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AN ATYPICAL ROTAVIRUS DETECTED IN A CHILD WITH GASTROENTERITIS IN RIO DE JANEIRO, BRAZIL

H.G. PEREIRA
J.P.G. LEITE
R.S. AZEREDO
VIVIAN DE FARIAS
FRITS SUTMOLLER

Particles morphologically identical to rotaviruses were found in the faeces of a nine week-old child with gastroenteritis. Analysis of the viral RNA genome by polyacrylamide gel electrophoresis revealed 10 bands (probably 11 segments) some of which differed in migration rate from those of the great majority of rotaviruses infecting man and other animal hosts. The virus was not detected by a highly sensitive enzyme immunoassay (ELISA) and therefore probably lacked the crossreactive antigen(s) shared by the majority of rotaviruses. This was the only strain with such behaviour among 230 rotaviruses of human origin examined in this laboratory since 1979.

The implications of the existence of non-crossreactive rotaviruses are discussed.

Rotaviruses have been shown to play an important role in the aetiology of gastroenteritis in man and animals (see reviews by Holmes, 1979 and Wyatt et al, 1981). They share certain properties which characterize them as a genus within the family reoviridae (Matthews, 1979). The virions are isometric and appear either as complete particles approximately 70nm in diameter with smooth contour conferred by an outer capsid layer, or as incomplete forms measuring 56nm, without the outer capsid and showing surface projections. Particles with dark centres due to penetration of contrasting stain are also observed. The virus genome consists of 11 separate segments of double-stranded ribonucleic acid (ds RNA) with molecular weights ranging from approximately 0.2×10^6 to 2.0×10^6 daltons. Most rotaviruses share at least one crossreactive antigen revealed by a variety of techniques such as enzyme- or radioimunoassays, immunofluorescence, immunodiffusion and complement fixation. In addition, they have subgroup-specific antigens detected by enzyme-linked immunosorbent assays (ELISA) and immuno-adherence haemaglutination assays (IAHA) as well as type-specific antigens identified by virus neutralization (Kalica et al, 1981). Agents identical to rotaviruses in morphological

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Instituto Oswaldo Cruz, Caixa Postal 926, 20000, Rio de Janeiro, RJ, Brazil.

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and biochemical characters but lacking the group-specific antigen have, however, been detected in chickens (McNulty et al, 1981), in pigs (Saif et al, 1980, Bridger, 1980, Bridger et al, 1982) and in man (Rodger, Bishop & Holmes, 1982). Rotaviruses can be identified and differentieted from each other by the electrophoretic behaviour of their genomic segments (Kalica et al, 1976, 1978; Espejo et al, 1977, 1980; Verly & Cohen, 1977; Rodger & Holmes, 1979; Rodger et al, 1981; Todd, McNulty & Allan, 1980; Lourenço et al, 1981). Human rotaviruses can be classified by this method into subgroups 1 and 2 in which the two smallest RNA segments (bands 10 and 11) of the first have slower migration rates than those of the second. Each subgroup can be subdivided according to differences in migration of other genome segments. Polyacrylamide gel electrophoresis (PAGE) of viral RNA extracted from faecal suspensions or from infected tissue cultures is a simple and sensitive method for the detection and differentiation of rotaviruses from human and other animal hosts.

Studies carried out in our laboratory revealed that, as in other parts of the world, human rotaviruses detected in the states of Rio de Janeiro, São Paulo and Pará in Brazil fall into subgroups 1 and 2, with a preponderance of the latter (Pereira et al, 1983). More recent studies revealed, in a single sample from a child with gastroenteritis, a rotavirus-like agent which differs markedly in electrophoretic behaviour from all strains previously investigated, and is not detectable by a highly sensitive ELISA. This finding is described in the present paper.

MATERIAL AND METHODS

1. The patient (M.S.R.) was a two months old female baby living in the township of Nova Iguaçu, just north of Rio de Janeiro city limits. She belongs to a family of low socio-economic level with six members living in a two bedroom brick house with two beds. Domestic drinking water is from a well located near a drainage ditch and there is no sewage system. The area is semirural and several neighbours raise pigs. On April 18th 1982 she was admitted to a ward in the Policlinica de Botafogo after a previous stay in another hospital where she had been treated for two days with intravenous fluids. On arrival she had a six day clinical history of diarrhoea with six or more yellowish stools per day, vomiting, fever and anarexia. Her condition was fair and the only abnormalities on physical examination were a slightly distended abdomen and a few rhonchy and rales in both lungs.

2. Laboratory investigations. Faeces were suspended at a concentration of 10% to 20% in 0.01M Tris-HCl buffer, pH 7.4 containing 15mM $CaCl_2$ (Tris/Ca++ buffer), homogenized by vigorous shaking with an equal volume of Freon 113 and centrifuged at 5000g for 15 minutes at 4°C. The aqueous phase was decanted and stored at −20°C. Virus concentrates were obtained by centrifugation of 4ml of the Freon-treated suspension over a 1ml cushon of 45% sucrose in Tris/Ca++ buffer, for 90 minutes at 1000000g. The pellet was resuspended in 0.5ml of the same buffer and tested by ELISA and RNA electrophoresis.

The enzyme immunoassay was performed by the double antibody sandwich ELISA technique (Voller, Bartlett & Bidwell, 1978) as described by Pereira et al (1983).

Electron microscopy was performed by the negative staining technique as described by Almeida et al (1979).

For polyacrylamide gel electrophoresis, faecal suspensions were incubated with 1% sodium dodecyl sulphate (SDS) for 30 minutes at 37°C, extracted with phenol-chloroform and precipitated with ethyl alcohol as described by Pereira et al (1983). Electrophoresis was carried out by Laemmli's (1970) technique except that no SDS was

added to the gel or the reservoir buffer. Slab gels 1.5mm thick, 160mm wide and 125mm high contained a polyacrylamide gradient ranging from 10% to 3.5%, covered by a 3% stacking layer. Electrophoresis was run for 4 to 6 hours at 30 to 40mA. After the run, the gels were immersed in a 0.5ug/ml solution of ethidium bromide in distilled water for 30 minutes followed by a wash in distilled water for at least one hour. They were then photographed in transmitted ultraviolet light.

RESULTS

The faecal sample obtained from patient M.S.R. on 15th April 1982 was shown by electron microscopy to contain particles with the typical morphology of rotaviruses (Figur- 1). Both the original suspension and a concentrate prepared by high speed centrifugation gave, however, negative results when tested by ELISA. Analysis of the original suspension by RNA electrophoresis revealed faint bands in the positions of segments 1 to 4 of other rotaviruses run in the same gel. Electrophoresis of RNA extracted from the pellet of 4ml of the same suspension sedimented through a sucrose cushion gave the pattern shown in channel B of Figure 2. The genome pattern of another human rotavirus known to belong to subgroup 2 is shown in channel A of the same figure. Although bands 1 to 4 in channel B cannot be clearly resolved due to overloading, it is obvious that the two samples differ markedly from each other. Particulary noticeable are the differences in bands 5, 6, 7 and in the two fastest-moving bands which by analogy with other rotaviruses, we assume to correspond to 10 and 11. Bands 8 and 9 in channel B are presumed to co-migrate.

Fig. 1 – Electron micrograph of faecal suspension obtained from patient M.S.R. on 15th April, 1982. Phosphotungstate negative contrast. Magnification 210.000 x.

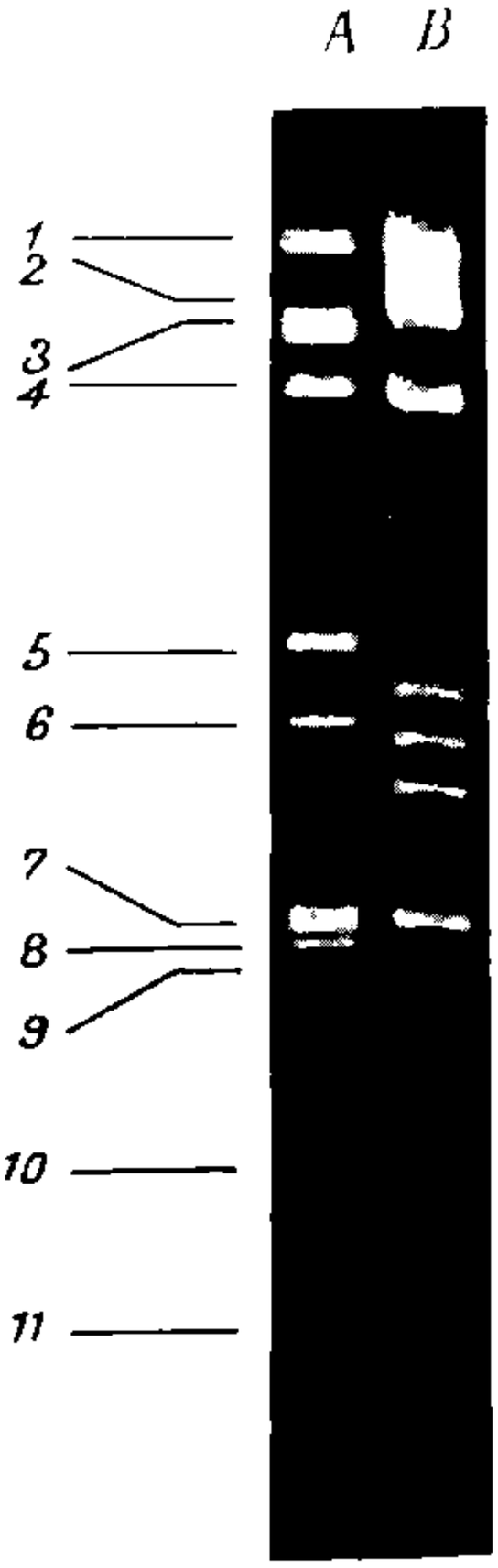

Fig. 2 - Polyacrylamide gel electrophoretic patterns of a subgroup 2 human rotavirus (channel A) and strain from patient M.S.R. (channel B).

No virus could be detected by any of the above procedures in a second faecal sample obtained from the same patient on 10th May 1982.

DISCUSSION

The virus detected in the faeces of patient M.S.R. resembles rotaviruses in morphology and in electrophoretic behaviour of its genome but could not be shown to contain the rotavirus group antigen. To our knowledge, the only other agent from a human host with a similar behaviour was found by Rodger, Bishop & Holmes (1982), in a patient with gastroenteritis in Melbourne, Australia. It is worthy of notice that these two strains are similar not only in the lack of group antigen but also in RNA electrophoretic behaviour. Thus, the two smallest genome segments of both strains migrate faster than those of other human rotaviruses. This separates them from subgroups 1 and 2. Furthermore, both have slow-migrating band 7 and apparently co-migrating bands 8 and 9. A strain showing some resemblance to these two in RNA electrophoresis and also lacking the group antigens has been found infecting pigs in the U.S.A. (Saif et al, 1980; Theil et al, 1980). The other non-crossreactive rotavirus isolated from chickens by McNulty et al (1980) differs from other avian rotaviruses in electrophoretic pattern and the authors raise the possibility that this strain may have derived from a mammalian host by cross-infection.

The existence of non-crossreactive rotaviruses poses a problem in relation to laboratory diagnosis. Serelogical methods most commonly used for diagnosis are based on the existence of crossreactive antigens. Therefore, viruses lacking such antigens are missed unless all samples are routinely examined by electron microscopy or by RNA electrophoresis. Although fairly sensitive, the latter technique is unlikely to detect virus with the same efficiency as that of electron microscopy or ELISA unless samples are large enough to be concentrated. This situation will remain until sufficient virus is available for the preparation of antigens and antisera with which serological techniques may be developed for diagnostic use. Such techniques will also be of value in the study of antigenic relationships between strains that do not possess the rotavirus group antigen. More refined method such as the fingerprinting of genome segments described by Clarke & McRae (1981) are likely to be of great value in this connection. Of particular interest will be the study of relationships between strains from different host species. The electrophoretic similarities between non-crossreactive human, porcine and avian strains suggest that virus may be transmitted from one species to another. Examples of this are already known, *e.g.* pigs can be experimentally infected with rotaviruses from calves, lambs, foals and children (see review by Wyatt et al, 1981).

RESUMO

Partículas morfologicamente idênticas a rotavirus foram encontradas nas fezes de uma criança de dois meses com gastroenterite. Análise do genoma viral por eletroforese em gel de poliacrilamida revelou 10 faixas (provavelmente 11 segmentos) de RNA, algumas das quais diferem em velocidade de migração das observadas na grande maioria de rotavirus de hospedeiros humanos e de diversas espécies de animais. O vírus não foi revelado por um ensaio imuno-enzimático de alta sensibilidade, o que sugere a ausência do antígeno de grupo que dá reações cruzadas entre a maioria dos rotavirus. O vírus descrito no presente trabalho foi o único com tal comportamento entre 230 amostras analisadas por nós desde 1979.

A relevância de existência de rotavirus não relacionados antigenicamente a outros membros do grupo é discutida.

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