Distribution of rotavirus G and P types in North and South Indian children with acute diarrhoea in 1998–99

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Abstract

VP7 and VP4 genotypes of 82 rotavirus strains from children with acute diarrhoea during November 1998–January 1999, in 4 Indian towns, were determined by reverse-transcription PCR. Overall, 68/82 (83%) could be VP7- and 52/82 (63%) VP4-typed. Geographical differences in rotavirus circulation have implications for future vaccination strategies.

Keywords: rotavirus, genotypes, PCR, diarrhoea, children, geographical distribution, India

Group A rotaviruses cause acute gastroenteritis in children worldwide, but are especially significant causes of morbidity and mortality in developing countries. They are classified into serogroups (A–G), based on the antigenic specificity of the inner capsid protein VP6 and into further types based on the 2 outer capsid proteins, VP7 and VP4. In recent years, reverse transcription polymerase chain reaction (RT-PCR) based on genotyping of VP7 (G-typing) and VP4 (P-typing) has replaced older serological techniques in molecular epidemiological studies. G-genotypes and serotypes are concordant but P types are not and have a double nomenclature because serotype designations are not unique. VP7 and VP4 proteins. VP7 and VP4. In recent years, reverse transcription polymerase chain reaction (RT-PCR) based on genotyping of VP7 (G-typing) and VP4 (P-typing) has replaced older serological techniques in molecular epidemiological studies. G-genotypes and serotypes are concordant but P types are not and have a double nomenclature because serotype designations are not available for all genotypes.

The commonest G types in human infections worldwide are G1–G4 and the commonest P types, the P1A[8] and P1B[4], but unusual isolates have been found in different parts of the world (KOSHIMURA et al., 2000) from different patient groups, with symptomatic and asymptomatic infections. A previous study from India studied the geographical variability of rotavirus strains, using a single-round PCR, and identified G9 strains in North India (RAMACHANDRAN et al., 1996). We now report the use of a nested K1–PCR and the identification of G9 rotaviruses in South India, with distinct variations in rotaviral circulation in 4 Indian towns during a single season.

This study was carried out during the winter (November 1998–January 1999) in 4 Indian towns (Vellore and Mysore in South India, Jalandhar and Yamunagar in North India). One primary care physician in each town was requested to collect samples from children aged <5 years, presenting with the symptoms of acute gastroenteritis during the winter period. A total of 365 faecal samples were analysed for identification of enteric pathogens and 82 were positive for rotaviruses by electron microscopy, latex agglutination (MERITEC, Meridian Diagnostics, Cincinnati, Ohio) and ELISA (Dako-EIA, Dakopatts, Copenhagen). The age distribution of the children ranged from 4 months to 5 years (mean 24 months) and was similar in all towns.

Genotyping of rotaviruses was performed using random priming as previously described (ITURRIZA-GOMARA et al., 1999). Sequencing of representative G types and all unusual isolates using an automated sequencer and sequence comparison was carried out as described earlier (ITURRIZA-GOMARA et al., 2000). Electropherotyping of viral RNA (BROWN et al., 1988) was done for representatives of each G and P type, and for all isolates that could not be typed by PCR.

Rotaviruses were identified in 82 (22.5%) of 365 faecal samples from children during the winter of 1998–99, with other bacterial and parasitic enteric pathogens in 71 (19.5%) samples, mainly Campylobacter spp. (n = 14, 4%), Shigella spp. (n = 14, 4%), and unknown (n = 1). A total of 8 strains (31%, 52 (63%) were P-typed. In each town, 75% strains typed by the G and P PCRs, with the exception of Mysore where 7 strains (32%) had a first-round product in the P typing PCR that did not amplify in the second round. The distribution of G and P types is shown in the Table.  

The commonest G: P combination seen was G1: P1A[8] (n = 14, 60% of all typed G1 strains) and this was followed by the unusual combination G1: P1B[4] (n = 9, 40% of all typed G1 strains) which was found in Vellore and Jalandhar. P2A[6] strains were found in Mysore and Jalandhar, in combination with G type 9 (n = 1) and G2A[8] (n = 1). A total of 8 strains (32%) from Mysore and 1 from Vellore (6%) (G4, 2 G1, 1 mixed, 1 G undetermined) gave a product in the first round of the nested PCR, but did not amplify in the second round, despite attempts at specific priming.

Four of 7 G9 strains were sequenced. On comparison of the entire 1062 bp of the VP7 gene, there was 99–100% sequence homology between the strains, and these were 96–99% similar to strains from the USA and the USA as has been previously reported, but were more distantly related to 116E, the Indian asymptomatic isolate (ITURRIZA-GOMARA et al., 2000). Electropherotyping of 6 G9s found 'short' patterns in all isolates, unlike the 'long' pattern reported for 116E. Other G and P untypable strains from North India had both 'short' and 'long' patterns.

In this study, G1 rotaviruses were the most frequently identified strains in 3 of 4 towns. In Vellore, the proportions of G1 and G4 were almost equal, similar to a previous hospital-based study from Vellore in the early 1980s (BROWN et al., 1988). Previous reports from North India have shown the presence of G types 1–4 and 9 (HUSAIN et al., 1996; RAMACHANDRAN et al., 2000), but no reports have indicated the presence of unusual types or G9 in this region.

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but no G9s were found in North India in this study. Distinct from our findings in South India, G3 were the second most common strains after G1 in both North Indian towns.

The most unusual findings were seen in Mysore where G9 strains (32% of all samples G typed) were the most common strains seen, although G1–4 were also isolated. It is also interesting to note that the P typing PCR used here amplified 7 isolates in the first round but not in the second round which may imply that these could be unusual VP4 types, potentially derived from cattle, since a recent report, also from Mysore, of a G8: P6[1] also supports the possibility of human–animal reassortment (JAGANNATH et al., 2000). In Vellore the high rate of isolation of G1: P1B[4] which have been reported as rare strains from India earlier (HUSAIN et al., 1996) suggests that reassortants continue to arise among human strains as well. A collection and analysis of circulating animal rotaviruses, potential human–animal and human–human reassortants may lead to valuable insights into the genes involved in host range restriction and pathogenicity.

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