.0		85.7	90	0.0		1.0	1
Darwin	141	2.8	4	0.7	1	16.3	23	0.0		75.2	106	0.7	1	4.3	6*
Western Diagnostic, NT	65	80.0	52	0.0		16.9	11	0.0		0.0		0.0		3.1	2
Perth	62	69.4	43	0.0		24.2	15	0.0		0.0		1.6	1	4.8	3
PathWest WA	217	53.9	117	2.3	5	27.7	60	0.0		10.1	22	0.5	1	5.5	12
Total	827	36.7	304	4.6	38	23.3	193	0.0	0	31.1	257	0.4	3	3.9*	32

An additional 175 specimens were omitted from analysis due to insufficient sample or because the specimen was not confirmed to be rotavirus positive.

* Two samples were identified as genotype G12 (Melbourne and Sydney) and one sample as genotype G8 (Darwin).

Serotype G2 strains were identified in four centres during the study, and represented less than 5% of the total strains identified. No serotype G4 strains were identified in any centre. Two genotype G12 strains were identified during the study, one in Sydney and one in Melbourne, while a single G8 strain was identified in Darwin.

P genotype was determined for 181 of the rotavirus positive samples. Sixty-four of the 65 G1 strains analysed were genotyped as P[8], and one sample was typed as P[6]. All of the G3 and G9 strains analysed were genotyped as P[8] (n= 41 and 70, respectively), while the five G2 strains analysed were all associated with P[4].

Less than 0.5% of the rotavirus samples contained multiple serotypes, and in 3.9% of the samples a serotype could not be identified. The latter could be samples with virus numbers below the detection limits of our assays, or could have contained inhibitors in extracted RNA that prevent the function of the enzymes used in RT and/or PCR steps. It is unlikely that these represent unusual serotypes not identified using standard methods, since none of the non-typeable isolates exhibited unusual PAGE patterns. Future studies will include further characterisation of the genes encoding the outer capsid proteins of these strains.

Discussion

In 2006–2007 the Australian Rotavirus Surveillance Program showed that serotype G1 continued to remain the dominant serotype nationally, comprising 36.7% of all strains characterised. It was identified in all centres, and continues to be the dominant type on both sides of the country, in particular in Sydney and Perth. This survey continues to highlight the importance of serotype G1 as a major cause of disease in Australian children. Similarly, serotype G1 continues to be reported as the dominant type in epidemiological studies conducted throughout the world.[10,11]

Similar to previous reports, multiple serotypes continue to circulate within the Australian population, causing significant disease in Australian children during the study period. This serotype diversity is illustrated by G1, G3 and G9 strains, that were each identified in at least six locations and were each the dominant serotype in at least one site during 2006–07. In all bar one instance, each strain was associated with the P[8] VP4 protein. Thus G1P[8], G3P[8] and G9P[8] combinations were the predominant strains identified in children during the current surveillance period.

The 2006–07 reporting period was also characterised by another large outbreak of acute gastroenteritis in the Northern Territory during March to May 2007. Similar to the large outbreak in 2001, a serotype G9P[8] strain was identified as the causative type. The importance of serotype G9 Australia-wide has been reflected with the continued increase in predominance, as well as the nationwide distribution of G9 strains during the past 2 years.[12,13]

In the Northern Territory, Rotarix® (GlaxoSmith-Kline) a live attenuated human G1P[8] virus rotavirus vaccine, had been introduced by the government into the immunisation schedule at 2 and 4 months of age for children born after 1 August 2006. This occurred 11 months prior to the introduction of rotavirus vaccines into the National Immunisation Program. This early vaccine adoption has provided an opportunity to determine how effective the vaccine is in an outbreak setting. Although Rotarix does not contain a G9 VP7 protein, protection against G9 serotypes has been previously demonstrated in phase III clinical trials, probably mediated via the VP4 P[8] protein.[4] Preliminary studies of children admitted to hospital during the rotavirus outbreak in Alice Springs suggest that children who were vaccinated were protected against severe disease (Dr Julie Graham, unpublished observations). However, continued assessment of rotavirus vaccine efficacy is required to fully understand the impact rotavirus vaccine has on rotavirus disease.

The prevalence of serotype G3 has slightly increased during the current survey, being present in seven of eight sites, but more significantly it was the predominant type identified in Melbourne. This emergence of G3 in Melbourne completes the eastward spread of G3 across Australia. During the past 4 years, G3 has slowly increased in prevalence across Australia, initially being dominant in Western Australia in 2003–04, then Western Australia and Northern Territory (including both Alice Springs and Darwin) in 2004–05, then Alice Springs in 2005–06.[6,12,13] While G3 has been identified in small numbers in eastern states since 2003–04, this year represents the first instance when it has predominated. The movement of serotype G3 across Australia is similar to that seen previously for serotype G9 in the early 2000s.[14,15]

Uncommon rotavirus types continue to be of worldwide interest because of the possible impact they may have on rotavirus vaccine programs. This year, two uncommon types have been identified in Australian children. Strains exhibiting a genotype G12 VP7 protein were identified in Melbourne and Sydney extending the previous identification of G12 in 2005–06 in Sydney, to a second location. Thus identification of G12 strains in Australia continues the worldwide identification of this emerging serotype.[16,17] The second uncommon type identified during this survey was a single genotype

G8 strain seen in Darwin. This strain represents the first report of a G8 strain in Australia since 1996.[18] Thus these reports of uncommon strains continue to highlight their existence in Australia.

The rotavirus serotyping results from this survey, together with those of previous years, highlight the unpredictable nature of changes in the prevalence of rotavirus strains across Australia and the potential for new and emerging strains to spread throughout the continent. In addition, the identification of genotype G8 and G12 further illustrate the diversity of strains capable of causing severe disease in Australian children. The introduction of the two rotavirus vaccines into the National Immunisation Program occurred after the conclusion of this year's surveillance. Understanding the fluctuations in rotavirus serotypes, using multi-centre national surveillance, will provide valuable insight into vaccine efficacy over the next 3–5 years.

Acknowledgements

The 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 and Medical Research Centre.

Rotavirus positive specimens were collected from numerous centres throughout Australia. The significant time and effort involved in the collection, storage, packaging, compiling data and forwarding of specimens was much appreciated.

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