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# CONCOMITANT ROTAVIRUS SEROTYPES 1 AND 4 INFECTIONS IN A DIARRHOEIC CHILD FROM BELÉM, BRAZIL

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## SUMMARY

Concomitant serotypes 1 and 4 infections were detected in a 15-month old female child with community-acquired diarrhoea which lasted 7 days and coursed with moderate dehydration. The evidence for dual rotavirus infection was offered by the following findings: a) enzyme-linked immunosorbent assay (ELISA) positive reactions to both 1 and 4 serotypes; and b) extra-migrating bands at electrophoresis of RNA in polyacrylamide gel (PAGE). These results suggest that children living under poor sanitation conditions are heavily exposed to rotavirus infections; in addition, the co-circulation of different serotypes in the same setting sustains the current concept that a rotavirus vaccine should be multivalent, in order to protect children against the four epidemiologically important rotavirus G serotypes.

**KEYWORDS:** Concomitant; rotavirus; Serotypes 1 and 4; Infections.

## INTRODUCTION

Rotaviruses are largely recognized as the most important enteropathogens causing acute gastroenteritis in both infants and young children worldwide. Because of the high mortality-rate associated with rotavirus diarrhoea, particularly in the developing countries, the availability of an effective vaccine is a goal to be pursued[13].

It is well established that human are mainly infected by rotaviruses belonging to group A, however, growing evidence also indicates that groups B and C may be involved in the aetiology of acute diarrhoea among both children and adults[8,15]. Most of group A rotaviruses are classified into either subgroup I or II, as specified by the major inner capsid VP6 protein[9,10]. To date, fourteen G serotypes have been identified on the basis of VP7 glycoprotein reactivity. In addition, eight P (of protease-sensitive) serotypes are currently recognized, as specified by VP4 protein reactivity[2,9]. Serotypes G1 to G4, G8, G9 and G12, and P1A, P1B, P2 and P3 are known to infect humans[24,28].

Apart from being classified on antigenic basis, rotavirus strains may also be analysed following the gel electrophoresis of their eleven RNA segments. This procedure essentially allows the identification of two genomic profiles (or electropherotypes): the long and short patterns, in which bands 10 and 11 migrate faster and lower than each other, respectively[4]. More recently, a "super short" electrophoretic profile was identified[22]. Previous studies conducted in Belém have assessed the diversity of electropherotypes co-circulating among diarrhoeic children[21].

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In the Amazon region, as in many other areas of Brazil, rotaviruses account for one-third of diarrhoeal episodes among hospitalized children and are associated with nearly 10% of cases of infantile, community-acquired acute diarrhoea[18,20,25]. All four epidemiologically important rotavirus G serotypes have been shown to infect children in our region, with apparent G1 and G2 "seasons"[11,19]. The co-circulation of different G types and the still prevailing local poor sanitation conditions, heavily expose children from low-income families to rotavirus infection. The present report deals with a case of acute diarrhoea affecting a 15-month old child, in which rotavirus G1 and 4 serotypes were identified in the same stool sample.

## PATIENTS AND METHODS

Our patient was a 15-month old female infant who was enrolled to participate in a survey for acute diarrhoea among hospitalized children in Belém, Brazil. This child was admitted with acute diarrhoea (defined as being the passage of three or more liquid or semi-liquid stools in a 24-hour period), also presenting vomiting and signs of moderate dehydration.

Diarrhoeic stool sample was obtained seven days after admission and placed on phosphate buffered saline (PBS), pH 7.4, for the detection of rotavirus antigen. The faecal specimen was also collected in two screw-caped vials of Cary-Blair medium (one vial containing antimicrobic supplement according to the formulation of Skirrow) for bacteriological examinations. Stools were placed on 10% formaldehyde for parasitological studies.

The assay for rotavirus antigen was performed by using the DAKOPATTS ELISA kits (Copenhagen, Denmark), essentially as described by FLEWETT et al.[5]. For subgrouping and G serotyping, monoclonal antibodies against subgroups I and II and each of the four epidemiologically important G serotypes were used, as described by TANIGUCHI et al.[26,27]. The electrophoresis of rotavirus RNA was carried out through a 5% polyacrylamide gel, using the discontinuous buffer system, as recommended by LAEMMLI[17].

Both bacteriological and parasitological procedures followed the specifications of the "WHO Manual for Laboratory Investigation of Acute Enteric Infections, Programme for Control of Diarrhoeal Diseases"[29]. The Auramine staining and the ZIEHL-NIELSEN modified technique were used for attempts of detection of *Cryptosporidium sp.*[7].

## RESULTS

The dual rotavirus infections were primarily demonstrated by the presence of 14 migrating RNA segments through electrophoresis in polyacrylamide gel (Fig. 1). The extra bands (indicating a second rotavirus strain) were noted in the first, second and third clusters, as follows: one band located between second and third segments of rotavirus strain which fully displays its complete (eleven genes) genomic profile; another extra band was visualized between segments 5 and 6 of this latter strain and other co-migrating with nine segment. Therefore, both electrophoretypes were found to be "long" (see control in Fig. 1), with eight co-migrating segments. No bacterial pathogen(s) could be isolated, however, *Cryptosporidium sp.* was identified in the stool sample.

The child was admitted with aqueous diarrhoea which lasted seven days. Vomiting was recorded in the first day of admission and moderated dehydration was identified during the first 48 hours of hospitalization. In addition, acute respiratory infection was diagnosed on days 6 through 8 of admission. The patient was released from hospital five days after diarrhoea was resolved.

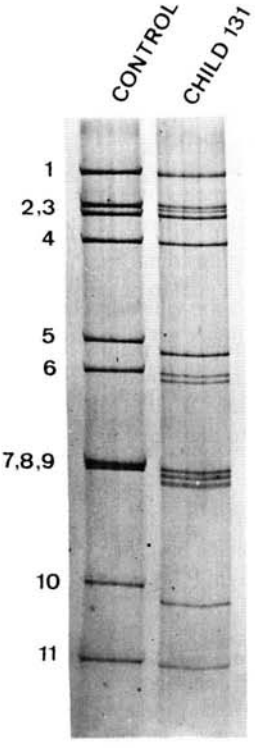


Fig. 1 - Electrophoresis of RNA in polyacrylamide gel (PAGE) in child nº 131 with dual rotavirus infection.

## DISCUSSION

Mixed infections involving two different rotavirus serotypes represent an unusual finding in our region, as based on previous local studies[19,20]. In fact this is the first description of concomitant, serotypes 1 and 4 infections in the same child, at least in the Amazon region. Previous investigations carried out by FREITAS et al.[6] and OLIVEIRA et al.[23] have demonstrated successive (but not concomitant) rotavirus infections affecting the same child, associated with different G serotypes. The late (seventh day of admission) collection of stool sample leads us to postulate the following, as to the origin of the two infecting rotavirus strains: a) it is possible that the child, at admission, was already excreting the two rotavirus strains, therefore suggesting that infection was acquired at community level; b) a second possibility would possibly involve a primary infection acquired outside the hospital and a secondary, nosocomial one, acquired within the hospital; it is known that rotaviruses account for one third of nosocomial, infantile diarrhoea in Belém[12]; and c) a third, less likely situation would involve infection by both strains within the hospital, therefore supporting the concept that rotaviruses are of major importance as aetiological agents of nosocomial diarrhoea[12].

Although rotavirus serotype 2 was largely prevailing over serotype 1, 3 and 4 in our region, at least during the period in which this case of dual rotavirus infection was identified, it has not been found to infect this specific patient.

As dehydration is regarded as an indicator of clinical severity, it could be postulated that dual rotavirus infection involving different G serotypes do not necessarily implies in a more severe illness, once the study child presented with only moderate dehydration that lasted two days.

Several studies throughout the world have demonstrated that dual rotavirus infections seem not be a common finding. Only 1% of children followed up by KIM et al.[16] in the USA were found to be infected by two different rotavirus strains, on the basis of electrophoresis of viral RNA. BISHOP et al.[3], on the other hand, in Australia, have reported cases of concomitant rotavirus infections among 3.2% of diarrhoeic children, involving serotypes 1 (subtypes b and c) and 4, therefore similar to our findings. In Bangladesh, AHMED et al.[1] were able to demonstrate cases of rotavirus infections in which strains had simultaneously serotypes 1 and 4 specificities, apart from belonging to subgroup II and displaying a "long" electrophoretic profile; this combination is similar to that found for the presently reported case of dual infection in Belém.

Although dual rotavirus infection constitutes a rare event in nature, it clearly indicates that a further effective rotavirus vaccine should not be monovalent. Indeed, it is currently accepted worlwide that a future rotavirus vaccine must protect infants and young children against all four epidemiologically relevant rotavirus serotypes. This is supported by the fact that predominance of a specific serotype seems to be a transient phenomenon, possibly mediated by the proportion of susceptible persons in a due population[14].

## RESUMO

### Infecção concomitante por sorotipos 1 e 4 de rotavírus em uma criança diarréica de Belém, Brasil

Infecções simultâneas por sorotipos 1 e 4 de rotavírus foram observadas em uma criança de 15 meses de idade, do sexo feminino, internada com dirréia aguda contraída na comunidade que perdurou por 7 dias, evoluindo com desidratação moderada. As evidências dessas infecções foram inferidas baseadas em testes tais como: a) ensaio imunoenzimático (ELISA), evidenciando-se reação positiva para os sorotipos 1 e 4; e b) migrações extras de segmentos de ARN visualizados à eletroforese em gel de poliacrilamida (EGPA). Esses resultados sugerem que as condições precárias de higiene e saneamento em que vivia essa criança propiciam a infecção maciça por esses agentes virais. Além disso, a co-circulação de diferentes sorotipos no mesmo ambiente sustenta a necessidade de utilizar-se, no futuro, uma vacina polivalente, que proteja as crianças contra os quatro sorotipos G, epidemiologicamente importantes.

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