

Shift in genomic RNA patterns of human rotaviruses isolated from white children in South Africa

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Summary

The molecular epidemiology of rotavirus infection in white children in Pretoria was investigated over a 2-year period. Rotavirus-positive specimens from 322 infants and young children submitted to private pathology laboratories were analysed by polyacrylamide-gel electrophoresis of the viral RNA. A predominance of long RNA profiles occurred and a temporal shift in the genomic patterns was identified. An epidemic of the classic shorter RNA profiles (suggestive of sub-group I rotaviruses) was observed in the winter of 1987, although these viruses were found less frequently than the sub-group II isolates (with a long RNA profile). Most neonatal isolates of rotaviruses exhibited a similar RNA electrophoretic type, indicating that this strain of rotavirus was nosocomially acquired in different maternity units in the city.

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The antigenic relatedness of human and animal strains of rotavirus and initial difficulties in cultivating human rotaviruses hampered early attempts at serological characterisation of human rotavirus strains. Analysis of electrophoretic mobility of the 11 segments of double-stranded RNA by polyacrylamide-gel electrophoresis (PAGE) yields a pattern that is both constant and characteristic for a particular rotavirus isolate,¹ and this has become the most commonly used method for epidemiological studies of rotavirus infection.^{1,2}

Rotavirus RNA migration pattern analyses from several countries have revealed considerable genomic diversity.³⁻⁵ Reports from South Africa have concentrated on the black population,^{6,7} although rotavirus infection has been reported to be more common among white infants and young children.⁸

The use of RNA gel electrophoresis to identify human rotaviruses is reported and their electrophoretotypes from hospitalised neonates and non-hospitalised white children with diarrhoea in Pretoria over an 18-month period are described.

Materials and methods

Rotavirus-positive stool specimens were received from 322 white children between April 1986 and December 1987. The specimens were processed at Niehaus and Botha Pathology Laboratories, which are situated in central Pretoria. The specimens were from 229 non-hospitalised infants and young children < 4 years old with diarrhoea and from 93 neonates with diarrhoea in different maternity hospitals. The screening test for rotavirus was performed using a latex agglutination assay as specified by the manufacturers (Rotascreen, Mercia Diagnostics).

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Extraction of the viral RNA genome was performed as described previously.⁷ Electrophoresis of the extracted RNA was conducted in 10% polyacrylamide gels using the discontinuous buffer system of Laemmli.⁹ The gels were stained with 0,5 µg/ml ethidium bromide for 40 minutes and photographed under UV light.

Results

An RNA electrophoretic profile was observed in 74,2% of the rotavirus-positive specimens (239/322), including both the classic long and the short electrophoretotypes. A predominance of long profiles occurred (200/239) with 8 patterns being evident, while 3 short patterns were identified (Fig. 1). The electrophoretic patterns were classified according to the 'Rotacode' devised by Moosai *et al.*¹⁰ The electrophoretotypes were listed alphabetically (Z, Y, X, etc.) in chronological order of detection with the prefix 'L' for long strains and 'S' for short strains. (Previously rotavirus electrophoretotypes isolated from black children were listed A, B, C, etc., in chronological order of appearance.)

Genomic shift in the occurrence of rotavirus strains, as identified by electrophoretic profiles, is illustrated diagrammatically in Fig. 2. Four of the long strains (LZ, LY, LX, and LW), which were found to occur during 1986, all decreased in number during 1987 and eventually were no longer observed at all. One electrophoretic strain (LV) only appeared in 1986 while 2 others (SY and LU) were only seen in 1987 and may represent the 'herald wave' of a newly emerging strain(s). The predominant strain of 1987 (LA) first appeared in low numbers in 1986 becoming the most prevalent form during the winter of 1987. This strain was previously reported to occur in black children in Ga-Rankuwa,¹¹ first in 1984 and tailing off in 1986.

An epidemic of the short RNA profiles (suggestive of human sub-group I rotaviruses) occurred between March and September 1987 when 27 of the 39 short strains appeared (69,2%).

A specific long RNA profile was observed from rotavirus-positive stools isolated from neonates. This RNA profile (designated LN) was observed to occur only in neonates over the whole period of the study, indicating that it was nosocomially acquired in the maternity units.

Discussion

Despite the difficulties in interpretation, electrophoretic analysis of the human rotavirus genome RNA has been widely used in studies of the epidemiology of rotavirus infection. The results reported in this study agree with those of other workers³⁻⁷ in that they demonstrate extensive variation at the molecular level between human rotavirus isolates.

During the study period 11 different electrophoretotypes circulated in the population, 4 of these making up the majority of the virus strains identified (144/239). Two of these electrophoretic strains, occurring in equal numbers, were the predo-

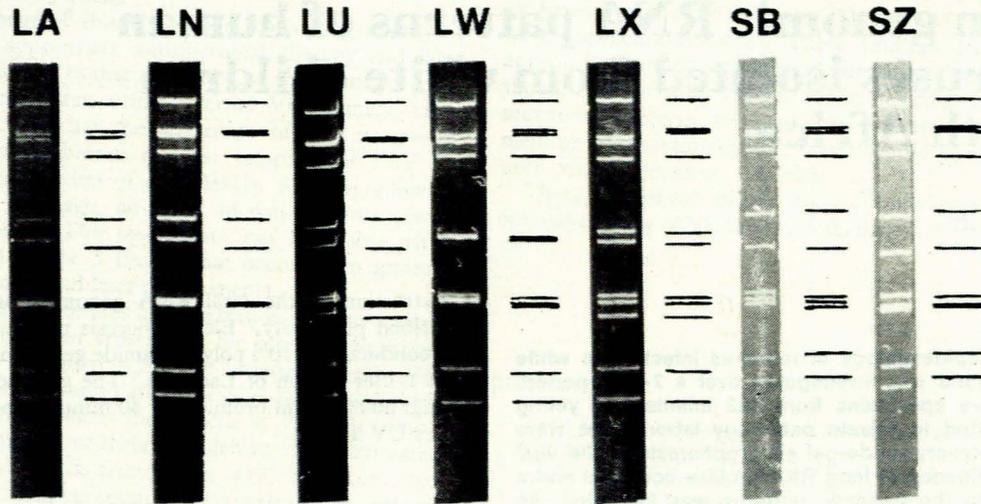


Fig. 1. Representative rotavirus electrophoretotypes isolated from children in Pretoria. The gels were run in 10% polyacrylamide gels overnight at 100 V and run from top to bottom. The figure is a composite from different gels.

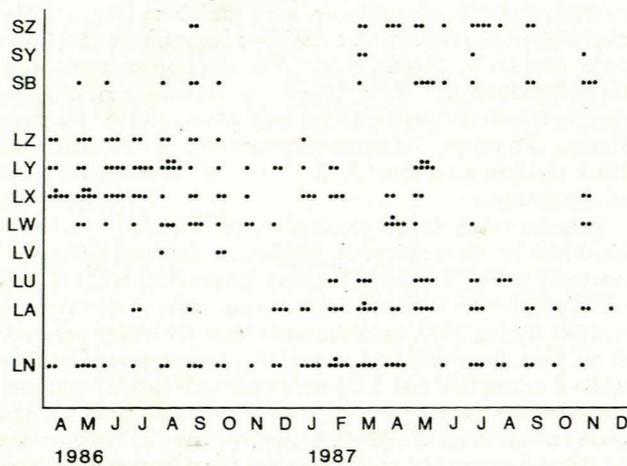


Fig. 2. The temporal distribution of electrophoretic strains of rotavirus observed over a 2-year period in Pretoria. Each dot represents a single isolate of that strain. Long profiles are presented by 'L' and short patterns by 'S'. The strain LN is the conserved neonatal strain found in at least 4 different maternity units.

minant strains during 1986. A third occurred in low numbers during 1986 but swiftly became the most numerically dominant strain in the following year. However, the most numerically dominant electrophoretotype identified over the 18-month period was that observed to occur in neonate stools; this probably indicates nosocomial acquisition of the virus.

Neonatal infection with rotavirus is usually asymptomatic, although it is not yet clearly understood whether this is related to host factors that restrict the efficient propagation of the virus, to passively acquired immunity from the mother or due to avirulent strains of the virus.¹² The most commonly occurring neonatal strain (LN) was observed in the vast majority of neonates with observable RNA electrophoretotypes and demonstrates the presence of an endemic and presumably conserved strain of rotavirus in several different maternity units. A few rotavirus isolates from neonates exhibited differing patterns, and may represent rotavirus acquired from outside the hospital setting. It is also of interest that the neonatal specimens were received from 4 separate maternity units and these results

indicate that all 4 units have a common, conserved (in terms of RNA electrophoretotype) strain of rotavirus. This would indicate that the nursing staff, or more probably the doctors themselves, are carrying the virus between different hospitals.

Some studies have suggested that a major shift in the most prevalent electrophoretotype occurs every 2 - 3 years.^{5,13} This study, although not extensive in duration, has highlighted some important factors. During the second half of 1986, the rotavirus strain that was to become the most numerically dominant during 1987 was first observed. This strain was previously reported to occur in black children attending Ga-Rankuwa Hospital (about 35 km from Pretoria), where it was one of only two strains that were common to both black and white infants in these geographically close areas.¹¹ The rapid increase in the numbers of this strain isolated from the white children of Pretoria indicates that an epidemic of this strain has occurred and that the Pretoria community of children was immunologically naive to this strain.

It has been proposed that new rotavirus strains occur in one of two ways; (i) via antigenic drift whereby small sequential changes in the genome eventually result in a new virus strain; or (ii) via reassortment of two viruses infecting the same host (corresponding to antigenic shift).¹⁴ A prerequisite for gene reassortment to occur is the simultaneous infection of 1 individual with more than 1 strain of rotavirus. In this study 6 children had mixed rotavirus infections, as evidenced by the appearance of more than 11 strands of RNA, and may represent cases from which reassortment rotavirus strains could emerge. The occurrence of dual infections would appear to be reasonably common with reports of up to 10%¹⁴ (2,5% in this study); this is probably the major mode by which new virus strains are generated with the subsequent potential for changes in antigenicity and pathogenicity.

In this study, as in most others, it has been assumed that virus strains with similar RNA migration patterns (i.e. in which all the RNA segments co-migrated) were in the same electrophoretic group. While this may be valid when viruses are closely related (e.g. isolates from the same localised outbreak or from the same patient), it may not be true of isolates from widely separated occurrences. This significance of the extensive genomic variation observed for rotaviruses is unclear, although the possibility that the genomic variation reflects alterations in viral pathogenicity or transmissibility has still to be fully investigated.¹⁵

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