CHARACTERIZATION OF ROTAVIRUSES ISOLATED FROM PIGS WITH DIARRHOEA IN VENEZUELA

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ABSTRACT

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The prevalence of porcine rotavirus infection was studied in 15 different herds located in the north-western region of Venezuela. The presence of rotavirus was studied by direct electron microscopy (EM) and by an enzyme-linked immunosorbent assay (ELISA). From 136 samples analyzed during the six months of the study (September 1983—February 1984), 38 (27.9%) were found to be positive for rotaviruses, with infection more common in animals that were 4—6 weeks old. Atypical rotaviruses were not detected in any of the samples examined. Most rotavirus positive specimens were subgrouped using specific monoclonal antibodies in an ELISA test. The majority of the samples (26 out of 38) were found to exhibit Subgroup I antigenicity. Only two specimens, collected from the same herd in two consecutive months, were found to belong to Subgroup II. To characterize further the circulating rotaviruses, electrophoretic analysis of the RNA genome was performed on samples selected from nine different herds. Great variability in the RNA electropherotypes was observed. No correlation was found between subgroup specificity and the migration of the two smaller segments (Genes 10 and 11), as has been described for human rotaviruses.

INTRODUCTION

Rotaviruses have emerged as an important group of viruses etiologically associated with neonatal diarrhoea in a variety of animal species, including man (Flewett and Woode, 1978; Babiuk et al., 1985). Rotaviruses have been detected in faeces from diarrhoeic piglets in many parts of the world, and probably have a worldwide distribution. Available information indicates that

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infection is very common, with most animals becoming infected early in life. It has recently been reported that rotaviruses were associated with 21% of the cases of porcine diarrhoea in Venezuela, being frequently observed in pigs that were 2—6 weeks old (Utrera et al., 1984). In addition, most adult pigs showed serological evidence of previous rotavirus infection.

Rotaviruses possess a genome composed of 11 segments of double-stranded RNA, surrounded by two concentric protein shells formed by at least six structural polypeptides (Esparza et al., 1984). Current research focuses on the identification of antigenic variations among rotavirus isolates (WHO Scientific Committee on Viral Diarrheas, 1984). The major polypeptide of the inner shell (p45K) carries a group-specific antigen, which is common among rotaviruses isolated from different animal species. However, the same polypeptide also carries one of two subgroup specificities (I or II) which is easily detected by using subgroup-specific monoclonal antibodies (Greenberg et al., 1983). Neither the group-common nor the subgroup-specific anti-genicities are found on some "atypical" rotaviruses ("pararotaviruses") which have been isolated from different animal species, including pigs (Bohl et al., 1982; Bridger et al., 1982). Subgroup antigens do not appear to be related to neutralization or immunoprotection, but they provide useful markers for epidemiological studies. The other important antigen of rota-viruses appears to be located on the outer-shell glycoprotein (p38K), is re-lated to the neutralization reaction, and defines the different rotavirus sero-types. Thus far, seven different serotypes have been described, and recent findings have demonstrated that certain human and animal rotaviruses may share subgroup or serotype antigens (Hoshino et al., 1984).

Bohl et al. (1984) reported the existence of at least two different sero-types among porcine isolates, with the O.S.U. strain as the prototype of what they called "porcine Serotype 1", and the Gottfried strain as the proto-type of "porcine Serotype 2". The same antigenic difference was found by Hoshino et al. (1984), with the Gottfried strain belonging, in their classifica-tion, to rotavirus Serotype 4 (together with some human isolates), and the O.S.U. strain to their Serotype 5 (together with equine rotavirus H-1). It should be mentioned that the O.S.U. strain shows a Subgroup I specificity, whereas the Gottfried strain is a Subgroup II virus. However, various com-binations of subgroup and serotype specificities can be expected as a result of the segmented nature of the viral genome. It is known that Gene 6 codes for the 45K protein, and that one of the genes in the triplet 7-8-9 codes for 38K glycoprotein (Mason et al., 1983). In vivo reassortment of such genes may result in the generation of new antigenic variants, a phenomenon that can be studied by analyzing the electrophoretic patterns of genomic RNA (Estes et al., 1984).

In this paper results of the subgroup characterization and RNA electro-pherograms of porcine rotaviruses identified in herds with a neonatal diar-rhoea problem are presented. The role of pathogenic *Escherichia coli* in the etiology of such neonatal diarrhoea has also been studied (García et al., 1986).

MATERIALS AND METHODS

Source of specimens

A total of 136 faecal samples were collected between September 1983 and February 1984 from diarrhoeic piglets belonging to 15 different herds located within a 6-mile radius in the north-western region of Venezuela (Mara county of the Zulia State). In general, herds were of small size, with poor sanitary conditions and with continuous problems of neonatal diarrhoea. Samples were obtained only from diarrhoeic piglets 1—6 weeks of age, and only one specimen was obtained from each animal during the study period.

Electron microscopy

Faecal samples were diluted 1:5 in distilled water, placed on formvar carbon-coated copper grids, stained with 2% potassium phosphotungstate (pH 6.8) and examined with a JEOL 100-B electron microscope (EM) at 60 kV at a magnification of 27 000 ×.

Detection of rotavirus antigens by enzyme-linked immunosorbent assay (ELISA)

Rotavirus antigens were demonstrated in stools by a confirmatory ELISA test (Kapikian et al., 1979). For this, polyvinyl chloride microtiter plates were coated with an optimal dilution of "capture" serum obtained before or after immunization of a rabbit with CsCl-purified double-shelled O.S.U. rotavirus (Theil et al., 1977). Faecal suspensions (10%) were incubated in duplicate wells precoated with either of the two "capture" sera. A hyperimmune serum from mice immunized with the O.S.U. rotavirus was used as detection antibody. The conjugate was horseradish peroxidase linked anti-mouse immunoglobulin, using 2,2'-azino-di-3-ethylbenzthiazoline sulphonate (ABTS) as substrate (Amersham International, code N. 931).

Rotavirus subgrouping

Subgrouping of positive samples was performed by ELISA essentially as described by White et al. (1984), using hybridoma fluids specific for Subgroup I (255/60) or Subgroup II (631/9), generously provided by Dr. H. Greenberg (Greenberg et al., 1983).

Electrophoresis of genomic RNA

Samples selected for electropherotyping were resuspended in phosphate buffered saline, extracted with trichlorotrifluoroethane (Freon 113) and pelleted at 100 000 × g for 1 h through a 45% sucrose cushion. For RNA extraction, partially-purified viruses were resuspended in 1% sodium dodecyl

sulfate (SDS) and incubated at 55°C for 1 h, followed by phenol-chloroform extraction. Samples were electrophoresed in 10% polyacrylamide slab gels, using the discontinuous buffer system described by Laemmli (1970). Electrophoresis was conducted at 30 mA (140 V) for 16 h at room temperature. RNA bands were stained with silver by the method of Offit et al. (1983).

RESULTS

Pattern of rotavirus excretion

Of the 136 samples analyzed during the 6 months of the study, 38 (27.9%) were found to be positive for rotaviruses, as assayed by ELISA test or by direct EM observation (Table I). Each sample was studied by both methods, in attempts to identify antigenically distinct "atypical" rotaviruses. These are morphologically similar to "classical" rotaviruses, but lack the group-specific antigen detectable by the ELISA test. Overall, the ELISA test proved to be more sensitive than routine examination of samples by EM. Of the 38 positive samples, only 28 were easily diagnosed by EM, whereas no sample positive by EM was negative by ELISA. Atypical rotaviruses were not detected in the specimens examined. At variance with the early infection observed with pathogenic *E. coli* (Garcia et al., 1986), rotavirus infection was found to be more prevalent in animals that were 4—6 weeks old (Table I).

TABLE I

Frequency of rotavirus excretion in relation to age

Age group (days)	Positive/total	Positive (%)
0— 7	0/4	0
8—15	3/45	6.7
16—23	10/33	30.3
24—30	15/31	48.4
31—37	8/18	44.4
≥37	2/5	40.0
Total	38/136	27.9

Subgroup characterization

The distribution of rotavirus-positive samples collected from diarrhoeic piglets from the 15 different herds is presented in Fig. 1. Most specimens were also subgrouped using monoclonal antibodies in an ELISA test. The majority of the samples (26 of 28 samples which could be subgrouped; 93%) were found to exhibit Subgroup I reactivity. Only two specimens (7%) collected from different animals in the same herd (No. 12) in two con-

Fig. 1. Distribution of rotavirus positive samples from diarrhoeic piglets from 15 different herds located in north-western Venezuela. Herds within brackets (2, 3, 4 and 10, 11, 12) are located in close vicinity. Empty squares represent rotavirus-negative samples. Shaded squares represent rotavirus-positive samples, indicating also in some cases the subgroup antigenicity (I or II). Superscript letters (A—I) on subgrouped rotavirus-positive samples are used to indicate individual samples analyzed on Figs. 2 and 3.

secutive months (January—February, 1984) were found to belong to Subgroup II.

RNA electropherotypes

To characterize further the circulating rotaviruses, RNA electrophoretic analysis was performed on samples selected from nine different herds. Comparison of electropherotypes was made with a standard RNA, extracted from the O.S.U. strain of porcine rotavirus (Fig. 2). The diversity of electropherotypes present in a relatively closed population is evident. With the possible exception of the two Subgroup II rotaviruses, for which comparisons were difficult due to the scarcity of one of the samples, no two electropherotypes were identical. With regard to the mobilities of the two smaller genes (10 and 11), all of the samples, regardless of subgroup specificity, showed similar patterns of migration, in contrast to the "short" (Subgroup I) and the "long" (Subgroup II) patterns described for human rotaviruses (Kalica et al., 1981).
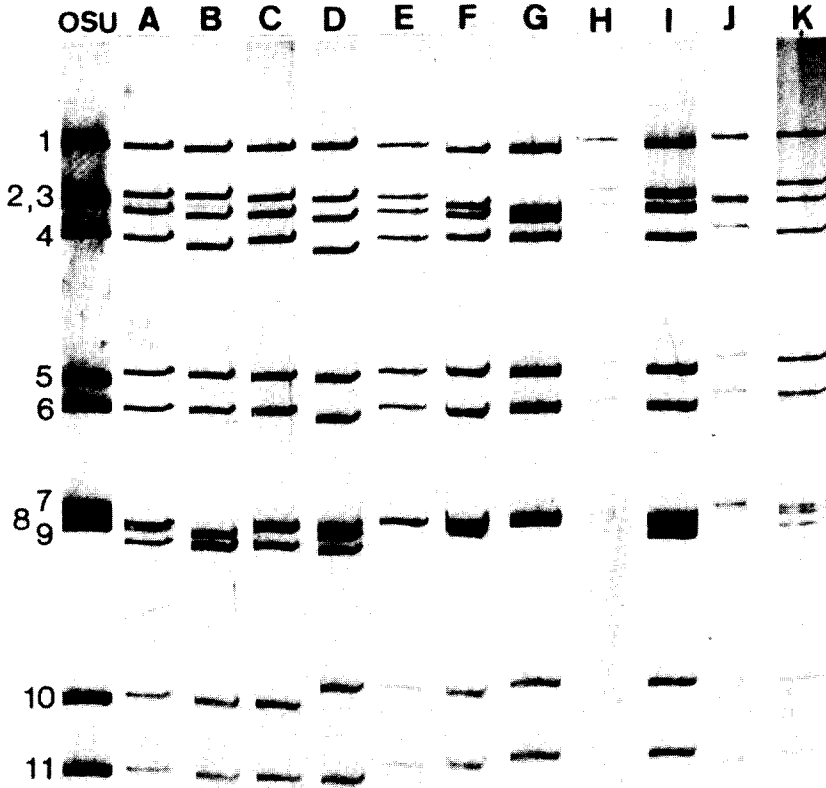
Fig. 2. Comparison of migration patterns of porcine rotavirus electropherotypes in 10% polyacrylamide slab gels, stained with silver. OSU, standard RNA extracted from the OSU strain of porcine rotavirus. Lanes A—K, electropherograms of different samples of porcine rotavirus. Each letter indicates the corresponding sample on Fig. 1. Samples A—G, J and K correspond to the Subgroup I rotaviruses, and Samples H and I correspond to the two Subgroup II viruses.

A better comparison of different electropherotypes can be made by co-electrophoresis of the two samples. Such analysis of six selected samples is shown in Fig. 3. The first co-electrophoresis, performed with samples obtained during October 1983 from two neighbouring farms (Samples B and C), showed doublet bands corresponding to different mobilities of Genes 4, 7 and 8. When Sample C was compared with a specimen obtained the following month, from a more distant herd (Sample D), differences in mobility were observed in Bands 4, 6, 8 and 10, suggestive of a more distant genetic relationship. The same observation was made when comparing Sample D (Herd 4, November 1983) with Sample F (Herd 2, January 1984), in which migration heterogeneity was observed in at least five bands (2, 4, 8, 9 and
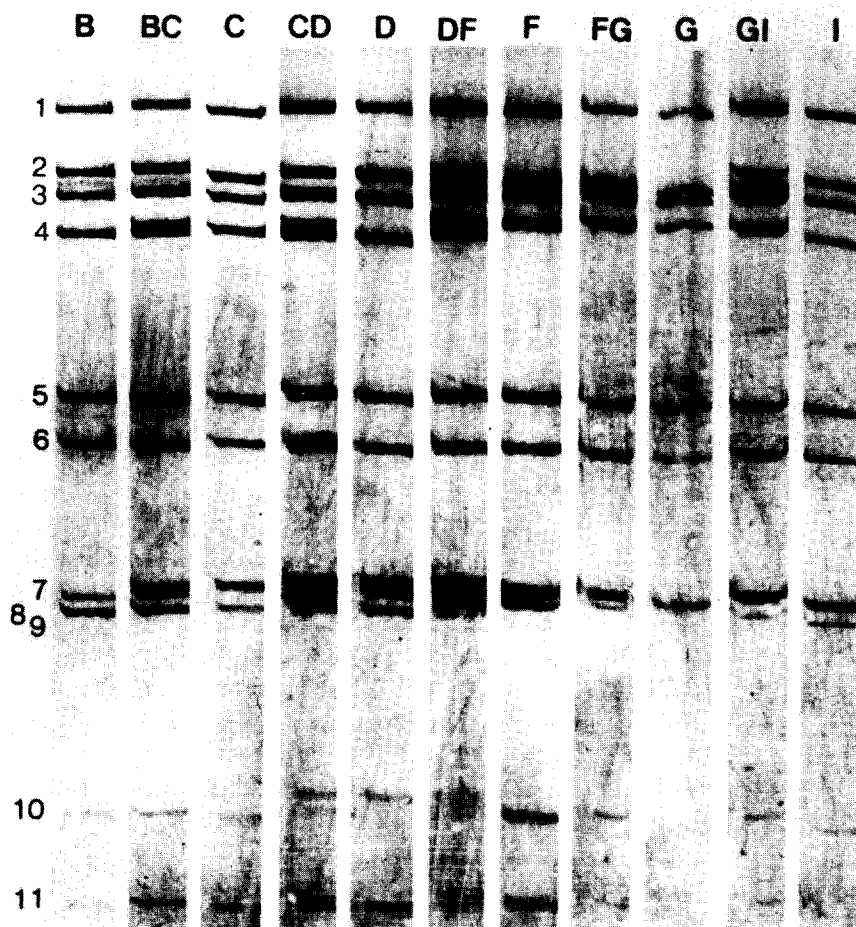
Fig. 3. Comparison by coelectrophoresis of selected samples from Fig. 2.

10). Comparison of epidemiologically related samples, such as F (Herd 2, January 1984) and G (Herd 2, February 1983) revealed changes in only one band, namely Gene 2. The electrophoretic comparison of Sample G (Subgroup I) with one of the two Subgroup II specimens (Sample I, from Herd 12, February 1984) revealed changes only in Bands 2 and 4.

DISCUSSION

Results presented in this paper confirm an earlier report of the high prevalence of porcine rotavirus infection in Venezuela (Utrera et al., 1984), comparable to the prevalence rates reported by others in temperate regions of the world. Bohl et al. (1978) have suggested that rotaviral infections are endemic in conventional swine herds, and Debouck et al. (1984) presented

evidence indicating that nearly all pigs examined had shed rotavirus before the fifth week of life. In their study they observed frequent subclinical rotavirus infections in suckling pigs, but in $\geqslant$ 50% of the diarrhoeic samples, rotavirus was the only enteropathogenic agent detected.

Rotavirus infection of humans in temperate countries occurs more frequently during the winter months, whereas in domestic animals they are more frequent at the times when a large number of susceptible individuals are born, usually during late winter or early spring (Babiuk et al., 1985). The age distribution of the rotavirus infection found in this study corresponds to that reported by others (Bohl et al., 1978; Utrera et al., 1984); very young animals appear to be protected by lactogenic immunity. However, Debouck et al. (1984) has reported porcine rotavirus excretion during the first days after birth, an observation that may have a parallel with the mild or asymptomatic rotavirus infections frequently observed in human newborns (Chrystie et al., 1978).

The recent identification of the atypical rotaviruses has been of considerable interest. At least three different strains have been studied in pigs: a Belgian isolate (Debouck and Peusaert, 1979), one from the United Kingdom (Bridger et al., 1982), and one from the United States (Saif et al., 1980; Bohl et al., 1982). A comparison of the U.K. and U.S.A. strains of atypical porcine rotaviruses revealed that they were unrelated as shown by cross-immunofluorescence studies (Pedley et al., 1983). That observation, also supported by genome profile and terminal fingerprint analysis, led to proposals for the existence of at least three separate rotavirus groups: A, that includes the classical rotaviruses, such as the O.S.U. strain; B, atypical rotaviruses with the group antigen of the British NIRO-1 strain; and C, atypical rotaviruses with the group antigen of the American Cowden strain. Antigenic differences among human atypical rotaviruses have also been reported (Puerto et al., 1984). Examination of porcine field sera indicate that rotaviruses belonging to all three antigenic groups are prevalent in pigs in the United Kingdom (Bridger et al., 1984). Moreover, Debouck et al. (1984), in Belgium, reported the frequent excretion of atypical rotaviruses in piglets over 18 days of age, most cases being subclinical infections. We were unable to detect atypical (Group B and C) rotaviruses in the diarrhoeic samples examined. All faecal samples containing rotavirus particles demonstrated the presence of Group A (O.S.U.) antigens by ELISA. Confirmation was made by genomic RNA electrophoresis, since atypical rotaviruses have very characteristic RNA electropherotypes. Group A rotavirus belonging to the two subgroup antigenic specificities were demonstrated, with most samples being classified as Subgroup I. Recent work in our laboratory (Palencia et al., unpublished observations) indicates that herds located in the same region may have both kinds of viruses circulating and even infecting the same animal.

The great variability in the RNA electropherotypes of the presently reported isolates could have two possible explanations. One is that rotaviruses do not readily spread from herd to herd, as suggested for human strains

of rotaviruses (Nicolas et al., 1984). The second explanation for the electropherotypic variation is based on a high mutation rate of the RNA polymerase, which could introduce point mutations, resulting in different mobilities of the RNA segments. An additional mechanism for rapid genetic evolution could be mediated by gene segment reassortments, a phenomenon that has been documented in vitro for rotaviruses (Greenberg et al., 1981). In favour of the point mutation hypothesis are the data recently presented by Konno et al. (1984), showing that at the beginning of a human rotavirus epidemic a single electropherotype is found, whereas later a great number of different electropherotypes are present. The authors suggested that a single strain of rotavirus is first introduced into a community, causing an outbreak of infection, and then changes under selective pressure so that genetically different strains emerge. The endemic nature of rotavirus infections in the tropics hampers the detection of "original" electropherotypes.

There is a correlation between "short" and "long" RNA patterns within Subgroups I and II of human rotaviruses (Kalica et al., 1981). It has been reported that all Subgroup I viruses possess the same serotype (1), whereas Subgroup II may belong to any of the other serotypes described for human rotaviruses (2, 3 and 4) (WHO Scientific Committee on Viral Diarrheas, 1984). The same may not be true for porcine rotaviruses (Hoshino et al., 1984). On the other hand, a complex pattern of one-way cross-reactivities has been found among human and animal rotaviruses (Bohl et al., 1984; Hoshino et al., 1984), making it difficult at the present time to present a definitive classification of serotypes, at least among animal rotaviruses.

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REFERENCES

Babiuk, L.A., Sabara, M. and Hudson, G.R., 1985. Rotavirus and coronavirus infections in animals. Prog. Vet. Microbiol. Immunol., 1: 80—120.
Bohl, E.H., Kohler, E.M., Saif, L.J., Cross, R.F., Agnes, A.G. and Theil, K.W., 1978. Rotavirus as a cause of diarrhea in pigs. J. Am. Vet. Med. Assoc., 172: 458—463.
Bohl, E.H., Saif, L.J., Theil, K.W., Agnes, A.G. and Cross, R.F., 1982. Porcine pararotavirus: detection, differentiation from rotavirus and pathogenesis in gnotobiotic pigs. J. Clin. Microbiol., 15: 312—319.
Bohl, E.H., Theil, K.W. and Saif, L.J., 1984. Isolation and serotyping of porcine rotaviruses and antigenic comparison with other rotaviruses. J. Clin. Microbiol., 19: 105—111.
Bridger, J.C., Clarke, I.N. and McCrae, M.A., 1982. Characterization of an antigenically distinct porcine rotavirus. Infect. Immun., 35: 1058—1062.

Bridger, J.C., Brown, J.F., Arnold, P.S., Pocock, D.H. and McCrae, M.A., 1984. Antigenic variation in porcine and bovine rotaviruses. In: S.D. Acres (Editor), Proceedings on the Fourth International Symposium on Neonatal Diarrhea, 3—5 October 1983. Veterinary Infectious Disease Organization, Saskatoon, Canada, pp. 48—55.

Chrystie, I.L., Totterdell, B.M. and Banatvala, J.E., 1978. Asymptomatic endemic rotavirus infections in the newborn. Lancet, 1: 1176.

Debouck, P. and Pensaert, M., 1979. Experimental infection of pigs with Belgian isolates of the porcine rotavirus. Zbl. Vet. Med. B., 26: 517—526.

Debouck, P., Callebaut, P. and Pensaert, M., 1984. The pattern of rotavirus and pararotavirus excretions in pigs on closed swine herds. In: S.D. Acres (Editor), Proceedings of the Fourth International Symposium on Neonatal Diarrhea, 3—5 October 1983; Veterinary Infections Disease Organization, Saskatoon, Canada, pp. 77—87.

Esparza, J., Gorziglia, M. and Liprandi, F., 1984. Studies on the molecular biology of rotaviruses: a review. In: S.D. Acres (Editor), Proceedings of the Fourth International Symposium on Neonatal Diarrhea, 3—5 October, 1983. Veterinary Infectious Disease Organization, Saskatoon, Canada, pp. 1—26.

Estes, M.K., Graham, D.Y. and Dimitrov, D.H., 1984. The molecular epidemiology of rotavirus gastroenteritis. Prog. Med. Virol., 29: 1—22.

Flewett, T.H. and Woode, G.N., 1978. Rotaviruses. Arch. Virol., 57: 1—25.

García, D., Cavazza, M-E., Botero, L., Gorziglia, M., Urbina, G., Liprandi, F. and Esparza, J., 1986. Preliminary characterization of *Escherichia coli* isolated from pigs with diarrhoea in Venezuela. Vet. Microbiol., in press.

Greenberg, H.B., Kalica, A.R., Wyatt, R.G., Jones, R.W., Kapikian, A.Z. and Chanock, R.M., 1981. Rescue of noncultivable human rotavirus by gene reassortment during mixed infection with *ts* mutants of a cultivable bovine rotavirus. Proc. Natl. Acad. Sci. USA, 78: 1420—1424.

Greenberg, H.B., McAuliffe, V., Valdesuso, J., Wyatt, R.G., Flores, J., Kalika, A.R., Hoshino, Y. and Sing, N., 1983. Serological analysis of the subgroup protein of rotaviruses, using monoclonal antibodies. Infect. Immun., 39: 91—99.

Hoshino, Y., Wyatt, R.G., Greenberg, H.B., Flores, J. and Kapikian, A.Z., 1984. Serotypic similarity and diversity of rotaviruses of mammalian and avian origin as studied by plaque-reduction neutralization. J. Infect. Dis., 149: 694—702.

Kalica, A.R., Greenberg, H.B., Espejo, R.T., Flores, J., Wyatt, R.G., Kapikian, A.Z. and Chanock, R.M., 1981. Distinctive ribonucleic acid patterns of human rotavirus subgroups 1 and 2. Infect. Immun., 33: 958—961.

Kapikian, A.Z., Yolken, R.H., Greenberg, H.B., Wyatt, R.G., Kalika, A.R., Chanock, R.P. and Kim, H.W., 1979. Gastroenteritis virus. In: E.H. Lennette and N.J. Schmidt (Editors), Diagnostic Procedures for Viral, Rickettsial and Chlamydial Infections, 5th Edn. American Public Health Association, Washington, pp. 927—995.

Konno, T., Sato, T., Suzuki, H., Kitaoka, S., Katsushima, M., Sakamoto, M., Yazaki, N. and Ishida, N., 1984. Changing RNA patterns in rotaviruses of human origin: demonstration of a single dominant pattern at the start of an epidemic and various patterns thereafter. J. Infect. Dis., 149: 683—687.

Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227: 680—685.

Mason, B.B., Graham, D.Y. and Estes, M.K., 1983. Biochemical mapping of the simian rotavirus SA 11 genome. J. Virol., 46: 413—423.

Nicolas, J.C., Pothier, P., Cohen, J., Lourenco, M.H., Thompson, R., Guimbaud, P., Chenon, A., Dauvergne, M. and Bricout, F., 1984. Survey of human rotavirus propagation as studied by electrophoresis of genomic RNA. J. Infect. Dis., 149: 688—693.

Offit, P.A., Clark, H.F., Stroop, W.G., Twist, E.M. and Plotkin, S.A., 1983. The cultivation of human rotavirus, strain "Wa", to high titer in cell culture and characterization of the viral structural polypeptides. J. Virol. Methods, 7: 29—40.

Pedley, S., Bridger, J.C., Brown, J.F. and McCrae, M.A., 1983. Molecular characterization of rotaviruses with distinct group antigens. J. Gen. Virol., 64: 2093—2101.

Puerto, F., Avendaño, L.F., Esparza, J. and Caul, E.D., 1984. Antigenic variation in Latin American pararotaviruses (atypical rotaviruses). J. Clin. Pathol., 37: 1416—1417.

Saif, L.J., Bohl, E.H., Theil, K.W., Cross, C.F. and House, J.A., 1980. Rotavirus-like, calicivirus-like, and 23 mm virus-like particles associated with diarrhea in young pigs. J. Clin. Microbiol., 12: 105—111.

Theil, K.W., Bohl, E.H. and Agness, A.G., 1977. Cell culture propagation of porcine rotavirus (reovirus-like agent). Am. J. Vet. Res., 38: 1765—1768.

Utrera, V., Mazzali de Ilja, R., Gorziglia, M. and Esparza, J., 1984. Epidemiological aspects of porcine rotavirus infection in Venezuela. Res. Vet. Sci., 36: 310—315.

White, L., Pérez, I., Pérez, M., Urbina, G., Greenberg, H., Kapikian, A. and Flores, J., 1984. Relative frequency of rotavirus subgroups 1 and 2 in Venezuelan children with gastroenteritis as assayed with monoclonal antibodies. J. Clin. Microbiol., 19: 516—520.

W.H.O. Scientific Committee on Viral Diarrheas, 1984. Nomenclature of human rotaviruses: designation of subgroups and serotypes. Bull. W.H.O., 62: 501—503.