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# A SEROLOGICAL INVESTIGATION OF ROTAVIRUS INFECTIONS IN A SHANTY TOWN POPULATION IN RIO DE JANEIRO

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## SUMMARY

The presence of antibodies against rotavirus was investigated by enzyme immunosorbent assay (ELISA) in two distinct groups of children living in a shanty town in Rio de Janeiro. One hundred and thirty six plasma samples were randomly collected from children of 0 to 33 months (first group) and 255 serum samples were collected from other 85 children at ages of 2, 6 and 9 months (second group). A high percentage of antibodies were found in the newborn children and this rate decreased progressively until the age of 11 months, after which it increased again. At the age of 7 months, geometric mean antibody titers increased indicating that infection had occurred.

**KEY WORDS:** ELISA; Serology; Rotavirus.

## INTRODUCTION

Rotavirus has been shown to be an important aetiological agent in infantile gastroenteritis in many countries[2,3,8]. Rotavirus diarrhoea is of particular importance in the Third World, where inadequate nutrition and severe dehydration account for a high fatality-rate per year[6].

The presence of rotavirus has been demonstrated in various States of Brazil[5,11,14,18]; faecal excretion of this virus has been observed in different socio-economic groups[12].

Serological investigations in both developed[7,8] and developing[9,10,17] countries have shown that many children acquire antibodies against rotavirus during the first three years of life.

In the present study we have determined the prevalence of rotavirus infection, using the ELISA test, in two distinct groups of children living in a shanty town within Rio de Janeiro.

## MATERIAL AND METHODS

### BLOOD SPECIMENS

The blood specimens were collected from children living in the shanty town of Manguinhos, located within the city of Rio de Janeiro[19]. Blood was collected randomly from 136 children with 0 — 33 months of age (first group) which visited the health unit (Unidade de Treinamento Germano Sinval Faria) for routine vaccination or medical consultation other than gastroenteritis. The specimens were collected between October 1981 and February

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1982. Capillary blood was collected from the finger in heparinized capillary tubes, centrifuged at 1,000g for 10 min. and 100µl plasma were separated and stored at -20°C until the performance of the test.

From other 85 children (second group), 255 serum specimens were collected between July 1982 and August 1983. The specimens were obtained at 2, 6 and 9 months of age. Capillary blood was collected directly into 13x100mm tubes and the serum was separated and stored at -20°C until use.

## ELISA TEST

Antigen: Calf rotavirus was propagated in bottles containing confluent monolayer cultures of MA104 cells. Each bottle was inoculated with virus suspension without serum. After total cytopathic effect, the virus was extracted by freezing (-70°C) and thawing (37°C), treated with fluorocarbon (Freon 113) and then sedimented by centrifugation at 100,000g for 1h at 4°C on 45% sucrose cushion. The sediment, resuspended in 0.01 M Tris-HCl pH 7.4 buffer containing 15 mM $CaCl_2$, was used as antigen.

Determination of rotavirus antibody titers was carried out using different modifications of double-antibody sandwich ELISA[15,16,20].

## RESULTS

The 136 plasmas were divided into four groups according to the age of the children. Fourteen (93.3%) of the 15 samples collected from the young children (0-2 months) had rotavirus antibodies (Table 1). In the age group of 2-6 months the positivity rate fell to 61.2% and the mean geometric titer also decreased for $10^{-2.8}$ to $10^{-2.4}$ (Table 2). The positivity rate continued to decline in the 7 to 11 months age group, but there was a slight increase in mean geometric titer. In the 12 to 23 months group the prevalence of rotavirus antibody increased to 69.8% and the mean geometric titer continued to increase to $10^{-2.9}$ (Tables 1 and 2).

TABLE 1

Distribution according to age and presence of rotavirus antibody of 136 children from a shanty town of Rio de Janeiro

| Age (months) | Number of children | Presence of rotavirus antibody | % with antibody |
|---|---|---|---|
| 0 – 1 | 15 | 14 | 93.3 |
| 2 – 6 | 49 | 30 | 61.2 |
| 7 – 11 | 29 | 15 | 51.7 |
| 12 – 33 | 43 | 30 | 69.8 |
| Total | 136 | 89 | 65.4 |

The 255 sera samples collected from 85 children at ages of 2, 6 and 9 months revealed the presence of rotavirus antibodies in the following manner: 91.8%, 57.6% and 57.6%, respectively. Figure 1 compares the antibody response in two intervals, namely between 2 and 6 months and 6 and 9 months. No antibodies were detected in the sera samples of 8.2% of children collected during the first interval (2-6 months). During the second interval (6-9 months) the percentage of negative samples was 29.4%. There was no difference in antibody titer in 22 children (25.9%) and in 16 children (18.8%) during the first and second

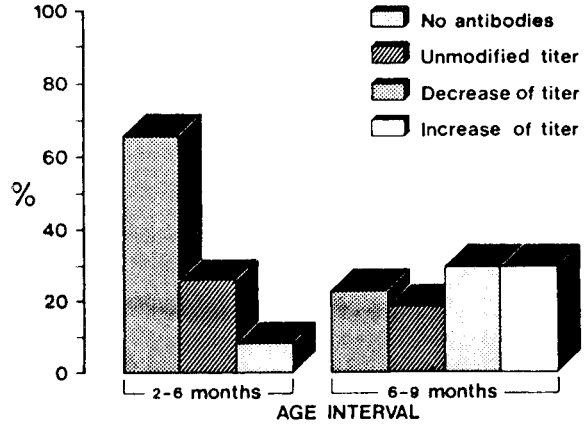
TABLE 2

Distribution according to age and titer of rotavirus antibody of 136 children from a shanty town of Rio de Janeiro

| Age (months) | Titer | | | | | | Mean geometric titer |
|---|---|---|---|---|---|---|---|
| | Neg. (+) | 2.0 | 2.5 | 3.0 | 3.5 | 4.0 | |
| 0 – 1 | 1 | 3 | 2 | 6 | 3 | 0 | 2.8(*) |
| 2 – 6 | 19 | 12 | 13 | 3 | 2 | 0 | 2.4 |
| 7 – 11 | 14 | 2 | 7 | 4 | 2 | 0 | 2.7 |
| 12 – 33 | 13 | 6 | 8 | 4 | 11 | 1 | 2.9 |
| Total | 47 | 23 | 30 | 17 | 18 | 1 | 2.7 |

(+) Negative samples.
(*) Inverse of last dilution found positive (determined by log)

**Figure 1: PROSPECTIVE STUDY OF ROTAVIRUS ANTIBODY IN SERA OF 85 CHILDREN**




interval, respectively. A decrease in antibody titer occurred specially during the first interval (65.9%) versus the 22.4% of the second interval. The increase in antibody titer occurred only in the children between the ages of 6 and 9 months. The titers of these children (29.4%) are compared in Table 3.

TABLE 3

Sera analysis from 25 children with increased antibody titer

| Number of sera | Titer | |
|---|---|---|
| | at 6 months | at 9 months |
| 4 | Neg. | 2.0(*) |
| 5 | Neg. | 2.5 |
| 1 | Neg. | 3.0 |
| 1 | Neg. | 3.5 |
| 5 | 2.0 | 2.5 |
| 2 | 2.0 | 3.5 |
| 6 | 2.5 | 3.0 |
| 1 | 3.0 | 3.5 |

(*) Inverse of last dilution found positive (determined by log)

## DISCUSSION

Studies performed by YOLKEN et al.[20] have shown that ELISA assay is more sensitive for the detection of rotavirus antibodies than complement fixation (CF), immunofluorescence (IF) and neutralization tests. In a study envolving a rotavirus epidemic in a school, it was also demonstrated that ELISA is a sensitive method for the detection of antibodies when compared to CF and counter immuno-electrophoresis[18]. This is the reason for which ELISA was chosen to make this study.

Our results show that in the first two months of life a high percentage of children had antibodies directed against rotavirus, probably of maternal origin. After the second month the incidence of children with these antibodies decreased progressively (Table 1 and Figure 1). Between 7 and 11 months, the percentage of children with antibodies was lower than in the case of younger children. However, the mean geometric titer was higher in this group than the one of 2-6 months (Table 2), suggesting that rotavirus infections occurred during this period. Figure 1 and Table 3 show that some children had small titer increases, between 6 and 9 months, while others had important increases, demonstrating the occurrence of infection. Out of 19 children (22.4%) that had a decrease in antibody titer, 11 became negatives. On the other hand, 25 children (29.4%) showed an increase in antibody titer and from these 11 became positives (Figure 1). The mean geometric titer increases even more during the second year of life (Table 2).

Results obtained by ELIAS in England[7], ISHAK et al. in Goias[9] and LAMPE in Rio de Janeiro[10], have shown that the maximum infection incidence occurred between 12 and 36 months of age. In a serological study performed in a rural area of Bangladesh[17] the highest percentage of sera with antibodies against rotavirus was between 19 and 24 months (95%). In a study with 0-5 year-old children made in Belém, the highest percentage of positivity has been detected in sera from 4 year-old children[13]. Our observations showed that infection occurred after the age of 6 months (Table 3) and the highest antibody titer was observed after the first year (Table 2).

AZEREDO et al.[1], LAMPE[10] and SUTMOLLER et al.[18] have shown that rotavirus is an important aetiological agent in infantile gastroenteritis in Rio de Janeiro. A study carried out with the same population in this shanty town has shown that the number of diarrhoeal episodes associated with rotavirus was relatively low compared to the serological response (AZEREDO et al., unpublished

results). Subclinical rotavirus infection has been demonstrated previously[2,3,4,16] and it seems probable that rotavirus infection occurs frequently in the population referred to in this paper. Therefore we think that the relative importance of rotavirus infection will not be exclusively recognized by studying the faeces of clinical cases.

## RESUMO

**Investigação sorológica de infecção por rotavirus em favela do Rio de Janeiro.**

Cento e trinta e seis plasmas coletados aleatoriamente de crianças entre 0 e 33 meses (primeiro grupo) residentes na favela de Manguinhos, Rio de Janeiro, RJ, e 255 soros coletados de 85 crianças aos 2, 6 e 9 meses de idade (segundo grupo), residentes na mesma área, foram analisados para a presença de anticorpos (classe IgG) para rotavirus por ELISA. Anticorpos foram detectados em alto percentual de crianças recém-nascidas, declinando até o 11º mês. Elevação dos anticorpos foi observada a partir do 12º mês de vida, mas houve evidências de infecção a partir do 7º mês.

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