

Report of the Australian Rotavirus Surveillance Program, 2000/2001

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Abstract

The National Rotavirus Reference Centre together with 15 collaborating laboratories Australia-wide conducted rotavirus surveillance from June 1999. The serotypes of rotaviruses that are responsible for the hospitalisation of children with acute diarrhoea were determined for the period June 2000 to May 2001. We examined 1108 rotavirus specimens using a combination of monoclonal antibody immunoassay, reverse transcription-PCR, and Northern hybridisation. Serotype G1 strains were the most prevalent overall (49.5%), and found in all centres. Serotype G9 rotaviruses, which were first identified in 1997, were second in importance (18.1%). Serotype G2 viruses were next (12.5%), followed by the re-emergence of serotype G4 viruses (9.7%). The findings of this study have implications for vaccine development strategies where protection against serotypes additional to G1-G4 may be required. *Commun Dis Intell* 2001;25:143-146.

Keywords: rotavirus, surveillance, serotype, vaccine, gastroenteritis

Introduction

Rotaviruses are the single most important cause of severe gastroenteritis in young children worldwide. The virus is responsible for the hospital admission of up to 10,000 children per annum Australia-wide.¹ The national surveillance program was designed to monitor the serotype variation of rotaviruses prior to and after anticipated rotavirus vaccine release in Australia. It supplements data from existing notification schemes which simply record the presence of the virus. Surveillance relies upon the co-operation and participation of sentinel laboratories from all States and the Northern Territory. This report covers the period June 2000 to May 2001.

Methods

Collaborating laboratories from each State and the Northern Territory undertook rotavirus detection by enzyme immunoassay (EIA) or latex agglutination. Rotavirus positive specimens were collected, stored frozen and forwarded to the Royal Children's Hospital (RCH) in 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 4 major group A human rotavirus serotypes (G1, G2, G3 and G4). Specimens with an absorbance value greater than 0.2 were considered positive for that serotype. Strains unable to be assigned a serotype were genotyped by reverse transcriptase/polymerase chain reaction (RT/PCR) using serotype specific oligonucleotide primers.² Northern hybridisation analysis utilising G type specific DNA probes under stringent hybridisation conditions was also employed to confirm serotype specificities.³ Polyacrylamide gel electrophoresis confirmed the sharing of electropherotypes between collaborating centres.

Results

Number of isolates

A total of 1391 specimens were received from 15 collaborating centres. Specimens containing insufficient specimen for testing or specimens that were not confirmed to be positive for rotavirus, were omitted from the serotyping data. A total of 1108 specimens were analysed for the June 2000 to May 2001 sampling period.

Rotavirus season

The peak months of rotavirus activity for all centres are shown in Figure 1. This combines the winter epidemics experienced in southern and eastern Australia with the irregular outbreaks in the Northern Territory.

Age distribution

The age distribution of rotavirus positive patients showed that the peak incidence occurred in children aged between 6 months and 24 months (Figure 2). The male:female ratio was 1.19:1. This result is similar to the findings of the 1999/2000 collection period.⁴

Serotype distribution

Rotavirus serotypes circulating in Australia from June 2000 to May 2001 are shown in Figure 3. Overall, serotype 1 was the most common serotype nationally, being responsible for 49.5 per cent of specimens, and was the predominant type in 8 of the 16 centres studied (Brisbane, Townsville, Adelaide, Perth, Northern Western Australia, Narrabri, Melbourne and Horsham). Serotype G9 was the second most common and was responsible for 18.1 per cent of infections. Type G9 viruses were present in Melbourne, Sydney, Brisbane, Perth, Horsham, Adelaide, northern Western Australia, Darwin and Alice Springs. Type G9 viruses were found to be the most important infecting rotavirus serotype in Alice Springs during a May 2001 rotavirus outbreak.

Figure 1. Reports of rotavirus, Australia, June 2000 to May 2001

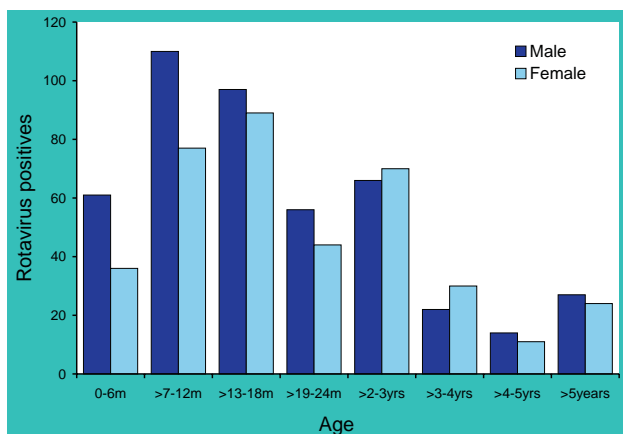


Figure 2. Reports of rotavirus, Australia, 1 June 2000 to 31 May 2001, by age

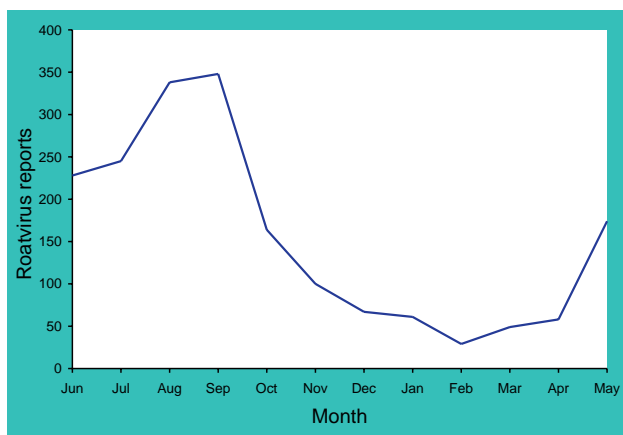
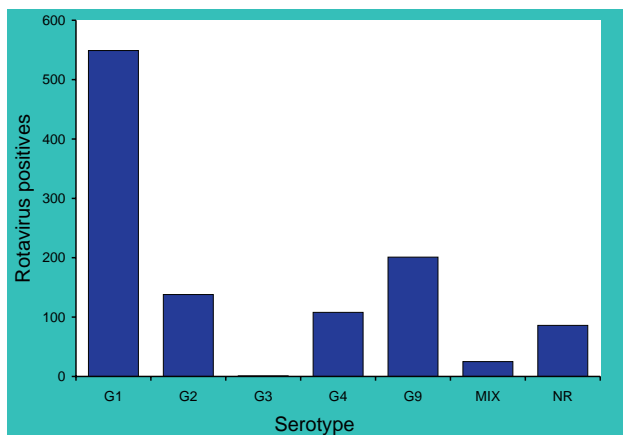


Figure 3. Reports of rotavirus, Australia, 1 June 2000 to 31 May 2001, by serotypes



Over 150 children were admitted to the Alice Springs Hospital. The epidemic was unusual in that there were 2 infecting serotypes circulating during the outbreak (G1 and G9), with G9 the most prevalent serotype (66% during May 2001). The G9 epidemic appears to have spread to Darwin at the time of writing this report (23 July 2001).

Serotype G2 was the next most common serotype, identified in all centres except Townsville and Alice Springs. Interestingly, this was the most common serotype in western

Sydney (46%). Serotype G4 was identified in 8 of the 15 centres (Sydney, Narrabri, Darwin, Gove, Perth, Adelaide and Melbourne) and was the predominant serotype in 2 centres, south east Sydney (62%) and Gove (88%). Significantly, serotype G4 strains were more prevalent in 2000/2001(9.7%) than the previous 1999/2000 season (1.9%) (Table).⁴

One serotype G3 virus was detected during the sampling period. Mixed infections (2.8%) were elevated when compared to the same sampling period for 1999/2000 (less than 1%).⁴ A serotype could not be assigned to 7.8 per cent of specimens. This result was lower than for the corresponding time period in 1999/2000⁴ due to increased use of molecular detection techniques.

These data illustrate some important differences between the distribution of serotypes in different parts of Australia, and map the occurrence of a 'new' serotype G9 which recently emerged worldwide.

Discussion

National rotavirus surveillance from 1 June 2000 to 31 May 2001 was highlighted by 2 outbreaks reported during the sampling period, a serotype G4 outbreak in Gove in the Northern Territory's 'Top End' in September 2000, and a G9 outbreak in Alice Springs in May 2001.

Analysis of rotaviral RNA by polyacrylamide gel electrophoresis of Darwin specimens collected in August 2000 and Gove specimens collected in September 2000, showed that the strains all shared the same electropherotype. The electropherotype appeared initially in Darwin in non-hospitalised children, and appeared to move east from Darwin to the isolated communities around Gove in Arnhem Land, where children were admitted to hospital with acute gastroenteritis. A similar electropherotype was circulating in Sydney earlier in 2000, suggesting the virus had moved from Sydney to Darwin.

Alice Springs experienced a large rotavirus outbreak in May 2001. Serotype G9 was detected in children from wide-spread remote locations as far as the Western Australian and South Australian borders. The outbreak put significant pressure on existing hospital facilities, and highlights the need for a rotavirus vaccine effective against serotype G9 as well as serotypes G1 to G4. The strain appears to have its origins in Perth where it was circulating from March to May 2001. Both the Alice Springs and Perth strains shared the same RNA electropherotype. The subsequent northward spread of serotype G9 strains to Tennant Creek, Katherine and Darwin, suggests the newly emerging strain is capable of causing widespread morbidity.

G1 viruses dominated in most centres and were present in all centres studied, This is consistent with previous studies undertaken in Australia (1993-1996),⁵ the United Kingdom (UK) (1996)⁶ and the United States of America (USA) (1996-1997).⁷

Serotype G2 specimens were identified by RT/PCR and Northern hybridisation because our serotype G2 MAbs no longer recognise the viruses. Gene sequence analysis of the MAb binding site of the G2 virus, showed the virus possessed an amino acid change at the same position as selected antigenic variants, that were unable to bind to the neutralising MAb. The change suggests G2 viruses

Table. Reports of rotavirus serotypes, Australia, June 2000 to May 2001, by centre

Centre	Serotype							Total
	G1	G2	G3	G4	G9	Mixed	NR*	
SE Sydney	10	6		33	2		1	52
W Sydney	31	65		30	5	8	2	141
Narrabri	10	2		1				13
Brisbane	33	5			3	2	9	52
Townsville	12							12
Darwin	15	1			6		10	32
Darwin WesternPath	1	1		9	1			12
Alice Springs	65				96		6	167
Gove	1	1		22		1		25
Perth	160	7		2	38	4	11	222
WA PathCentre	67	6		1	3		16	93
Adelaide	81	5		1	1		2	90
Hobart	2	12					3	17
Horsham		2			8			10
Melbourne	59	25	1	9	38	10	21	163
Total	549	138	1	108	201	25	86	1108

* Non reacting to G1, G2, G3 and G4 monoclonal antibodies

† Alice Springs/Darwin rotavirus epidemic still continuing at time of printing

circulating in 2000/2001 are different serologically from G2 viruses from 1983.

The antigenic similarities between G4 and G9 viruses were again apparent. G9 specimens reacted with both G4 and G9 serotyping MABs. The use of RT/PCR and Northern hybridisation confirmed serotype and clarified any serological cross-reactivities. Serotype G9 viruses were first described in Australia in 1999.⁸ G9 viruses which were previously reported in India (1993-1994),⁹ Bangladesh (1987-1997),¹⁰ Malawi (1997-1998),¹¹ the USA (1996-1997),⁷ and the UK (1996),⁶ appear to persist as a major infecting serotype in Australia. Its circulation and significance should be closely monitored because of the potential impact it may have on vaccine development strategies.

The first evidence of serotype diversity within the one geographical location was noted during the 2000/2001 sampling period. Rotavirus specimens received from southeast Sydney were predominantly G4 viruses, whereas those from western Sydney appeared to be mainly G2 viruses. The reason for this difference is unclear. Continued surveillance of Sydney rotavirus serotypes should provide greater insight into rotavirus serotype diversity within a single geographical location.

The increased reliance on RT/PCR as a diagnostic tool in the study, led to a decrease in the number of non-typable specimens and a corresponding increase in the number of specimens with mixed infections. Mixed infections have been reported up to a frequency of 18.8 per cent in Ireland (1997-1999),¹² where standard rotavirus serotypes were mixed with strains that had not been identified in that country before. Mixed infections have the potential to generate reassortants resulting in new or emerging strains that may be the causative agents in major rotavirus outbreaks of the

future. Judicious use of RT/PCR as an effective surveillance tool is justified.

Ongoing surveillance of rotavirus serotypes is warranted due to the impact of new and emerging rotavirus serotypes. This is particularly pertinent when considering the deleterious impact G9 rotaviruses have had on the communities in and around Alice Springs in 2001. Variation in the predominant strains in different centres is important when considering the potential impact of vaccination, and to inform the design of second and third generation vaccines.

Asia-Pacific collaboration

The National Rotavirus Reference Centre (NRRC) hosted Dr Janice Lo, a senior medical and health officer of the Department of Health for the Hong Kong Government. Janice spent 3 months with the NRRC examining the Australian Rotavirus Surveillance System. During her stay she undertook a laboratory based rotavirus surveillance course utilising a sample cohort from Hong Kong. Janice plans to submit her results to the Hong Kong Medical Journal and intends to initiate a rotavirus surveillance study in Hong Kong using the skills she acquired in Australia.

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