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A total of 614 fecal specimens were obtained during a survey for rotavirus infection conducted between May 1996 and May 1998 among 437 newborns admitted to special care nurseries at a public hospital in the urban area of Belém, Brazil. Routine stool samples were taken weekly from all babies up to the age of 28 days. Overall, 51 (11.7%) of the neonates excreted rotaviruses while in hospital, of whom 42 (82.3%) developed asymptomatic nosocomial infection; nosocomial infection was also proved in five of the nine patients with diarrhea. Three distinct RNA profiles were detected, of which one short electrophoretotyping pattern was far more frequent (>90% of the strains). Using monoclonal antibody-based enzyme immunoassays, 32 (62.7%) of the rotavirus-positive strains were classified as G2, and 1 (1.9%) as mixed G1 and G2. A G serotype could not be assigned to 18 (35.3%) of the isolates. A reverse transcription-polymerase chain reaction was used for determining the VP4 type-specificity of a subset of 28 rotavirus-positive samples. Characterization of the VP7-genotype specificity was also sought for 18 of these latter strains. Overall, P[6] and G2 genotypes were identified in 93% and 94% of tested samples respectively, with results being further confirmed by Southern hybridization. Although surveillance was conducted during a 25-month period, 50 (98%) of 51 rotavirus isolates clustered between January and December 1997. The earliest [P6]G2 rotavirus infections were detected by late January 1997, involving two (13- and 14-day-old) babies admitted with acute diarrhea. Thereafter, strains bearing these genotype specificities were identified among five infants with hospital-acquired gastroenteritis, followed by 16 others who were infected asymptomatically. This is the first report from Brazil describing nosocomial transmission of P[6]G2 rotavirus strains among neonates. J. Med. Virol. 67:418–426, 2002.

KEY WORDS: rotavirus; neonates; P and G types; transmission

INTRODUCTION

Group A rotaviruses are the most common worldwide cause of acute nonbacterial gastroenteritis in infancy and early childhood, accounting for an estimated 140 million diarrheal episodes and a minimum of 418,000 to 520,000 deaths each year [De Zoysa and Feachem, 1985; Institute of Medicine, 1986; Kapikian and Chanock, 1996; Miller and McCann, 2000]. The significant global burden of the disease highlights the urgent need for development and deployment of an effective rotavirus vaccine, especially for use in the less developed areas [Glass et al., 1994; Vesikari, 1997; Bresee et al., 1999; Linhares and Bresee, 2000].

Accumulating evidence from several investigations throughout the world indicates that rotavirus infection in newborns differs both clinically and epidemiologically from that occurring in older infants [Bishoop et al., 1983; Haffejee, 1991; Cicirello et al., 1994; Kapikian and Chanock, 1996]. Although rotaviruses are major pathogens causing life-threatening dehydrating gastroenteritis in infants and young children, the majority of infections that occur in neonates are either mild or asymptomatic. It has also been shown that neonatal infections are, in general, nosocomial and largely attributable to rotavirus strains that are distinct from those circulating among children with diarrhea in the external community [Haffejee, 1991; Bhan et al., 1993; Cicirello et al., 1994]. In addition, several studies conducted in both developed and developing countries have

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shown that neonatal infections confer protection against subsequent severe disease [Bishop et al., 1983; Velásquez et al., 1996].

Based on the dual typing system proposed by Estes and Cohen [1989], rotavirus serotypes have been defined by the outer capsid VP7 (glycoprotein) and VP4 (protease-sensitive) proteins, which are encoded by RNA genes 4 and 7 or 8 and 9, respectively. By primarily using enzyme-linked immunoassays (ELISA) with type-specific monoclonal antibodies, an overall 14 G serotypes were defined, 10 of which (G1–G4, G5, G6, G8–G10, G12) have been detected in humans [Coulson et al., 1987; Taniguchi et al., 1987; Estes, 1996]. Because of the limited availability of suitable routine antigenic VP4 typing methods, the reverse transcription-polymerase chain reaction (RT-PCR) and hybridization assays have been used routinely to characterize P genotypes [Larralde and Flores, 1990; Coulson, 1993; Masendycz et al., 1997]. Overall, 20 VP4 genotypes have been recognized, six of which (P[8], P[4], P[6], P[9], P[10], P[12]) are known to infect humans. With the exception of India, where P[6] strains with G1, G2, G3, G4, or G9 specificities are common [Ramachandran et al., 1996], worldwide studies indicate that rotavirus type-specificities are predominant [Timenetsky et al., 1994; Gentsch et al., 1996; Leite et al., 1996]. In Southeast Brazil, the frequent detection of P[8]G5 rotavirus strains is a matter of concern in view of future national vaccination strategies involving candidate rotavirus vaccines currently undergoing field evaluation [Leite et al., 1996; Gouvea and Santos, 1999]. Recent studies indicate that P[8]G5 strains occur in Belém, Northern Brazil at rates lower than those reported for other regions in the country [Mascarenhas et al., 2002].

Unlike rotavirus strains found commonly in the community, those infecting neonates usually bear type G1, G2, G3, or G4 specificity but in general possess a unique P[6] type [Hoshino et al., 1985; Flores et al., 1986; Gentsch et al., 1993]. Although this VP4 type-specificity is believed to render neonatal (nursery) strains avirulent [Gorzgilia et al., 1986], recent surveys conducted in several settings indicate that genotype P[6] strains may also be associated with a significant proportion of diarrheal episodes in the community [Timenetsky et al., 1994; Steele et al., 1995; Ramachandran et al., 1996; Adah et al., 1997; Cunliffe et al., 1999]. Contrasting with these findings, data from studies in India have shown that neonatal rotavirus strains, mostly of the unusual P[11]G9 genotype, cause asymptomatic infection and circulate at very low frequency in the community [Jayashree et al., 1988; Bhan et al., 1993; Das et al., 1993; Gentsch et al., 1993]. Because of these characteristics, a culture-adapted strain (116E) from this collection has been proposed as a candidate rotavirus vaccine [Bishop, 1993; Bresee et al., 1999].

The study deals mainly with the epidemiological features of rotavirus infection among hospitalized newborns in Belém, Brazil and provides characterization of nosocomially transmitted neonatal rotavirus strains using electropherotyping, G serotyping, G/P genotyping analyses and Southern hybridization.

### MATERIALS AND METHODS

#### Patients and Clinical Specimens

The survey was conducted between May 1996 and May 1998 at the major public and teaching hospital, Santa Casa de Misericórdia do Pará, located within the city limits of Belém, northern Brazil. The study included 437 newborn babies, up to 28 days of age, admitted to the hospital’s neonatal care unit. All neonates participating in this study presented with various problems, including prematurity, respiratory distress, and hyperbilirubinemia. The neonatal care unit was comprised of 44 beds (including 10 incubators) placed in six wards, as follows: 1) one external nursery gathering neonates admitted directly from the community; 2) three special-care nurseries; 3) one “non-infected” premature babies room; and 4) one intensive care unit. All babies admitted from outside the hospital were grouped in the external nursery after admission. Sick newborns requiring intermediate level medical care were distributed among the three special care nurseries, and critically ill patients were placed in the intensive care unit. Premature neonates showing no clinical evidence of infection shared a separate room.

Although all babies admitted to neonatal care units on Tuesdays and Thursdays were enrolled in this study, recruitment of neonates with diarrhea occurred at any day throughout the study period. Starting after 72-hr of birth, fecal samples were obtained serially at 7-day intervals, up to the age of 28 days of hospital stay. Stool samples were also obtained as soon as possible whenever diarrhea was detected. The definition of diarrhea was considered to be the occurrence of three or more looser-than-normal stools within a day.

Feces were collected in disposable diapers, placed in insulated boxes with cold packs, and transported to the laboratory within 4 hr. These samples were placed in sterile saline for rotavirus examination and in two screw-capped vials of Cary-Blair medium (one vial contained Skirrow antimicrobial supplement). An additional aliquot was placed in a vial containing methiolate, iodine, and formalin.

All babies admitted to the external nursery whose stools were negative on admission but positive for rotavirus antigen after 72 hr of admission were considered as being infected nosocomially. Detection of rotavirus antigen in stools before 72 hr of admission was regarded as a community-acquired infection.

#### Rotavirus Detection, G-Serotyping and Genotyping

Ten percent (w/v) fecal extracts were screened for the presence of Group A rotavirus antigen using monoclonal antibodies by a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Dakopatts, Denmark), according to the manufacturer’s instructions. The assay for the determination of rotavirus
G-serotypes has been described in detail previously [Taniguchi et al., 1987]. Briefly, this was a microtiter plate solid phase assay using monoclonal antibodies specific for G types 1, 2, 3, and 4, produced in Sapporo, Japan [Taniguchi et al., 1984; Urasawa et al., 1988].

Genomic double stranded rotavirus RNA (dsRNA) was extracted with the glass powder method described by Gentsch et al. [1992]. Electrophoresis of deproteinized dsRNA was carried out in 10% polyacrylamide slab gels with a 5% spacer gel, both containing sodium dodecyl sulphate. The runs were carried out at room temperature for 4–5 hr at 40 mA constant current, followed by silver nitrate staining [Laemmli, 1970; Linhares et al., 1993].

Determination of G and P genotypes was made using a multiplex reverse-transcriptase polymerase chain reaction (RT-PCR), as described previously [Gouvea et al., 1990; Gentsch et al., 1992; Das et al., 1994]. Briefly, G-genotyping involved a two-step amplification procedure. First, consensus primers 9con1 and 9con2 were used in a 30-cycle RT-PCR after denaturation of the dsRNA template; the resulting PCR product was then subjected to a second, 20-cycle amplification reaction that included 9con1- and type-specific primers 9T-1, 9T-2, 9T-3P, 9T-4, and 9T-9B for G types 1, 2, 3, 4, and 9, respectively. Characterization of P genotypes followed a strategy similar to that used for G typing: a 30-cycle amplification, using both consensus primers con2 and con3, was followed by a 30-cycle second amplification PCR using con3 and type-specific primers 1T-1 (P[8]), 2T-1 (P[4]), 3T-1 (P[6]), and 4T-1 (P[9]).

Southern Hybridization

Southern hybridization with nested PCR-products was carried out using digoxigenin (dig)-labeled genotype-specific hybridization probes, as described by Ando et al. [1995], with further modifications for rotavirus, as proposed by Ramachandran et al. [1996] and Leite et al. [1996]. Briefly, genotype-specific oligonucleotide probes labeled at 5' end with dig were designed to have high homology with strains of one type and low homology with strains belonging to other types. The G2 and P[6] sequences of the probes and the hybridization conditions were described previously by Leite et al. [1996] and Ramachandran et al. [1996].

Other Laboratory Analyses

All stool samples were examined in addition by commercial ELISA kits for the presence of enteric adenoviruses (Cambridge, Britich, Worcester, MA) and astrovirus (Dakopatts ELISA, Copenhagen, Denmark). The samples were also checked for the presence of bacteria and parasites according to techniques described in the WHO Manual for Laboratory Investigation of Acute Enteric Infections [Organisation Mondiale de la Santé, 1987].

RESULTS

Characteristics of Studied Patients

During the 25-month study period, 437 premature/sick newborn babies (59% male; ages ranging from 1–28 days) were admitted to one of the six neonatal care unit wards. Data on the birth characteristics and hospitalization of rotavirus-positive and rotavirus-negative babies are given in Table I. Taking these two groups together, over 60% of the babies were born to women whose gestational time was less than 9 months, and low birth-weights (<2.5 kg) were recorded in over 65% of these newborns. At least 80% of babies in both groups were breast-fed while in hospital. Although two-thirds of the newborns (either rotavirus-positive or rotavirus-negative) came to the neonatal care unit wards directly from the hospital's maternal unit, the remainder were referred from other public health centres in the Belém urban area. Most of the neonates (87%) were admitted within their first week of life and the duration of hospitalization exceeded 2 weeks for at least 35% of them in both groups.

A total of 614 fecal specimens were obtained from the 437 neonates, including 308 (70.5%) newborns from whom only one stool specimen was obtained and 129 (29.5%) with two or more samples collected. Overall, 54 neonates had either hospital- or community-acquired (n = 16) diarrhea; of these, only nine (16.7%) were shedding rotavirus. All rotavirus-related cases of diarrhea clustered in January and February (first half) 1997. The first two newborns with diarrhea were admitted to the external nursery from the outside community by mid-January 1997. Shortly thereafter, a few rotavirus-associated nosocomial diarrheal episodes occurred, mainly in the external nursery. From mid-March 1997 onward, all rotavirus infections were asymptomatic and involved mostly neonates admitted from the maternal unit.

Characterization of Rotavirus Strains

Among the 51 newborns excreting rotavirus, 42 (82%) developed non-diarrheal, nosocomial infection. Although all 51 rotavirus-containing samples from hospitalized neonates were examined for G-serotypes, P- and G-genotype-specificities were sought for 28 (54.9%) and 18 (35.3%) of these isolates, respectively (Table II). A G2 serotype was assigned to 32 (62.7%) of the rotavirus-positive strains and 18 (35.3%) of them were not typed when tested against a panel of G1–G4 type-specific monoclonal antibodies. P[6] and G2 genotypes accounted for over 90% of tested samples, and 18 (94.7%) of the 19 strains identified as having both VP4-genotype and VP7-serotype/genotype were P[6]G2.

Strains bearing P[6]G2 genotype-serotype specificity displayed short dsRNA profiles, with slight differences indicated by comparing the relative positions of bands 7, 8, and 9 (Cluster III); very few (2 or 3) P[6], G2 short electropherotypes displaying a difference in segments 10 and 11 were identified. An additional P[6], G
undetermined strain had a typical long electropherotype; only two strains displaying this long RNA profile could be detected, both of which originated from the community.

The G2- and P[6]-type specificities of rotavirus-positive samples were identified by the detection of DNA fragments with sizes of 244 base pairs (bp) and 267 bp, respectively. Identification of the RT-PCR amplified products was confirmed further by Southern hybridization, as indicated by hybridization with the G- and P-type specific corresponding probes.

The search for enteropathogens other than rotaviruses yielded the following isolation rates, mostly related to asymptomatic neonates: enteropathogenic Escherichia coli (0.81%), Salmonella Group B (0.16%), enteric adenoviruses (0.95%) and astrovirus (3.42%) (data not shown).

### Temporal Distribution of Rotavirus Infections

The monthly occurrence of rotavirus infections from May 1996–May 1998 is shown in Figure 1. Although no rotavirus infections occurred in the neonatal care unit during the first 8 months of surveillance, 50 (98%) of the 51 isolates clustered between January 1997–December 1997. Overall, 50 (21.1%) of the 237 newborn babies admitted during this “epidemic” period were found to excrete rotavirus.

The temporal distribution of 23 P[6]G2 rotavirus isolates is illustrated in Figure 2. There were only

### Table I. General Characteristics of Hospitalized Neonates Enrolled to Participate in the Study

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Rotavirus-positive</th>
<th>Rotavirus-negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infants’ gestational age&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 9 month</td>
<td>31 (64.6)</td>
<td>236 (63.4)</td>
</tr>
<tr>
<td>9 month or greater</td>
<td>17 (35.4)</td>
<td>136 (36.6)</td>
</tr>
<tr>
<td>Birth weight</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low (≤ 2.5 kg)</td>
<td>35 (68.6)</td>
<td>256 (66.3)</td>
</tr>
<tr>
<td>High (&gt; 2.5 kg)</td>
<td>16 (31.4)</td>
<td>130 (33.7)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>24 (47.1)</td>
<td>233 (60.4)</td>
</tr>
<tr>
<td>Female</td>
<td>27 (52.9)</td>
<td>153 (39.6)</td>
</tr>
<tr>
<td>Age at admission&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 week</td>
<td>42 (82.3)</td>
<td>326 (87.4)</td>
</tr>
<tr>
<td>2 weeks</td>
<td>6 (11.8)</td>
<td>23 (6.2)</td>
</tr>
<tr>
<td>3 weeks</td>
<td>3 (5.9)</td>
<td>24 (6.4)</td>
</tr>
<tr>
<td>Distribution among wards, at admission&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>External nursery</td>
<td>9 (18.7)</td>
<td>119 (31.3)</td>
</tr>
<tr>
<td>Special care units&lt;sup&gt;c&lt;/sup&gt;</td>
<td>38 (79.2)</td>
<td>241 (64.4)</td>
</tr>
<tr>
<td>Intensive care unit</td>
<td>1 (2.1)</td>
<td>20 (5.3)</td>
</tr>
<tr>
<td>Feeding type while in hospital&lt;sup&gt;b,d&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breastfed only</td>
<td>36 (70.6)</td>
<td>308 (86.3)</td>
</tr>
<tr>
<td>Breastfed plus bottlefed</td>
<td>7 (13.7)</td>
<td>43 (12.0)</td>
</tr>
<tr>
<td>Bottlefed only</td>
<td>8 (15.7)</td>
<td>6 (1.7)</td>
</tr>
<tr>
<td>Hospital stay&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td>32 (8.3)</td>
</tr>
<tr>
<td>&lt; 1 week</td>
<td>13 (25.5)</td>
<td>131 (33.9)</td>
</tr>
<tr>
<td>1 week</td>
<td>14 (27.4)</td>
<td>87 (22.5)</td>
</tr>
<tr>
<td>≥ 3 weeks</td>
<td>24 (47.1)</td>
<td>136 (35.2)</td>
</tr>
<tr>
<td>Infection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nosocomial diarrhoea</td>
<td>5 (9.8)</td>
<td>33 (8.5)</td>
</tr>
<tr>
<td>Community-acquired diarrhoea</td>
<td>4 (7.8)</td>
<td>12 (3.1)</td>
</tr>
<tr>
<td>Non-diarrhoeic, nosocomial infection</td>
<td>42 (82.4)</td>
<td>231 (68.1)</td>
</tr>
<tr>
<td>Not infected</td>
<td>—</td>
<td>341 (88.4)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Thirty-eight percent of mothers underwent cesarean section.
<sup>b</sup>Data were not available from all 437 participating babies.
<sup>c</sup>Comprises special care nurseries and “non-infected” premature babies’ room.
<sup>d</sup>Chi-squared of the trend, 17.6, P < 0.001.

### Table II. Rotavirus G-Serotypes and Genotypes in Hospitalized Neonates in Belem, Brazil, From May 1996 to May 1998

<table>
<thead>
<tr>
<th>Genotypes&lt;sup&gt;a&lt;/sup&gt;</th>
<th>G2</th>
<th>G1 + G2</th>
<th>G?</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>P[6], G2</td>
<td>11</td>
<td>1</td>
<td>3</td>
<td>15</td>
</tr>
<tr>
<td>P[6], G&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7</td>
<td>—</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>P[6], G&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>P[7], G2</td>
<td>13</td>
<td>0</td>
<td>10</td>
<td>23</td>
</tr>
<tr>
<td>Not tested for G or P</td>
<td>32</td>
<td>1</td>
<td>18</td>
<td>51</td>
</tr>
</tbody>
</table>

<sup>a</sup>No G3 or G4 strains were identified.
<sup>b</sup>Not tested.
<sup>c</sup>Tested, but not typed.
seven diarrheal episodes associated with this genotype, all of which were within the first trimester of 1997; the only two P[6]G2 strains from community-acquired diarrheal episodes were noted in January 1997. The remainder (n = 16) of the P[6]G2 rotavirus isolates were obtained from non-diarrhea babies who became infected nosocomially.

**DISCUSSION**

This is the first study on the epidemiological features of rotavirus infection among hospitalized neonates in Brazil. In general, the data presented above confirm those from several previous worldwide investigations showing that neonatal rotavirus infections are usually asymptomatic, and that rotaviruses spread efficiently within newborn nurseries [Vial et al., 1988; Haffejee, 1991; Cicirello et al., 1994].

Rather than involving a regular hospital nursery, where babies generally are roomed with their mothers, this cohort study was targeted at a communal, special-care unit where newborns are housed because of prematurity or other health problems. During the whole 25-month surveillance study period 11% of the enrolled neonates were shown to excrete rotaviruses while in hospital, and among these, only 18% developed diarrhea. These rates appear to be higher than those recorded elsewhere for regular hospital nurseries, where an average of 9% of neonates excreted rotavirus, usually without developing diarrhea [Perez-Schael et al., 1984; Srinivasan et al., 1984; Grillner et al., 1985]. Rotavirus detection rates in the present study were lower than those reported for previous studies involving special-care nurseries, however, where 42–77% of newborns became symptomatically infected by rotavirus [Cameron et al., 1978; Bryden et al., 1982]. This difference might be explained by the fact that in the present study rotaviruses were not readily detected throughout the entire 2-year study period but clustered from the 9th to 20th months of surveillance.

Surprisingly, rotavirus infections could not be recorded among babies admitted to the nursery in the period of May–December, 1996, when an annual, slight peak incidence of rotavirus infection is expected to occur at community level [Linhares et al., 1996]. In the present study, it seems likely that rotaviruses were introduced into this nursery from the external environment, as the only two cases of community-acquired diarrhea were recorded in January 1997, just before the commencement of nosocomial rotavirus infections among hospitalized neonates. The fact that rotaviruses transmitted nosocomially are a reflection of those strains circulating in the external environment has been demonstrated previously in studies conducted in pediatric wards [Pacini et al., 1987; Gusmão et al., 1999].

In the present survey, a comparison has been made between infected (n = 51) and uninfected neonates (n = 386) to assess whether or not specific factors might

![Fig. 1. The monthly prevalence of rotavirus infections among hospitalized neonates in Belém, Brazil.](image)
Affect the incidence of rotavirus infection within the special-care nurseries. These two groups did not differ significantly \((P>0.05)\) in relation to the infants' gestational age, birth weight, gender distribution, age at admission, room of the ward in which babies were admitted, and duration of hospital stay. Although findings similar to those of the present study were reported by Bhan et al. [1993] in New Delhi, studies conducted in London by Dearlove et al. [1983] indicate that smaller, sicker babies who stayed in the hospital longer are more likely to acquire infection. Also in contrast with local observations, other studies conducted in both developed and developing countries have shown that a longer hospital stay increases the likelihood of neonates having nosocomial rotavirus infection [Pacini et al., 1987; Haffejee, 1991; Omoigberale and Abiodun, 1995]. It is believed that local nursery crowding may have accounted for the rapid spread of rotavirus among our hospitalized neonates. In this context, it is likely that fomites and hospital personnel had a role in the rotavirus transmission across the six wards of the hospital's neonatal care unit. Regrettfully, a limitation of the present study was in not having obtained stool samples from the hospital staff.

In comparing neonates breast-fed only with those who are bottle-fed only (Table I), it could be suggested that breast-feeding may have a role in protecting against rotavirus infection. This is in accordance with reports from numerous researchers indicating that breast-fed infants are far less likely to shed rotaviruses than are formula-fed infants [Totterdell et al., 1976; Banatvala et al., 1978; McLean and Holmes, 1981; Duffy et al., 1986]. One possible explanation for the 8-month rotavirus-free period in the beginning of the study is that breast-feeding might have protected against infection by rotavirus strains other than P[6]G2, which was introduced presumably into the neonatal unit environment in January 1997.

A major finding in the present survey was that nearly 95% of typed rotavirus strains belonged to P[6]G2 genotype. Strains bearing these genotype specificities circulated throughout eleven successive months, infecting around 20% of the babies who were admitted to one of the 6 neonatal care unit wards. The hypothesis of a common source for nosocomial transmission of the P[6]G2 rotavirus genotype is sustained by the fact that over 90% of strains displayed a single short electrophoretic profile.

Worldwide, a variety of rotavirus strains have been isolated from neonates, and a high proportion have been associated with asymptomatic infections [Haffejee, 1991; Das et al., 1994; Gentsh et al., 1996; Kilgore et al., 1996]. The present data showing the transmission of P[6]G2 genotype among hospitalized neonates agree with other findings indicating that, in general, the rotaviruses designated commonly as newborn

![Temporal distribution of 23 P[6], G2 rotavirus strains excreted by diarrheic- and non-diarrheic hospitalized neonates in Belém, Brazil during 1997. ANI, asymptomatic nosocomial infection; CAD, community-acquired diarrhea; ND, nosocomial diarrhea.](image)
avirulent strains have a unique P[6] type and either G1, G2, G3, or G4 specificity [Flores et al., 1985; Hoshino et al., 1985; Gentsch et al., 1996]. There have been recent investigations in India, however, showing that P[6] strains with common G types may account for over 40% of typed strains detected from stools of older infants and young children with diarrhea [Ramachandran et al., 1996]. Similarly, the P[6] allele has been identified among 8% of South African pediatric patients with diarrhea [Mphalele and Steele, 1995]. Studies conducted in Brazil have also shown that P[6] rotavirus strains bearing G1, G2, and G3 specificities may be a cause of acute gastroenteritis among older infants and children [Timenetsky et al., 1994; Leite et al., 1996]. Although largely recognized as a cause of asymptomatic infection among newborns, as observed in the present study, P[6] genotype rotaviruses are emerging currently as a frequent cause of diarrhea among older infants and children.

It was not possible to search for the presence of P[11] type among the rotavirus strains of the present study because this specific primer was not available. This uncommon P genotype has been found recently in neonatal rotavirus outbreaks in India, bearing either G3, G9, or G10 specificity [Dunn et al., 1993; Cicirello et al., 1994; Das et al., 1994; Gentsch et al., 1996]. It is of interest that mixed P type infections could not be detected among hospitalized neonates who participated in the present survey, although it does appear to be a common finding when investigations are carried out in the external community [Timenetsky et al., 1994; Leite et al., 1996; Mascarenhas et al., 1998, 1999].

Although several studies have indicated that rotavirus strains infecting hospitalized neonates differ from community strains found in children infected at home [Steele et al., 1995; Kilgore et al., 1996], the present data suggest that a P[6]G2 rotavirus strain may have been introduced into the hospital's neonatal care unit from the external community. Indeed, as demonstrated in Figure 2, two cases of rotavirus P[6]G2-related community acquired diarrhea were identified among children admitted to the neonatal care unit by early January 1997, when the “outbreak” was just beginning. Interestingly, there were only five nosocomial diarrhea cases associated with the P[2]G6 strain, all of which clustered in the months of January, February, and March 1997. Thereafter, all hospital acquired P[6]G2 infections were asymptomatic, leading us to postulate that an attenuation of the originally introduced strain might have occurred during spread among the newborns. Further evidence is needed to support such a hypothesis, however, such as carrying out nucleotide-sequencing determinations to see if deduced amino acid sequence differences existed between P[6]G2 strains circulating during the “outbreak” period.

Because rotavirus-positive samples were tested primarily for G serotypes by ELISA, several of the specimens were exhausted, and a lower number available for further genotyping by RT-PCR with subsequent confirmation of results by Southern hybridization. Nevertheless, it is noticeable that whereas 35% of strains could not be G serotyped by G1 to G4 ELISA monoclonals, only 5% of samples were not typed by RT-PCR including genotype-specific primers for G1–G4 and G9. These results support previous findings showing that RT-PCR is much more sensitive than ELISA for the detection of rotaviruses [Buesa et al., 1996]. An additional advantage of the RT-PCR (and the hybridization with oligonucleotide probes) is its extensive current application to P genotyping.

With the exception of astrovirus, which were found in 3.4% of samples, enteropathogens other than rotaviruses (either bacteria or parasites) were isolated at low rates among local hospitalized neonates, and these agents appeared not to be related to the very few diarrheal cases identified in the present study cohort.

The data presented above should be regarded as an initial approach toward a better understanding of the molecular epidemiology of rotavirus infection among neonates in our region. To broaden our knowledge, it would be worth looking further for the occurrence of rotavirus-infecting-types among neonates in the external community, namely those newborns with diarrhea who are treated at primary care centres.

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