

ATYPICAL ROTAVIRUS AMONG DIARRHOEIC CHILDREN LIVING IN BELÉM, BRAZIL

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Atypical rotaviruses were detected in faeces from two diarrhoeic children living in Belém, Pará, Brazil. Rotavirus particles were detected by electron microscopy and the RNA electrophoresis showed patterns which were compatible with group C rotaviruses. Tests for the presence of group A antigen by enzyme-linked-immunosorbent assay (ELISA) were negative. The two children had three successive rotavirus infections and in both cases the atypical strains were excreted at the time of the third infection, causing a mild and short-lasting disease.

Key words: atypical rotavirus – reinfection – Belém

Rotaviruses have been shown to be a major cause of infantile diarrhoea throughout the world in both temperate and tropical regions (Davidson et al., 1975; Paul & Erinle, 1982; Kapikian et al., 1976; Linhares et al., 1983). They belong to the Reoviridae family and infect a wide variety of animals (Flewett et al., 1973). Man is mainly infected by those rotaviruses belonging to group A, commonly detected by conventional, widely used techniques (Kapikian & Chanock, 1985).

More recently, other atypical groups have been detected in chickens (McNulty et al., 1981) piglets (Bohl et al., 1982) and rarely in man (Pereira et al., 1983; Nicholas et al., 1983). These atypical viruses are morphologically similar to the group A rotavirus, but antigenically unrelated; in addition, the RNA electrophoresis shows a genomic configuration which markedly differs from those described for the conventional ones of group A rotavirus (Pedley et al., 1983, 1986).

The importance of these atypical rotavirus strains as a cause of acute diarrhoea has been assessed by some authors (Rodger et al., 1982; Hung et al., 1984). In Brazil, the first detection of atypical rotavirus in man was recorded in

Rio de Janeiro by Pereira et al. (1983). They were also detected in other countries including France (Nicholas et al., 1983), Australia (Rodger et al., 1982), Bulgaria (Dimitrov et al., 1983), México (Espejo et al., 1983) and Argentina (Sorrentino et al., 1986). Recently, in China, an atypical strain (Group B) was responsible for an extensive and severe epidemic of diarrhoea affecting adults (Hung et al., 1984; Chen et al., 1985).

Rotaviruses have been associated with both endemic and epidemic gastroenteritis in the Amazon (Linhares et al., 1985; 1981). All strains studied to date, however, belong to group A. The present article describes the occurrence of an atypical strain of rotavirus (probably Group C) in two diarrhoeic children.

MATERIAL AND METHODS

Area of study and population involved – Faecal samples were collected during a longitudinal study of rotavirus infections in the peripheral area of Belém, Pará, Brazil described elsewhere (Linhares et al., 1985). The children population belonged to very low social-economic group and were followed from birth to three years of age.

Laboratory tests – EM: electron microscopy was performed using a direct staining method as described by Flewett et al. (1973) A Jeol 1000 EM model was used for searching viruses in stools. PAGE: analysis of rotavirus RNA

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by polyacrylamide gel electrophoresis was performed as described by Laemmli (1970). Viral RNA was extracted from crude faecal suspension by treatment with sodium dodecyl sulphate (SDS) followed by deproteinization with phenol. Electrophoresis was carried out in 10% acrylamide and 5% bisacrylamide slab gels, and running was performed at 30-35 mA for 6-8 hours. Staining with silver nitrate solution followed the method described by Herring et al. (1982). ELISA: the Enzyme-Linked Immunosorbent Assay was carried out using the World Health Organization kit prepared at the Regional Virus Laboratory, East Birmingham Hospital, Birmingham, England (Beards et al., 1984).

RESULTS

Two atypical rotaviruses were detected in the faeces of two male children aged 33 (child A) and 35 (child B) months. The infections caused by the atypical rotaviruses were preceded by two other infections caused by group A strains. Illness was characterized, in both patients, by aqueous diarrhoea, 3-4 evacuations per day and, in one, vomiting and nausea.

EM examination of the faecal samples showed particles with typical rotavirus morphology (Fig. 1). Suspensions tested by a conventional ELISA technique (Beards et al., 1984), however, gave negative results.

The comparison of the genomic pattern of the two samples with those of subgroup II human rotavirus (Fig. 2), showed that the former lacks the tight triplet of medium molecular weight, corresponding to segments 7, 8 and 9, present in group A rotaviruses; in addition, three genomic segments of our atypical strains migrated in the corresponding region of segments 5 and 6 of group A rotavirus. Finally, one pair of segments migrated in the corresponding region of segments 7, 8 and 9 of group A. These findings are compatible with the association of the two samples with the group C rotaviruses. In one sample it was difficult to detect the last two bands.

Entamoeba histolytica was found in the faeces of both children.



Fig. 1: electron micrograph of atypical rotavirus in a faecal suspension (child A). Phosphotungstate negative contrast. Magnification 207.500X.

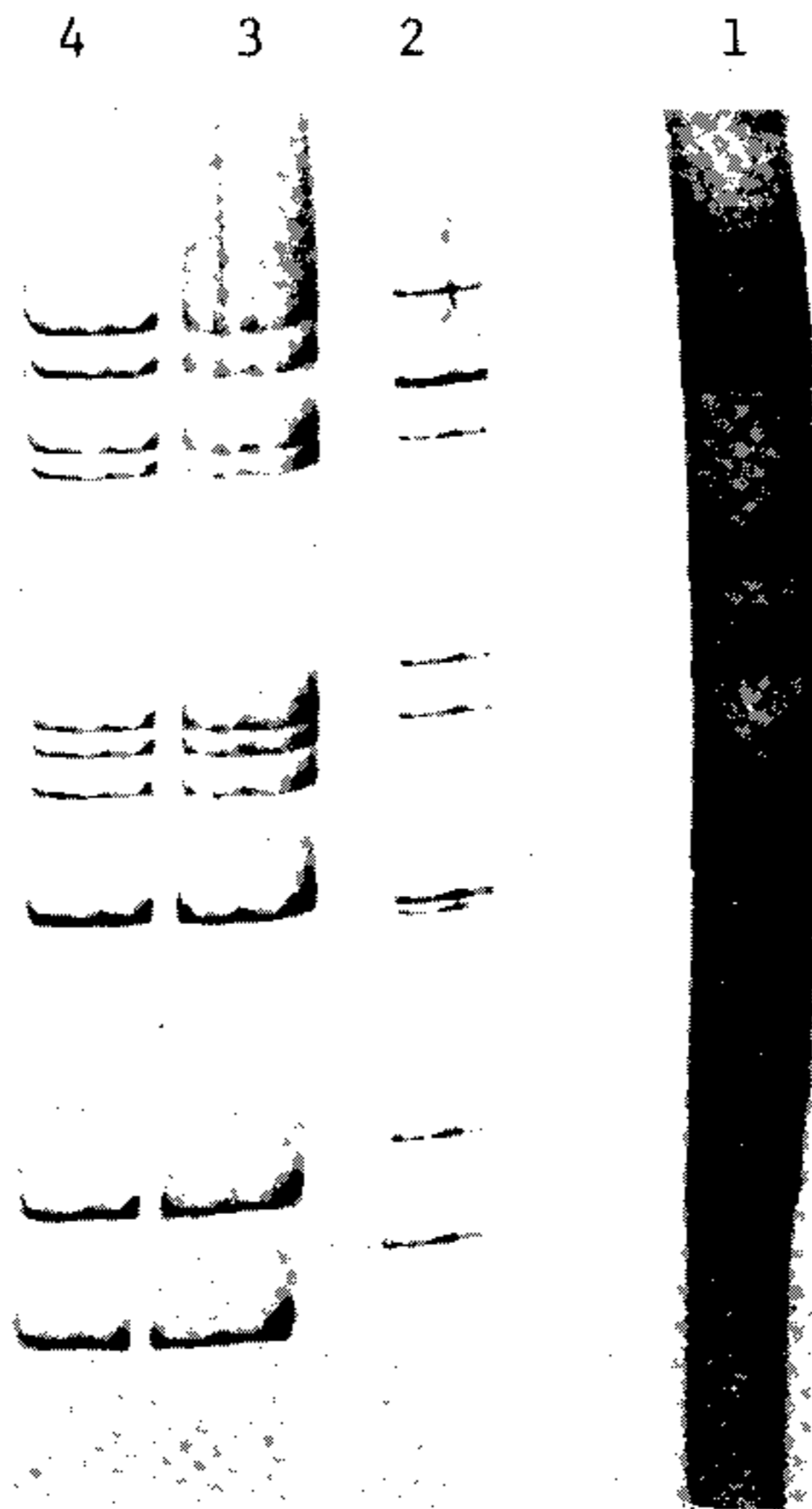


Fig. 2: RNA electrophoretic pattern of: a simian rotavirus SA11 (1), sub-group 2 human rotavirus (2), and strain from child A (3, 4). Staining with silver nitrate.

DISCUSSION

Atypical rotaviruses have been detected by many authors in different countries (Pereira et al., 1983; Nicholas et al., 1983; Rodger et al., 1982; Espejo et al., 1983) and in our study they were responsible for clinical infections in two children, who had previously showed two successive infections with rotavirus group A.

In child B all infections were symptomatic, the first one being more severe than the others. In child A the first two infections were asymptomatic, and the third was associated with a symptomatic infection with the atypical strain. Several authors (Pereira et al., 1983; Nicholas et al., 1983; Dimitrov et al., 1983; Sorrentino et al., 1986) have also associated the infection of atypical rotavirus with cases of acute or prolonged diarrhoea affecting young children.

The genomic analysis of our two atypical rotaviruses indicated that they belong to group C, according to previous characterization made by Pedley et al. (1983). It should be mentioned that a rotavirus strain detected in Rio de Janeiro, Brazil, has also been found to belong to Group C (Bridger et al., 1986).

E. histolytica was found in the same specimens in which the atypical rotaviruses were detected. This makes difficult to establish which pathogen was the primary agent of diarrhoea.

Several other ELISA - negative specimens collected during our longitudinal study are being processed by electrophoresis, in the hope of detecting additional atypical strains: in spite of its high sensitivity and specificity, the ELISA method does not detect these strains, and it is important to use techniques such as the PAGE that allows identification of strains (including pathogenic ones) that do not belong to group A.

Observations on atypical rotavirus have, to date, clearly indicated their importance as human pathogens, and future studies need to be broadened, in view of their importance related to the production of candidate-vaccines against the rotaviruses.

RESUMO

Rotavírus atípicos detectados em crianças diarreicas, em Belém, Brasil - Rotavírus atípi-

cos foram detectados nas fezes de duas crianças diarreicas residentes em Belém, Brasil. Partículas de rotavírus foram visualizadas por microscopia eletrônica nos espécimens fecais de ambos os pacientes, tendo a eletroforese do ácido ribonucleico (ARN) exibido padrões compatíveis com rotavírus do grupo C. Testes imunoenzimáticos (ELISA) foram negativos quanto à presença de antígenos do grupo A. As duas crianças apresentaram três infecções sucessivas por esse agente, sendo que, em ambos os casos, os rotavírus atípicos foram excretados por ocasião da terceira infecção, produzindo sintomas brandos e de pouca duração.

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