Monitoring of rotavirus infection in a paediatric hospital by RNA electrophoresis

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During the spring of 1987 and the autumn of 1988, stool specimens were collected from infants and young children in the paediatric unit at H. F. Verwoerd Hospital, Pretoria, and examined for the presence of rotaviruses to assess the potential for hospital-acquired infection in the paediatric wards. Stool samples were also collected from children admitted to the hospital for causes unrelated to gastro-enteritis to investigate the possible asymptomatic carriage of rotavirus in this population. Hospital-acquired rotavirus infection was determined in only 9% of cases. Very little asymptomatic carriage of the virus was identified. Electrophoretic analysis of the rotavirus strains showed that the majority of the infections (20 of 42) were associated with a particular strain with a long RNA profile, while 7 minor strains co-circulated (5 with a long electrophoretype and 2 with a short one). An apparent small outbreak of nosocomial infection with a single strain was observed to occur in one of the paediatric wards during the spring and early summer.

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Rotavirus is well known as an important cause of acute infantile gastro-enteritis resulting in the hospitalisation of young children with the infection.[1] However, the virus has also been reported to be a leading cause of nosocomial infection, especially in newborn nurseries[2,3] and paediatric units.[4-6] Recent reports have highlighted the tremendous impact of nosocomial infection with diarrhoeal agents, causing an inestimable increase in morbidity, a potential increase in infant mortality and a significant increase in hospital costs.[7,8]

Rotavirus is ideally suited to spread in the hospital setting owing to: (*i*) the large numbers of infectious particles excreted in the stools of infected individuals,[1,9] (*ii*) the low infectious dose of virus;[4,10] (*iii*) the resistance of rotavirus to commonly used disinfectants;[11,12] (*iv*) the often asymptomatic carriage of the virus;[13,14] (*v*) the ability of the virus to remain

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viable for long periods of time;[15,16] and (*vi*) the potential for transmission of the virus via hands or fomites (see reference 17 for a recent review). These factors, coupled with the congregation of susceptible hosts in a closed setting, make the problem of cross-infection a perennial one for hospital staff.[18]

Electrophoresis of the rotavirus RNA genome has been used to monitor the transmission and spread of rotaviruses in the field[3,19,20] and in the hospital.[4-6] The rotavirus genome consists of 11 segments of double-stranded RNA which can be separated by electrophoresis through polyacrylamide gels. Electrophoresis of the viral genome allows the detection of a recognisable RNA profile or 'electrophoretype' for each distinct viral strain. This RNA profile is both constant and characteristic for the particular strain,[19] and is therefore ideal for the identification and monitoring of rotavirus infection and, in particular, nosocomial or single-source outbreaks.

This study was initiated to examine the prevalence of rotavirus infections in children admitted to H. F. Verwoerd Hospital, Pretoria, and to examine the incidence of hospital-acquired rotavirus infection as recognised by the molecular epidemiology of the rotavirus strains. We also attempted to examine the prevalence of asymptomatic carriage of the virus in children admitted to the hospital with symptoms unrelated to gastro-enteritis.

Materials and methods

Patient sample

Between August and November 1987 and between March and June 1988 stool specimens were collected at random from children under 6 years of age in two paediatric wards at H. F. Verwoerd Hospital. Ward 1 has 20 beds and admits children older than 3 years, including those with infectious diseases. Patients with gastro-enteritis are housed in one of the four cubicles of this ward when sufficient beds are available, or in an open area of the ward with cot beds. Ward 2 has 21 beds and admission is limited to small babies and toddlers. Very ill infants are housed in one of the four cubicles in the ward.

Previous reports have demonstrated a seasonal distribution of rotavirus infection in this sub-continental region,[21,22] and to examine this possibility specimens were collected during the spring and early summer of 1987 and again during the following autumn and early winter (Table I). Furthermore, owing to the asymptomatic carriage of rotavirus infection reported by others[13,14] and the lack of knowledge of levels of asymptomatic carriage in our region, it was decided to collect stools from children both with and without symptoms of gastro-enteritis.

Hospital-acquired rotavirus infection was defined as rotavirus infection first found to occur more than 72 hours after admission to the hospital or within 48 hours after discharge.[6,7,23]

Rotavirus detection

The specimens were diluted in phosphate-buffered saline to produce 20% suspensions which were then stored at –20°C until further analysis was performed. The presence of rotavirus antigen was detected using a commercially available enzyme immunoassay (Rotavirus EIA, International Diagnostic Laboratories, Israel), which was used as specified by the manufacturers. Each specimen was tested in duplicate.

Table I. Patient sample showing numbers of children with rotavirus infection and of those infected in the hospital*

Season	No. of patients	No. positive for rotavirus	No. infected in hospital
Sep - Nov 1987	192	18	14
< 1 mo.	8	2	2
1 - 12 mo.	91	12	9
13 - 24 mo.	51	1	1
> 24 mo.	30	3	1
Apr - June 1988	146	26	14
< 1 mo.	6	1	0
1 - 12 mo.	68	13	8
13 - 24 mo.	41	8	3
> 24 mo.	23	4	3

* Ages were not recorded for all children.

Polyacrylamide gel electrophoresis

The viral RNA genome was extracted from all rotavirus positive specimens according to previously published methods.[24] Briefly, viral RNA was extracted from the faecal suspension by deproteinisation with a phenol-choloroform mixture followed by ethanol precipitation. Electrophoresis of the extracted RNA was performed in 10% polyacrylamide vertical slab gels at 100 V overnight.[25] The gels were subsequently fixed and silver-stained for visualisation of the RNA bands.[26]

Results

Patient sample

Overall, 828 stool specimens from 338 patients were analysed for the presence of rotavirus. This represented over 20% of the total admissions to the wards during the study period. The children included in the sample were aged between 4 days and 6 years (mean age 21 months). During the warmer months (August - November 1987) 462 specimens were received from 192 children admitted to the two paediatric wards. During the cooler months (March - June 1988) 416 specimens were received from 146 patients.

Rotavirus infection

Rotavirus infection was identified in 44 of the 338 patients (13%). More children were found to be excreting rotavirus during the autumn/winter season (26 of 146 children) than during the spring/summer season (18 of 192 children). This was statistically significant at $P < 0,05$ (χ^2-test). Fig. 1 shows the clustering of rotavirus-positive patients observed during the study period.

Asymptomatic infection

Only 8 children were identified to be asymptomatically infected with rotavirus. These included a newborn baby with a congenital heart defect and 5 children over 2 years of age (two 2-year-olds, one 3-year-old and two 4-year-olds) and therefore considered to be outside the target age of rotavirus infection.

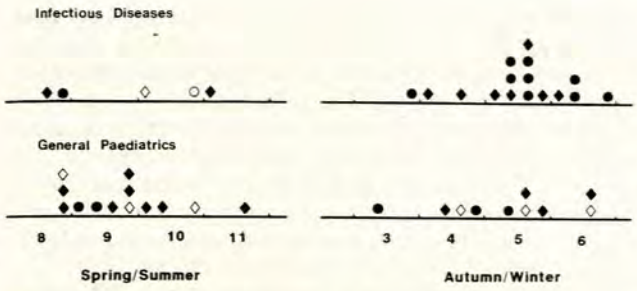

Fig. 1. Diagrammatic representation of the distribution of rotavirus positive cases between ward 1 (infectious diseases) and ward 2 (general paediatric) identified during the study period. Spring and early summer was August - November 1987 and autumn/early winter March - June 1988. Each symbol represents a single patient. Community-acquired infection is demonstrated by a closed circle, nosocomial infection by a closed diamond, and asymptomatic infection by the open symbols.

Autumn/winter

Of the 26 children with rotavirus infection identified during this period, 16 were housed in ward 1, and 9 of these had acute rotavirus gastro-enteritis. However, a further 7 children in this ward developed nosocomial rotavirus infection while in the hospital. Four of the 7 children with nosocomial rotavirus infection were initially admitted with gastro-enteritis caused by agents other than rotavirus, and the rotavirus infection occurred as a super-infection on average 11 days after admission (range 9 - 15 days). The remaining 3 children had been admitted with meningitis and the rotavirus infection occurred on average 6 days after admission (range 5 - 7 days).

In ward 2, 10 infants who were excreting rotavirus were identified. Three of these children were admitted to this ward despite symptoms of acute diarrhoea (1 case) or vomiting (2 cases), which were all later identified as being caused by acute rotavirus infections. Two of these children were under 3 months of age. Seven cases of nosocomial rotavirus infection (3 of which were asymptomatic) were detected in children originally admitted with a wide range of clinical problems (pneumonia, congenital heart disease, mental retardation and child abuse).

Spring/summer season

A marked difference was observed in the incidence of rotavirus infection between the two seasons, since only 1 of 5 children admitted to ward 1 with acute infantile gastro-enteritis was excreting rotavirus on admission during this period. A second child (48 months of age), who was admitted with tonsillitis, was found to be excreting rotavirus asymptomatically at the time of admission. Three further children, 1 of whom was also asymptomatically infected, were identified as having acquired the infection in the hospital. Two of these children had been admitted to the hospital with bronchopneumonia and the third with bacterial gastro-enteritis. None was excreting rotavirus on admission.

Thirteen rotavirus-infected infants were identified in ward 2. Only 2 of these children were admitted to the hospital with rotavirus infection, and both these were admitted with pyrexia of unknown origin. Of the 11 patients with nosocomial rotavirus infection, 1 was a week-old neonate with congenital heart disease who was found to be excreting rotavirus for 6 days, although only 'loose stools' had been reported. All but 2 of the 10 other patients developed diarrhoea after being infected in the ward.

Polyacrylamide gel electrophoresis

A rotavirus RNA profile was observed from the stools of 42 of the 44 rotavirus-positive patients (95%). The extraordinarily high rate of identification of an RNA profile for each infectious episode can be attributed to the fact that there were multiple specimens from each patient. The 2 patients who were enzyme-linked immunosorbent assay (ELISA)-positive and RNA-negative had a limited number of rotavirus-positive specimens (1 and 2 only). Both these were defined as nosocomial infections, which may also mean that the load of virus excreted was limited. In all cases where more than one rotavirus-positive stool specimen was received from a single patient the rotavirus RNA profile was found to be the same.

Eight distinct RNA profiles were observed and were labelled to correspond to previous reports from this laboratory.[24,27] Six long RNA profiles were observed (LY, LX, LW, LF, LK, LT). One of these profiles (LK) occurred in far greater numbers than the other electrophoretic strains (20 of all cases) and was determined to be the predominant strain circulating in the community outside the hospital during 1988 (our own observations). Two short electrophoretypes were detected (SZ and SB) in 8 children. The RNA patterns proved useful in examining the cases which were nosocomially acquired, although no one RNA pattern was associated solely with nosocomial infection, as described below. Fig. 2 shows examples of the pertinent electrophoregrams.

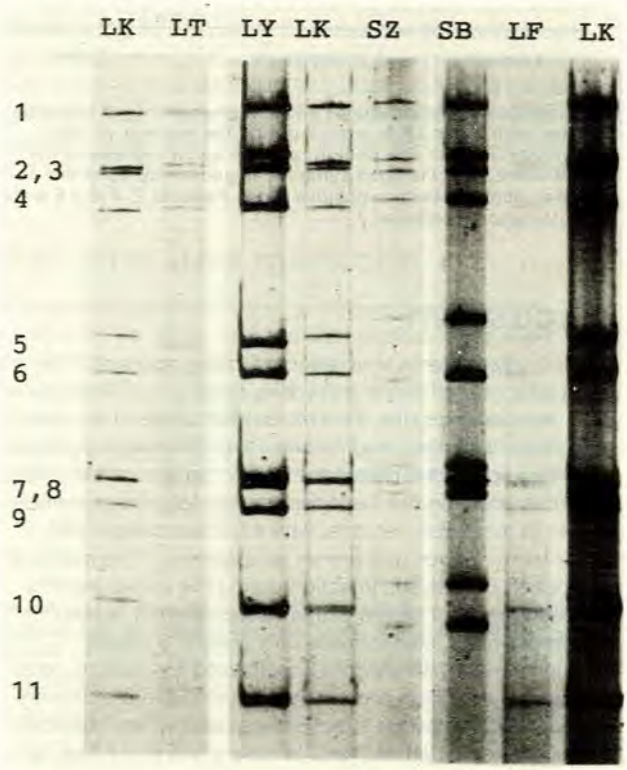

Fig. 2. RNA profiles of rotavirus electrophoretypes identified at H. F. Verwoerd Hospital. LK was the predominant strain during the course of the study. Migration was from top to bottom and segments are numbered from largest to smallest.

Nosocomial rotavirus infection

Nosocomial rotavirus infection was found to occur in 28 (9%) of the patients investigated. Seven of these infections (25%) were asymptomatic and would not have been identified as positive for rotavirus except for the screening effected by this study.

During the spring/summer season of 1987, 11 of 13 young children in ward 2 with rotavirus infection were nosocomially infected. The facts that these cases occurred in the ward which did not usually admit gastro-enteritis cases and that this was not the 'high' rotavirus season suggested a common-source outbreak of the infection. On examination of the electrophoretic migration profiles of these rotavirus strains, it was observed that 4 electrophoretic strains with both long and short patterns occurred, indicating that this was not a localised outbreak of infection. However, strain LK, which was predominant during 1988 but not 1987, was detected in 6 children and it is believed that these cases were probably spread from a single common source, although the index case was not identified and may have been admitted to the ward before the study commenced (Fig. 3).

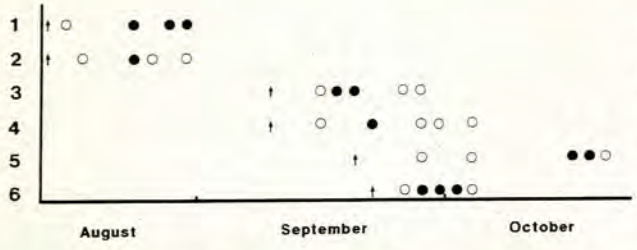

Fig. 3. Temporal relationship of a localised epidemic of rotavirus infection with strain LK in ward 2 during the summer of 1987. Each line represents one patient, and the arrow represents the date of admission, the open symbol a negative stool and the closed symbol a rotavirus-positive stool. Patients 2, 4 and 5 were asymptomatically infected.

Discussion

In this study rotavirus was detected in the stools of 13% (44 of 338) of the infants and young children admitted to H. F. Verwoerd Hospital. This rate varied between the two seasons investigated, and rotavirus infection was observed to be more prevalent during the cooler months of the year. Rotavirus infection has been reported to follow a seasonal pattern in temperate regions, where it is associated with cooler temperatures and a drier atmosphere.[17] The seasonal distribution of rotavirus infection during the cooler months has been reported before in this subcontinental region.[21,22]

Asymptomatic infection was only seen in 8 cases, although almost 200 children hospitalised for reasons other than gastro-enteritis were examined for rotavirus excretion. Asymptomatic rotavirus infection has been widely reported in neonates,[2,3,20] in adults[28] and in older children.[13,14] The median age of the children included in the study fits very closely with the expected target age group of children susceptible to rotavirus infection,[1] and this might be a reason why little asymptomatic infection was seen. Of the 8 children with asymptomatic infection, 1 was a newborn baby and 5 of the other 7 were over 2 years of age (i.e. older than the target age of rotavirus infection). The remaining children were 11 and 12 months of age. Asymptomatic infection in children slightly older than the accepted target group could probably be explained by their having some degree of protection from clinical disease due to previous infection with rotavirus.[29]

Electrophoresis of the rotavirus genome yielded an RNA profile in 42 of the 44 rotavirus-positive children, and 8 different electrophoretypes were identified. One of these strains, LK, was the most predominant and accounted for 20 cases, while minor variants, with a short or a long electrophoretype, were also circulating. This pattern of simultaneous circulation of several different rotavirus electrophoretic strains with a single type predominating is typical of rotavirus epidemiology and has been reported before.[3,20,24] The fact that the pattern of rotavirus infection resembled that of the community outside the hospital indicates that the viruses found in the hospital are introduced from outside the hospital. On the other hand, endemic nosocomial rotavirus infection identified in neonates in the maternity unit is characterised by the persistence and constancy of the viral strain.[2,3,20,27]

In this study, very little nosocomial rotavirus infection was observed overall (9%). The low level of hospital-acquired infection is probably due to the great emphasis placed on infection control procedure by both nursing staff and paediatricians in the hospital. The exact mode of transmission of rotavirus infection within the institutionalised setting is still a matter of some debate; however, the role of contaminated hands in the transmission of rotavirus is considered important.[17] Other studies have shown that strict compliance with hand-washing techniques was responsible for a decrease in the levels of nosocomial infection.[5,17]

It is of interest that most of the nosocomial rotavirus infections in this study were observed in ward 2 and not in ward 1, where most children with acute gastro-enteritis were admitted. As identical infection control procedures are utilised in both wards, this implies that the staff on ward 1 are more consistent in maintaining strict hygienic standards, with better compliance to hand-washing procedures between patient contacts, possibly because of greater day-to-day awareness of the problem. Also, nosocomial infection with rotavirus in ward 1 was recorded as occurring, on average, more quickly in children admitted with meningitis than with non-rotaviral gastro-enteritis (6 days v. 11 days). Possibly this is due to closer vigilance on the part of the staff caring for the seriously ill children with meningitis, resulting in more frequent contact and thus a greater risk of transmission.

Electrophoresis of the viral genome has proved a convenient and reliable means of investigating the epidemiology of rotavirus infection in the hospital. The results from this study showed that the same electrophoretic strains of rotavirus were associated with both community-acquired and hospital-acquired infection and that the same rotavirus strains were associated with both symptomatic and asymptomatic infection. The rapid diagnosis of rotavirus infection in patients admitted to the hospital with symptoms of gastro-enteritis would enable better management of the patient, such as isolation or discharge (as in many cases effective rehydration can be achieved at home, and most

rotavirus infections are self-limiting). Even vomiting alone as a symptom should result in investigation for rotavirus and potential isolation of the patient.[30] Monitoring of the infection within the hospital can also prove valuable, since it would allow detection of areas where there has been a breakdown in infection control procedures. In this study, as in others, the importance of strict adherence to standard infection control procedures has been indicated by the low level of hospital-acquired infection detected.

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