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SEROLOGICAL EVIDENCE OF ROTAVIRUS INFECTION IN A GUINEA PIG COLONY

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Antibodies reacting with simian rotavirus SA11 were detected by enzyme immunoassay (EIA) and Western blot assay (WBA) in sera from guinea pigs bred for experimental use at the Fundação Oswaldo Cruz, Rio de Janeiro, Brazil. The proportion of antibody-positive animals and the antibody titres rose sharply in 1985, were maintained at a high levels in 1986 and declined in 1987. There were no obvious signs of disease coinciding with serological evidence of infection. Results of WBA suggest that the virus involved belongs to subgroup 1 of group A rotaviruses.

Key words: guinea pig – rotavirus – serology

Susceptibility of guinea pigs to rotaviruses is suggested by serological evidence (e.g. Woode et al., 1976; Panel report, 1978). In our own experience we have found rotavirus antibodies detectable by enzyme immunoassay (EIA) in the pre-immunization serum of only one of 50 guinea pigs bled between January 1981 and December 1984 but in subsequent years we observed a sharp rise followed by a decrease in the proportion of animals showing such antibodies. These results were confirmed by Western blot assay (WBA) and although we failed to demonstrate the presence of virus in the intestinal contents of a few animals tested, we feel that the evidence obtained is convincing enough to deserve reporting.

MATERIALS AND METHODS

The guinea pigs were from a colony maintained in the Oswaldo Cruz Foundation (FIOCRUZ) with an average holding of 5000 guinea pigs. Other experimental animals held in separate rooms included mice, rabbits, hamsters and rats. The mouse colony was shown (unpublished) to be infected with the rotavirus of epidemic diarrhoea of infant mice (EDIM).

Guinea pigs received for experimental use in the Department of Virology were bled from the heart and assayed for rotavirus antibodies before immunization.

Enzyme immunoassays (EIA) were performed in polystyrene microtitre plates with previously described reagents (Pereira et al., 1985) adapted for a double sandwich antibody assay in which rotavirus SA11 antigen was added to wells coated with goat anti-SA11 serum, followed by serial dilutions of guinea pig sera under test, followed by rabbit anti-guinea pig IgG conjugated with horseradish peroxidase and detection with tetra-methyl-benzidine.

Western blot assays (WBA) were performed as described by Towbin et al. (1979) using purified rotavirus SA11 as antigen.

RESULTS

The Table and Fig. 1 show the results of EIA and WBA. The most striking findings are the rise in the proportion of antibody-positive sera in 1985, the persistence of antibodies in 1986 and their decline in 1987.

There were few discrepant results when the two assays were compared. Of the 120 sera tested, 41 were positive and 65 were negative in both assays (90% agreement) but 12 were positive by WBA and negative by EIA. The strongest reaction observed in WBA was given by the major group-specific inner capsid protein VP6 (see Fig. 2). All but 7 of 58 positive sera showed this reaction. Another internal protein (VP2) was revealed by 19 of the positive sera, this being the only antigen

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TABLE

Rotavirus antibodies detected by EIA and WBA in guinea pig sera collected between 1981 and 1987

Year	Number of sera	EIA titres*			WBA			
		<100	100-1000	>1000	negative	VP2 only	VP6 only	VP2+ VP6
1981	27	27	0	0	19	4	3	1
1982	7	6	1	0	6	0	1	0
1983	7	7	0	0	7	0	0	0
1984	9	9	0	0	9	0	0	0
1985	11	3	4	4	1	1	2	7
1986	24	2	13	9	2	21	17	4
1987	33	24	7	2	20	1	12	0

* Reciprocal of serum dilution edpoint.

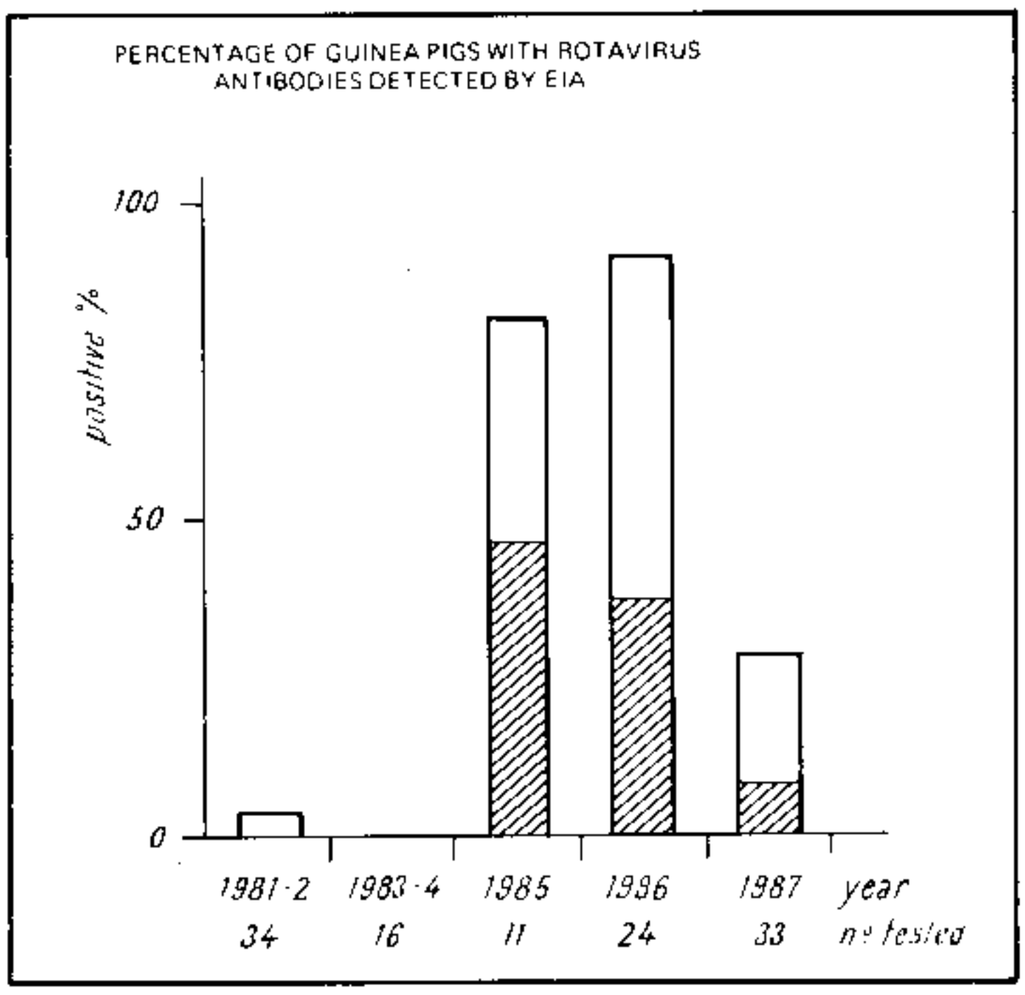

Fig. 1: proportions of guinea pigs with rotavirus antibodies in different years. Hatched columns: sera positive by both WBA and EIA. Empty columns: sera positive by WBA only.

detected by 7 of these sera whereas the remaining 12 reacted with both VP6 and VP2. Only 2 of the 53 positive sera reacted with the type-specific outer capsid glycoprotein VP7 but this reaction was too faint to be considered significant. Of the 12 sera that were positive by WBA only, 2 reacted with both VP2 and VP6, 3 with VP2 only and 7 with VP6 only. These reactions were unequivocal and not significantly different from those observed with sera that were positive in both assays. WBA was therefore slightly more sensitive than EIA.

No signs of disease or any abnormality were reported by the animal handlers amongst the guinea pigs at any time during the period of study.

DISCUSSION

Our results suggest the occurrence of a wave of rotavirus infection without obvious signs of disease, lasting at least two years, with a peak between 1985 and 1986. The detection of anibodies in a small proportion of animals before 1985 suggests that infection may have been present at low level before that time. The first animal with high antibody level was bled in January 1985 but the exact time when the incidence of infection started to rise cannot be established as the precise dates of the immediately preceding bleedings were not recorded.

The antigen most frequently revealed by WBA was the major inner capsid protein VP6 which carries sub-group specific antigenic determinants (Greenberg et al., 1983). As the simian SA11 strain used as antigen in this assay belongs to subgroup 1 of group A rotaviruses our findings suggest that a rotavirus of this subgroup was responsible for the epidemic. The fact that some sera reacted only with VP2 may mean that an additional rotavirus may have been involved. It is noticeable that reactions with VP2 only were particularly common in 1981, i. e. well before the start of the major wave of infection. However no conclusion can be reached on the exact nature of the virus or viruses involved purely on serological evidence. Attempts to demonstrate the presence of rotavirus in the guinea pig colony and to correlate it or not with disease are in progress.

Our results point to the need for monitoring guinea pig colonies for rotavirus infection, especially when these animals are used for the preparation of immune sera.

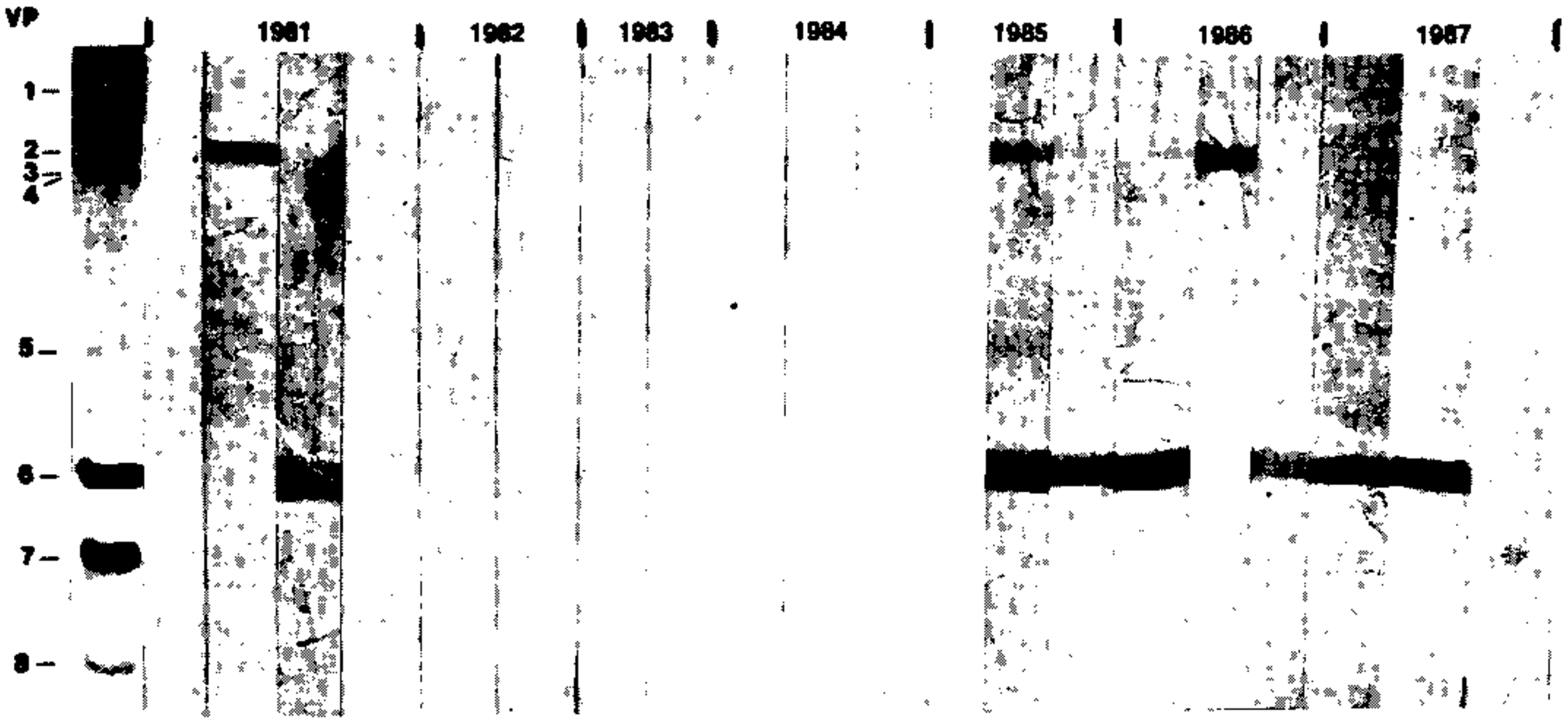

Fig. 2: Western blot assays in guinea pig sera representative of different years. The first lane from left shows the polypeptides of purified rotavirus SA11 fractionated by polyacrylamide gel electrophoresis and stained with Coomassie blue. Remaining lanes show WBA patterns of representative sera colected during the years indicated.

RESUMO

Evidência sorológica de infecção por rotavírus em uma colônia de cobaios – Anticorpos reagindo com rotavírus símio SA11 foram demonstrados por ensaio imuno-enzimático (EIE) e por "Western blot assay" (WBA) em soros de cobaios mantidos para fins experimentais na Fundação Oswaldo Cruz, Rio de Janeiro, Brasil. A proporção de animais soro-positivos e os níveis de anticorpos subiram rapidamente em 1985, mantiveram-se altos em 1986 e baixaram em 1987. Não foram observados sinais de doença coincidente com a elevação de anticorpos. Resultados de WBA sugerem que o rotavírus responsável pela resposta sorológica pertence ao subgrupo 1 do grupo A.

Palavras-chave: rotavírus – cobaios – sorologia

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