Rotaviruses as a Cause of Nosocomial, Infantile Diarrhoea in Northern Brazil: Pilot Study

Rosa Helena P Gusmão, Joana D'Arc P Mascarenhas, Yvone B Gabbay, Zéa Lins-Lainson, Francisco Lúzio P Ramos, Talita AF Monteiro, Sebastião Aldo Valente, Alexandre C Linhares

Instituto Evandro Chagas, Fundação Nacional de Saúde, Av. Almirante Barroso 492, 66090-000 Belém, PA, Brasil

Faecal samples were obtained from 190 children, aged 0 to 5 years, admitted to a public hospital in Belém, Pará, Brazil. These patients were placed in a pediatric ward with 40 beds distributed in six rooms. Cases were classified into three groups: (a) nosocomial: children who developed gastroenteritis 72 hr or later after admission; (b) community-acquired: patients admitted either with diarrhoea or who had diarrhoea within 72 hr following admission; (c) non-diarrhoeic: those children who had no diarrhoea three days before and three days after collection of formed faecal sample. Specimens were routinely processed for the presence of rotaviruses, bacteria and parasites. Rotaviruses were detected through enzyme-linked immunosorbent assay (ELISA) and subsequently serotyped/electrophoretyped. Rotaviruses were the most prevalent enteropathogens among nosocomial cases, accounting for 39% (9/23) of diarrhoeal episodes; on the other hand, rotaviruses occurred in 8.3% (11/133) and 9% (3/34) of community-acquired and non-diarrhoeic categories, respectively. Mixed infections involving rotavirus and Giardia intestinalis *and rotavirus plus* G. intestinalis *and* Entamoeba histolytica *were detected in frequencies of 8.6 and 4.3%, respectively, in the nosocomial group. The absence of bacterial pathogens in this category, and the unusual low prevalence of these agents in the other two groups may reflect the early and routine administration of antibiotics following admission to this hospital. Rotavirus serotype 2 prevailed over the other types, accounting for 77.8% of isolates from nosocomial diarrhoeal episodes. In addition, at least five different genomic profiles could be observed, of which one displayed an unusual five-segment first RNA cluster. Dehydration was recorded in all cases of hospital-acquired, rotavirus-associated diarrhoea, whereas in only 57% of nosocomial cases of other aetiology. It was also noted that nosocomial, rotavirus-associated diarrhoeal episodes occur earlier (7 days), following admission, if compared with those hospital-acquired cases of other aetiology (14 days).*

Key words: rotavirus - nosocomial - infantile diarrhoea

Diarrhoeal disease represents a leading cause of both morbidity and mortality among infants and young children in developing countries. It has been reported that approximately 500 million children are affected annually, yielding an estimated 3.3 million deaths from acute diarrhoea. An additional hazard in the tropical regions of the world is that malnutrition makes children more prone to severe disease and, conversely, repeated episodes of diarrhoea may lead to malnutrition (Bern et al. 1992).

In the context of the aetiology of acute gastroenteritis, several studies throughout the world have assessed the importance of rotaviruses as major enteropathogens in childhood, either in developed (Gurwith et al. 1983) or developing countries. It is estimated that 140 million cases of rotavirus diarrhoea occur each year in the so called "Third World", causing almost one million deaths (Brandt et al. 1979, Steinhoff 1980, De Zoysa & Feachem 1985).

With regards to the epidemiology of rotavirus infection, it should be emphasized the major role of this agent as a cause of nosocomial diarrhoea among pediatric populations. Pacini et al. (1987), for instance, studying the incidence of gastroenteritis in a pediatric hospital in Ohio, USA have demonstrated that rotaviruses account for 40% of cases of hospital-acquired diarrhoea. In addition, similar studies conducted in other regions have also stressed the role of rotaviruses as agents of nosocomial diarrhoea. On the other hand, it has been demonstrated that prolonged hospitalization significantly increases the risk for rotavirus infection (Ryder et al. 1977, Koopman et al. 1984).

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Studies carried out in Belém, Brazil by Linhares et al. (1983) indicate that rotaviruses account for at least 30% of all diarrhoeal episodes among children who attend local clinics either as inpatients or outpatients. At community-level, in the same area, these agents are associated with 10% of cases of infantile acute diarrhoea (Linhares et al. 1989). All four epidemiologically important rotavirus serotypes occur in our region, serotype 1 being the most prevalent (50% of strains) (Linhares et al. 1988). Recent studies in other regions of Brazil, however, have shown that serotypes 2 and 3 are predominant (Pereira et al. 1993, Castro et al. 1994, Rácz et al. 1994).

In spite of the already established importance of rotaviruses as agents of infantile diarrhoea in the Amazon region, no local data were available to date that could assess its role in the aetiology of nosocomial infection.

In this report we essentially discuss both clinical and epidemiological aspects of nosocomial, infantile diarrhoea associated with rotaviruses and other enteropathogens in Belém, Brazil.

MATERIALS AND METHODS

From November 1992 to July 1993 faecal specimens were obtained from 190 children, less than five years old, who attended the "Hospital da Santa Casa de Misericórdia do Pará", a public hospital in Belém, Brazil. These children were placed in a pediatric ward with 40 beds, distributed in six rooms. With the aim of properly controlling the present study, cases were classified as follows: (a) nosocomial: children who developed diarrhoea at least three days after admission; (b) community-acquired: patients who were either hospitalized with diarrhoea or developed it within 72 hr of admission; (c) non-diarrhoeic: situations in which no diarrhoea was recorded at least three days before and three days after collection of sample. Children in the three groups were age- and sex-matched, and diarrhoeal episode was defined as three or more liquid or semi-liquid motions in a 24-hr period.

Enrollment of children occurred at admission to the hospital, when signed consents were obtained from parents. Nutritional, anthropometric and socio-economical data were routinely recorded in special forms, once the child was recruited to participate in the study. The surveillance for diarrhoeal episodes was carried out through daily visits to the pediatric ward. Whenever diarrhoea was detected, daily visits were made to children, until the episode ended.

Stool specimens were obtained as soon as possible following the detection of a diarrhoeal episode. Faeces were then placed in phosphate buffered saline (PBS), pH 7.4, for rotavirus examination; in two screw-caped vials of Cary-Blair medium (one containing antimicrobic supplement according to the formulation of Skirrow), for bacteriological tests (we routinely searched for *Salmonellae*, *Shigellae*, enteropathogenic and enterotoxigenic *Escherichia coli* and *Campylobacter*); and in 10% formadehyde solution for parasitological examination. Samples were assayed for rotavirus antigen by using DAKOPATTS ELISA kits (Copenhagen, Denmark), as previously described (Flewett et al. 1989). Monoclonal antibodies against each of the four human serotypes were kindly provided by Dr Shozo Urasawa, from the Department of Hygiene and Epidemiology, Sapporo Medical College, Sapporo, Japan. The serotyping of strains was routinely performed, essentially as described by Taniguchi et al. (1987). Electrophoresis of deproteinized, rotavirus RNA was performed through a 5% polyacrylamide slab gels using the discontinuous buffer system, as previously described (Laemmli 1970, Linhares et al. 1993).

Samples were tested for the presence of bacteria and parasites essentially according to techniques described in the "WHO Manual for Laboratory Investigation of Acute Enteric Infections (Organisation Mondiale de la Santé1987).

RESULTS

Of the 190 enrolled children, 23 (12%) belonged to the nosocomial group, 133 (70%) had community-acquired diarrhoea and 34 (18%) were non-diarrhoeic inpatients.

Overall, rotaviruses were the most frequent enteropathogen found, occurring in 39% (9/23), 8.3% (11/133) and 9% (3/34) of the nosocomial, community-acquired diarrhoea and non-diarrhoeic categories, respectively. Specifically with respect to the nosocomial group, *E. histolytica* and *G. intestinalis* were demonstrated in 13% and 30% of cases, respectively. No enteropathogens could be found in 4 (17%) out of the 23 cases of hospital-acquired diarrhoea (Table I). *E. histolytica* (17%; 23/133) and *G. intestinalis* (23%; 8/34) were the most frequent enteropathogens detected in the community-acquired diarrhoea and non-diarrhoeic groups, respectively. *Cryptosporidium* sp. was encountered in 14% (19/133) and 9% (3/34) of community-acquired diarrhoea and non-diarrhoeic categories, respectively. The only isolated bacterial pathogens were *Shigella* sp. and *Salmonella* sp.: eight (6%) of the former among children with community-acquired diarrhoea and one of the latter pathogen in the non-diarrhoeic patients.

TABLE I

Occurrence of rotaviruses, bacteria and parasites in faeces from 190 children attending a public hospital in Belém, Brazil

Agents	Groups[a] Nosocomial acquired	Community-diarrhoeic	Non-
Rotaviruses	9/23 (39%)	11/133 (8.3%)	3/34 (9%)
Salmonella sp.	0/23 (0%)	4/133 (3%)	0/34 (0%)
Shigella sp.	0/23 (0%)	8/133 (6%)	1/34 (3%)
Entamoeba histolytica	3/23 (13%)	23/133 (17%)	6/34 (18%)
Giardia intestinalis	7/23 (30%)	17/133 (13%)	8/34 (23%)
Cryptosporidium sp.	0/23 (0%)	19/133 (14%)	3/34 (9%)

a: no. positives/ no. tested (%)

TABLE II

"Pure" and mixed rotavirus infections in 190 children attending a public hospital in Belém, Brazil

Agents	Groups[a] Nosocomial acquired	Community-diarrhoeic	Non-
Rotavirus - only	6/23 (26%)	8/133 (6%)	1/34 (2.9%)
Rotavirus + *Giardia intestinalis*	2/23 (8.6%)	1/133 (0.7%)	1/34 (2.9%)
Rotavirus + *Cryptosporidium* sp.	0/23 (0%)	1/133 (0.7%)	0/34 (0%)
Rotavirus + *Entamoeba histolytica*	0/23 (0%)	0/133 (0%)	1/34 (2.9%)
Rotavirus + *Entamoeba histolytica* + *Giardia intestinalis*	1/23 (4.3%)	1/133 (0.7%)	0/34 (0%)

a: no. positives/ no. tested (%)
"Pure": no other enteropathogens than rotaviruses found

Table II shows that no enteropathogens other than rotaviruses were detected in 26% (6/23), 6% (8/133) and 2.9% (1/34) of nosocomial, community-acquired and non-diarrhoeic groups, respectively. Associations of rotavirus and other pathogens in the same specimen were identified as follows: *G. intestinalis* (4 cases), *Cryptosporidium* sp. (1), *E. histolytica* (1) and *G. intestinalis* plus *E. histolytica* (2). No enteropathogens could be demonstrated in 49% of the 190 cases investigated.

The correlation of electrophoretypes with serotypes for the 23 rotavirus strains, according to the classification of cases as nosocomial, community-acquired and non-diarrhoeic, is demonstrated in Table III. Long and short electrophoretic profiles were detected in 52% (12/23) and 48% (11/23) of cases, respectively. Twenty-one (91.3%) out of the 23 strains could be serotyped, whereas absence of Vp7 was noted in two (8.7%) of the samples. Among serotyped strains, 17 (81%) were identified as type 2, of which six (35.2%) showed the unusual combination with the long RNA pattern; three strains were classified as serotype 1 and mixed infection, involving serotypes 1 and 4, was detected in one diarrhoeic child belonging to the community-acquired category.

TABLE III

Rotavirus serotypes and electropherotypes in 23 positive samples from children attending a public hospital in Belém, Brazil

Group	Serotype	Electrophoretype	No. cases (%)
Nosocomial	2	short	5 (55)
	2	long	2 (22)
	1	long	2 (22)
Community-acquired	2	short	5 (45)
	2	long	3 (27)
	1 and 4	long	1 (09)
	N.D.[a]	long	2 (18)
Non-diarrhoeic	2	long	1 (33)
	1	long	1 (33)
	2	short	1 (33)

a: serotype not determined because of lack of outer capsid

Fig. 1 shows five distinct rotavirus RNA electrophoretic profiles corresponding to eight nosocomial diarrhoeal cases (one nosocomial rotavirus strain, code 209, is not shown in Fig.). It is notable that all, but one (code 019) strains detected from December 1992 to March 1993 displayed a long genomic pattern, correlating either with serotype 1 or 2; on the other hand, all three rotavirus strains occurring in July 1993 had short electro-phoretype and belonged to serotype 2. In addition, one atypical strain (code 212) displayed five (instead of the usual four) segments in the first dsRNA size class.

The monthly incidence of rotavirus-positive cases, according to the category is presented in Fig. 2. It is notable that four (44.4%) out of the nine nosocomial diarrhoeal cases occurred in July 1994.

The clinical symptoms in 23 episodes of nosocomial diarrhoea, in relation to the presence of rotavirus in faeces is shown in Table IV. The percentage of dehydration was significantly higher in the rotavirus-related infections than in those of other aetiology (100% *vs.* 57%; $p < 0.05$). It was also observed (data not specified in Table) that rotavirus-associated diarrhoeal episodes were of earlier onset, in relation to the date of hospital admittance, if compared to those of other aetiology: 7 and 14 days, respectively.

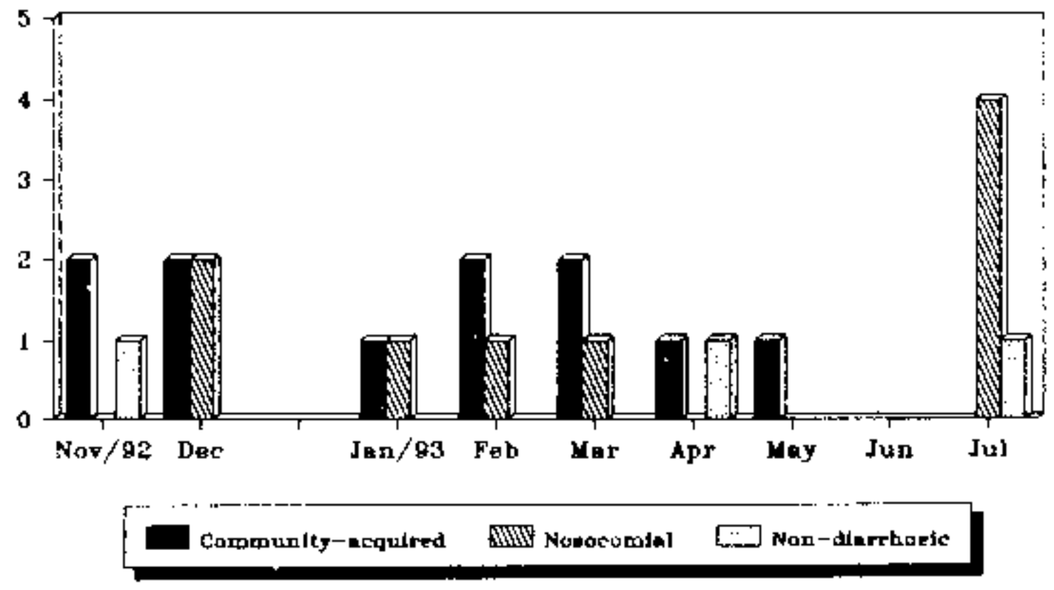

Fig. 2: temporal distribuition of rotavirus-positive cases among hospitalized children in Belém, Brazil.

DISCUSSION

Although preliminary, our data clearly indicate the major importance of rotaviruses in the aetiology of nosocomial gastroenteritis in our region. The incidence rate of rotavirus-related nosocomial diarrhoea in Belém, Brazil (nearly 40%) is comparable to those of other studies conducted throughout the world (Pacini et al. 1987). On the other hand, the fact that almost 10% of the non-diarrhoeic children were excreting rotaviruses sustains the concept that these viruses are highly transmissible agents in hospital envi-

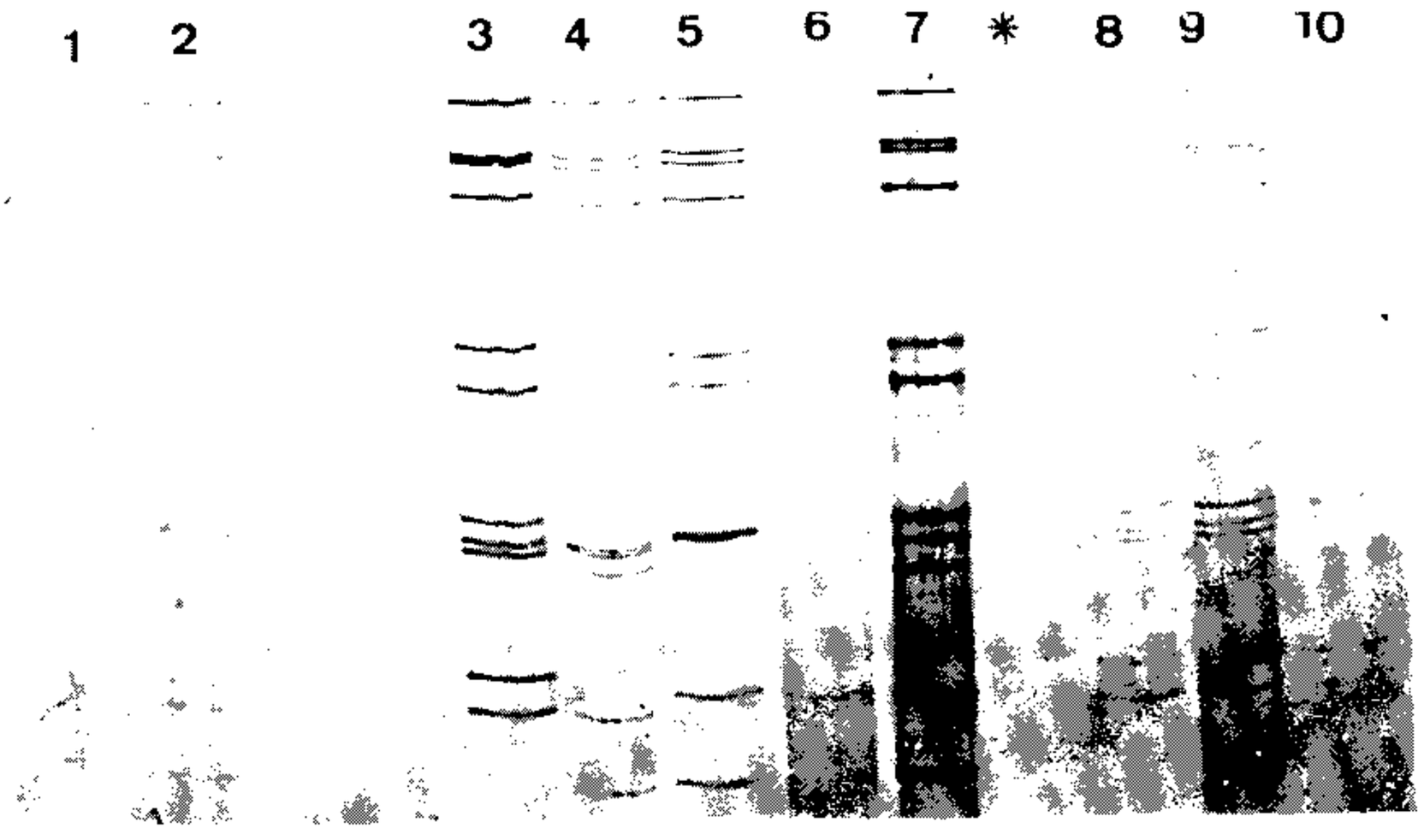

Fig. 1: RNA genomic profiles of eight rotavirus strains obtained from children with nosocomial diarrhoea in Belém, Brazil
1: control, human serotype 1; 2: control, SA11 strain

Nosocomial strains

	Code	Collection	Serotype
3.	019	December 92	2
4.	021	December 92	1
5.	037	January 93	2
6.	068	February 93	2
7.	120	March 93	1
8.	212	July 93	2
9.	217	July 93	2
10.	223	July 93	2

* Electrophoretype not evident, probably due to low concentration of RNA: corresponds to code 209, collected in July 93 and serotype 2.

TABLE IV

Clinical symptoms in 23 episodes of nosocomial diarrhoea, according to the presence of rotavirus in faeces, among children attending a public hospital in Belém, Brazil

Symptoms	Rotavirus - positive (9)	Rotavirus - negative (14)
Mean number of evacuations/days	5.1	5.2[a]
Mean duration of diarrhoea in days	4	4.2[a]
Dehydration (%)[c]	100	57[b]
Vomiting (%)	55	36[a]
Fever (temp.≥38°C (%)	78	57[a]
Mucus in faeces (%)	44	57[a]
Blood in faeces (%)	0	7[a]

( ): number of children
a: not significant
b: significant ($p < 0.05$)
c: need for parenteral rehydration in 89% and 57% of rotavirus-positive and negative cases, respectively ($p < 0.05$)

ronments (Pacini et al. 1987). Constrasting with this, during a community-based, longitudinal study carried out in Belém, Brazil, Linhares et al. (1989) have demonstrated that only 0.5% of non-diarrhoeic children were excreting rotaviruses, whereas infections occurred in 8% of cases of gastroenteritis. In our study, as in others conducted in temperate regions (Pacini et al. 1987), it is likely that the hospital staff had a role in the transmission of rotavirus among children; however, this is certainly facilitated by the lack of nursery hygienic conditions, which often occurs as a result of constant crowding in local public hospitals.

The occurrence of rotavirus as the only-pathogen found in 26% and 6% of nosocomial and community-acquired diarrhoeal episodes, respectively, emphasizes its epidemiological magnitude at hospital environment (Noone & Banatvala 1983, Matson & Estes 1990). In addition, this latter rate is similar to that obtained in a previous, local investigation (Linhares et al. 1989).

The specific role as enteropathogens of both *G. intestinalis* and *E. histolytica* could not be evaluated in the context of nosocomial infections, as these parasites were detected in stools from children who were also excreting rotaviruses. Although *Cryptosporidium* sp. had not been detected among children with nosocomial diarrhoea, it has occurred in 14% of diarrhoeic children from the community-acquired group. This rate is significantly higher than that recorded previously in Northern Brazil (Loureiro et al. 1989). The low frequencies of bacterial pathogens in the present study may reflect the early, routine administration of antibiotics to children, following their admission to hospital. It is therefore plausible to assume that the isolation of both *Shigella* sp. and *Salmonella* sp. indicates the possible emergence of resistant strains into hospital environment.

Although clinical specimens from diarrhoeic patients had routinely been examined for the presence of rotaviruses, bacteria and parasites, enteropathogens could not be identified in nearly 50% of the situations. In this context, it is likely that a proportion of cases had been primarily associated with bacterial enteropathogens that could not be isolated, as a result of the early, routine administration of antibiotics. In addition, viruses other than rotaviruses (eg. enteric adenoviruses, Norwalk and Norwalk-related agents, and astroviruses) may also have been implicated in the aetiology of cases of diarrhoea. The occurrence of the latter agents deserves further, particular investigation.

While in the present study rotavirus serotype 2 accounted for 80% of isolates, in a previous (1983 to 1985), longitudinal investigation carried out by Linhares et al. (1988) in Belém, Brazil rotavirus serotype 1 has largely prevailed over the other types. As most of diarrhoeal episodes recorded in the present investigation belong to the community-acquired group, it could be postulated that rotavirus serotype 2 is likely to be more often associated with severe cases (i.e. those requiring hospitalization). However, it should not be ruled out the possibility of rotavirus serotype 2 being also predominant at the external community, as fluctuations in serotype-specific prevalences, over time, have been demonstrated in Belém (Linhares et al., 1993). This latter hypothesis is supported by recent findings of Castro et al. (1994) and Rácz et al. (1994) indicating that serotype 2 is predominant among infants attending a day care nursery and an outpatient clinic, respectively. Further, community-based studies including episodes of various clinical severities would be needed, in order to elucidate this particular aspect.

Our data concerning the frequencies of both long and short genomic profiles differ from previous findings in our region. In the latter studies (Linhares et al. 1989, 1993) it has been demonstrated that long electrophoretypes largely predominate over the short profiles either in hospital or community environments. In the present investigation, however, both long and short RNA patterns were detected at comparable rates: 52.2% and 47.8%, respectively. The occurrence

of 6 (26.1%) out of 23 rotavirus strains, belonging to serotype 2 and having long electrophoretype constitutes an unusual finding, at least in the light of previous findings in our region (Linhares et al. 1989).

Data shown in Fig. 1 present at least five distinct RNA patterns in the nosocomial group and suggest that rotavirus strains which circulate in hospital environment essentially reflect those in the community. This is also sustained by the fact that rotavirus serotype 2 was highly prevalent in both nosocomial and community-acquired diarrhoea groups, yielding rates of 77.8% and 88.9%, respectively. The detection of an atypical nosocomial strain (code 212) displaying an unusual, "avian-like" electrophoretype raises the question of whether this reflects a naturally occurring genomic rearrangement of a human strain or, less likely, an evidence for interspecies transmission. Further hybridization assays will be performed regarding this particular finding.

The comparison made between clinical severities of rotavirus-related episodes of acute diarrhoea and those with other aetiologies (Table IV) indicates that dehydration is much more frequent among children belonging to the former than to the latter category. This is in accordance with previous observations made during a prospective study (Linhares et al. 1989) conducted in Belém, Brazil. In addition, the shorter period of time between hospital admission and the onset of rotavirus-associated diarrhoeal episodes (if compared with nosocomial diarrhoea of other aetiologies) indicates the high potential for transmission of these viral agents in pediatric wards.

In the light of the above described findings, assessing the importance of nosocomial rotavirus infections, practical recommendations aiming at prevention and control should be emphasized such as: thorough handwashing (particularly by members of hospital-staff), appropriate control for external visits and adequate number of beds per ward.

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