

REINFECTIONS AND ROTAVIRUS SEROTYPES IN BELÉM, BRAZIL (PRELIMINARY REPORT)

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SUMMARY

Repeated infections involving different rotavirus serotypes were detected in four children living in Belém, who were followed up since birth to three years of age. In one child (Reg. 23.983) three successive symptomatic infections (one of them associated with serotype 2) were noted: the first, at four months of age, the second at 20 months and the third at 27 months. Another child had two subsequent infections, the first one by rotavirus serotype 1, and the second by a not identified rotavirus serotype. In this case two episodes of rotavirus-related diarrhoea were recorded, occurring eight months apart. Apparent infections were detected on two occasions involving the same child (Reg. 24.004), the first being associated with serotype 1, and the second with serotype 2. The fourth child (Reg. 24.097) had two successive infections by not determined rotavirus serotypes, without clinical manifestations; the first occurred at 24 months and the second at 28 months.

KEY WORDS: Rotavirus; Reinfections; Children; Serotypes.

INTRODUCTION

Rotaviruses have been shown to be major enteropathogens among children aged 0 to 5 years in both temperate⁶⁻⁷ and tropical⁴⁻¹⁶ countries. In the latter areas, where malnutrition is a common finding, severe dehydration following rotavirus diarrhoea certainly accounts for a high case fatality rate among young children. An effective rotavirus vaccine would, therefore, significantly reduce the overall infantile mortality rate in developing countries. For that, however, longitudinal studies are needed on the serotype-epidemiology of rotavirus infections, in order to determine which serotypes are prevalent in different tropical regions, apart from establishing if they cause sequential infections in the same child. Successive rotavirus infections have been reported by several authors³⁻¹⁰ throughout the

world, however, most deal with sub-groups I and II and not with serotypes. It is known that in general human subgroup I rotaviruses correlate with serotype 2, however, there have been recent evidence indicating that sub-group I includes a new serotype too¹². So, sub-grouping does not constitute a reliable basis to determine serotypes.

We report here partial results concerning a community-based longitudinal study carried out in Northern Brazil, involving children followed since birth to three years of age. This investigation was aimed at elucidating clinical and epidemiological aspects of rotavirus infections, apart from dealing with sub-group/serotype epidemiology study. Data presented below represent preliminary results obtained from four

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children who were reinfected by rotavirus during their first three years of life.

PATIENTS AND METHODS

Patients — About seventy children living in the peripheral area of Belém, Brazil, were followed up since birth to three years of age from December 1982 to March, 1986. Fortnightly home-visits were regularly made, when faeces were collected; these specimens were also obtained throughout any diarrhoeic episodes. Diarrhoeic children were always visited by a physician from our team who, apart from taking both clinical and dietary records, routinely administered oral therapy if required. Maternal milk, acute and convalescent serum samples were also collected whenever diarrhoea occurred. The four children studied here have previously been investigated with respect to the occurrence of reinfections and sub-groups (10).

Methods — All faecal samples were examined for the presence of rotavirus antigen by enzyme-linked immunosorbent assay (ELISA), as basically described by BEARDS *et al.*². Briefly, micro plates (polystyrene, NUNC 239454) were sensitized with rabbit anti-rotavirus serum (100 μ l per well) diluted: 10,000 in carbonate buffer pH 9.6. After an overnight incubation at 4°C plates were washed with PBS containing Tween 20 (polysorbate) (PBS-T) at a final concentration of 0.1% v/v. 25 μ l of stool suspension (20% v/v) was added in duplicate to the pre-coated wells, plus 75 μ l of PBS-T, containing EDTA at a final concentration of 0.01 M (PBS-T-EDTA). The plates were incubated again overnight at 4°C and then washed in PBS-T. 100 μ l of guinea-pig anti-rotavirus serum, diluted 1:100,000 in PBS-T containing bovine serum albumin (BSA) at a final concentration of 1% (PBS-T-BSA) was added and plates incubated at 37°C for 2.5 hours; after washing, a 100 μ l sample of alkaline phosphatase labelled goat antiguine-pig globulin, diluted 1:200 in PBS-T-BSA, was then added and plates incubated for 1.5 hours at 37°C. Plates were washed again and 100 μ l of p.nitro-phenyl phosphate (Sigma 104-105; 1 mg/ml) in diethanolamine was added to each well. After a 20 min incubation at 37°C the reaction was stopped by adding 50 μ l per well of a 3M sodium hydroxide solution. The optical densities (OD) were determined by

using a Flow ELISA — reader Multiskan (405 nm filter). All samples reaching OD greater than 0.1 were tested by means of a blocking test using both pre- and post-immune rabbit anti-rotavirus sera.

All rotavirus-positive samples were sent to the WHO Collaborating Centre for Reference and Research on Rotaviruses, East Birmingham Hospital, Birmingham, United Kingdom, where serotyping was performed by using monoclonal antibodies (1).

The genomic study of rotavirus RNA was carried out basically by the method described by LAEMMLI⁹ with minor modifications. The electrophoresis of RNA in polyacrylamide (PAGE) was preceded by nucleic acid extraction by sodium-dodecyl-sulphate and conventional partial purification. After running, gels were stained with silver nitrate. The "Rotacode", proposed by MOOSAI *et al.*¹³, was used as the system for classifying the different patterns of RNA profile.

RESULTS

Fig. 1 shows the temporal distribution of reinfections by rotavirus among four children within their first three years of life. In one case (child n° 23.983) we recorded three successive apparent infections (two by not determined rotavirus serotypes and one by rotavirus serotype 2), which occurred at 4, 20 and 27 months of life. Two successive symptomatic infections, one by serotype 1 and the other by a not determined rotavirus serotype were observed in another child (n° 24.384). First apparent infection caused by serotype 1 followed by symptomatic serotype 2 infection were detected in a third child (n° 24.004). In a fourth child (n° 24.097) two asymptomatic infections caused by not identified rotavirus serotypes were noted. Four different electrophoretic patterns of rotavirus RNA were detected; in two rotavirus-positive samples the unclear profiles did not allow us to classify the electropherotypes.

Table I presents the clinical symptoms recorded in the four cases of reinfections by rotavirus. The patient n° 23.983 who suffered from three successive diarrhoeic episodes associated with rotavirus, developed clinical symptoms of com-

TABLE 1
Clinical symptoms among four children who had successive rotavirus infections

Patient Number	Infections	Sero-type	Duration of clinical symptoms (in days)					Degree of dehydration	Other pathogens detected
			Aqueous diarrhoea	Nausea	Vomiting	Abdominal pain.	Fever		
23.983	1 st	-Vp ⁷	4	3	3	—	3	1	Shigella sonnei and enterotoxigenic (LT) + E. coli
	2 nd	-Vp ⁷	6	5	5	5	—	1	
	3 rd	1**							
24.004	1 st	1	4	—	—	—	1	—	Fungi
	2 nd	2	5	2	2	5	2	1	
24.097	1 st	-Vp ⁷ *							NE
	2 nd	-Vp ⁷ *							
24.384	1 st	1	5	—	—	5	5	1	Fungi
	2 nd	-Vp ⁷	1	—	—	—	1	—	

*Asymptomatic

NE Not examined for other enteropathogens than rotavirus

** Not followed for clinical observation

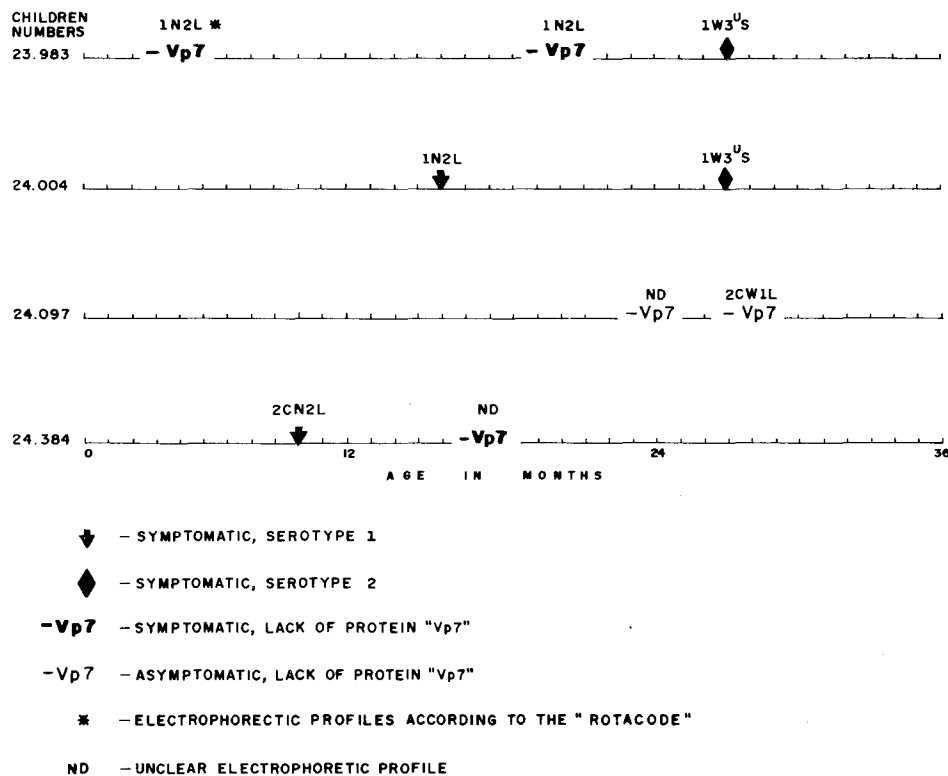


Fig. 1 — Temporal distribution of reinfections by rotavirus in relation to age and serotypes.

parable severities in two of these episodes; *Shigella sonnei* and LT+ *E. coli* were detected in the first infection. In two other patients (n° 24.004 and 24.384) the severity of symptoms was different in comparing both the first and second infections. Not determined rotavirus serotypes were involved in repeated inapparent infections affecting a fourth child (n° 24.097).

It should be mentioned that plasmatic proteins were measured by conventional methods in serum samples collected from the child (n° 23.983) who suffered three successive apparent rotavirus infections, yielding normal patterns.

DISCUSSION

During three years about seventy children were followed to study both clinical and epidemiological aspects of rotavirus infections. This investigation allowed us to detect cases of reinfections by rotavirus within the first three years of life, and also to establish whether or not the same child can be infected by the same or different rotavirus serotype. Only part of our serotyping are at present available, but they clearly indicate that sequential rotavirus infections may occur.

Two rotavirus serotypes were recorded in our study, including serotype 1 (subgroup II) which has been shown to be widely distributed throughout the world, according previous studies³⁻¹⁰.

Sequential rotavirus infections caused by the same serotype were not detected among the four children studied. Serotype 1 and 2 were involved with symptomatic infections. The greater pathogenicity of serotype 1, if compared with serotype 4, has already been demonstrated by some authors¹⁸. The latter one, however, was not detected in our preliminary investigation. Serotype 3, which has been reported¹⁸ to be associated with either apparent or mild infections was not also detected in our specimens.

Our results indicate that infection by serotype 1 did not prevent a further infection by serotype 2.

SERUM SAMPLE
COLLECTED AT:

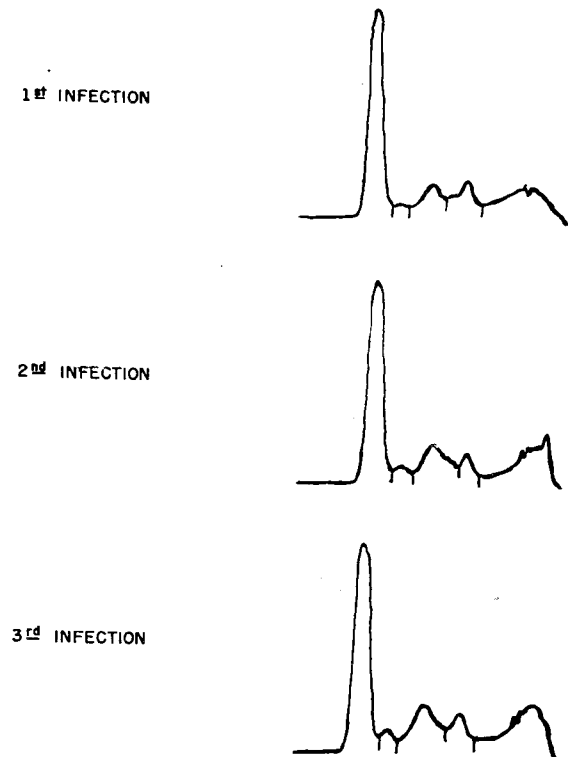


Fig. 2 — Patterns of plasma proteins in serum samples from child n° 23.983.

BISHOP et al³ showed that neonatal rotavirus infection does not confer immunity against reinfection, but does protect against the development of clinically severe disease during reinfection. Our preliminary results, however, suggest that reinfection may give rise to a more severe illness in the second occasion, if compared with the first infection (Reg. 24.004).

In five cases rotavirus serotype was not determined because of lack of protein "Vp7". As faecal specimens were kept frozen for several months until processing for serotyping by using monoclonal antibodies, we believe that viruses may have lost external capsid where this protein is located, so making impossible serotype-specificity.

Four different electrophoretotypes were detected and even the same pattern was observed during reinfections affecting one child. Because of the small number of cases in our preliminary study, we could not correlate serotypes and subgroups (according to the electrophoretic pattern).

It is difficult to explain on an immunological basis, why reinfections should occur. One possible reason is that specific IgA in the human bowel does not last for sufficiently long period. In fact, it seems that this class of Ig and other nonimmune factors are more important than IgG in protecting against rotavirus infections¹⁷.

The examination of plasmatic proteins suggests that children were not immunocompromised. In spite of more accurate immunological studies are necessary, our results led us to postulate that reinfections were not related with a possible immune defect.

Further and broader studies on reinfection by rotavirus serotypes are needed, particularly in relation to the development of an effective rotavirus vaccine for human use.

RESUMO

Reinfecções e sorotipos de rotavírus em Belém, Brasil (nota prévia)

Infecções sucessivas causadas por rotavírus foram detectadas em quatro crianças habitantes de Belém, acompanhadas desde seu nascimento até os três anos de idade. Em uma delas (Reg. 23.983) três infecções sintomáticas foram observadas, duas por sorotipo não especificado e outra pelo 2: a primeira aos 4 meses, a segunda aos 20, e a terceira aos 27. Outro indivíduo (Reg. 24.384) apresentou dois episódios diarreicos, com intervalo de oito meses, o primeiro por sorotipo 1 e o segundo por sorotipo não identificado. Infecções também aparentes foram observadas em duas ocasiões envolvendo a mesma criança (Reg. 24.004), a primeira por sorotipo 1 e, doze meses após, uma segunda pelo 2. Em uma outra criança (Reg. 24.097) duas infecções, ambas inaparentes e por sorotipo não especificado, foram

detectadas: a primeira aos 24 meses de vida e a segunda, aos 28.

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