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Antigenic Characterization of Swine Rotaviruses in Argentina

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Fecal samples from 156 diarrheic piglets were collected from several herds located in two main breeding areas of Argentina. Rotaviruses were detected in 60 samples (38.4%) by polyacrylamide gel electrophoresis and in 55 samples by a group A-specific enzyme-linked immunosorbent assay (ELISA). All samples which were positive by polyacrylamide gel electrophoresis and negative by ELISA had elicited atypical electropherotypes resembling those of group B or C. ELISA-positive samples showing genome rearrangements were also detected (R. C. Bellinzoni, N. M. Mattion, O. R. Burrone, S. A. González, J. L. La Torre, and E. A. Scodeller, J. Clin. Microbiol. 25:952–954, 1987; N. M. Mattion, S. A. González, O. R. Burrone, R. C. Bellinzoni, J. L. La Torre, and E. A. Scodeller, J. Gen. Virol. 69:695–698, 1988). By subgrouping with monoclonal antibodies, it was found that of 32 positive samples, 13 belonged to subgroup I, 2 belonged to subgroup II, 2 samples had both specificities, and 15 samples were neither subgroup I nor subgroup II (non-I/II). In addition, 10 samples were adapted to grow in tissue culture, cloned, and serotyped by means of neutralization assays. Two samples were classified as serotype 5, and none of them were classified as serotype 4. The other strains showed only a one-way relationship with serotype 5 and can be tentatively classified as new porcine serotypes. Two samples with rearranged genomes had a one-way relationship with antiserum to human strain 69M, which displays a supershort electropherotype and was classified as a new human serotype (S. Matsuno, A. Hasegawa, A. Mukoyama, and S. Inouye, J. Virol. 54:623–624, 1985). At one farm, similar rearranged strains were detected during three successive years. Serotype changes were found between the isolates of the first and the second year, suggesting that a high degree of antigenic variability went on during continuous circulation of these strains in the field.

Rotaviruses are one of the major etiological agents of acute viral gastroenteritis in the young of a large number of animal species and humans (6, 27). In previous studies, it has been demonstrated that rotavirus infections are common in swine (2, 18, 25, 26). Two serotypes of porcine rotaviruses have been described and extensively characterized (4, 13). One of these strains had been designated Gottfried strain and was included together with human rotaviruses within serotype 4. The other one was designated OSU strain and is not related to any of the existing human serotypes, and it was included together with equine rotaviruses, within serotype 5. Recently, the presence of new porcine serotypes has been reported (19, 20).

Early serological studies indicated that rotaviruses recovered from different species shared a common group antigen (24, 27). However, since 1980, rotaviruses lacking the common group antigen were isolated from humans and animals and several antigenically distinct serogroups (B, C, etc.) of these atypical rotaviruses have been reported. Swine rotaviruses belonging to groups A, B, C, and E have been described (3, 21–23). A growing body of evidence indicates that atypical rotaviruses are very common in pigs, and antibodies against groups B and C are usually detected in adult animals (5).

Group A rotaviruses are further divided into subgroups. Two subgroups have been demonstrated for humans (8, 14), and the existence of a unique avian subgroup antigen was suggested (13). Recently an equine group A rotavirus carrying both subgroup I and II antigens (11) and an equine rotavirus (strain H-2) with both subgroup specificities have been recognized (13).

This report describes the genomic and antigenic characteristics of different swine rotaviruses recently isolated in Argentina.

One hundred and fifty-six stool samples from diarrheic piglets (1 to 45 days old) were obtained from nine swine herds; five of the herds were located in the province of Buenos Aires, and four were located in the province of Córdoba. Viral double-stranded RNA was extracted from feces of diarrheic piglets or from infected cell culture fluids as previously described (1). The RNA was electrophoresed on 10% polyacrylamide gels (15) and stained with silver nitrate as described by Herring et al. (10). Samples were processed for enzyme-linked immunosorbent assay (ELISA) with polyclonal group A-specific rotavirus antiserum as previously described (22). For adaptation of viruses to tissue culture, monolayers of MA104 cells were grown in tubes (10^5 cells) or in 25-cm^2 flasks (10^6 cells) at 37°C in Glasgow minimal essential medium (GIBCO Laboratories) supplemented with 10% fetal calf serum, 10% tryptose phosphate broth, and antibiotics. Before inoculation, monolayers were washed three times with phosphate saline. Ten-percent suspensions of feces were pretreated with 20 μg of trypsin per ml at 37°C for 30 min and inoculated on top of the monolayers. After 1 h of incubation, the inoculum was discarded and the cells were incubated with serum-free growth medium containing 2 μg of trypsin or pancreatin per ml. The tubes were incubated for 48 to 72 h on a roller drum. Cell cultures were then frozen and thawed three times, and several (7 to 10) additional subpassages were performed as described above, using undiluted cell culture fluids as inocula. Inoculated monolayers were examined for cytopathic effect at each passage or were tested by the immunoperoxidase technique (17). In all cases, the isolates were cloned four times by plaque-to-plaque dilution or by the limited dilution method and passaged at a low multiplicity of infection (0.1 PFU per cell).

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TABLE 1. Presence of rotavirus in fecal samples of diarrheic piglets as detected by PAGE and ELISA

Province	No. of samples	No. positive by:		Total (%) positive samples
		PAGE	ELISA	
Córdoba	23	10	6	10 (43.4)
Buenos Aires	133	50	49	50 (37.5)
Total	156	60	55	60 (38.4)

The following viruses were used for antisera production: OSU and Gottfried strains (kindly provided by K. Theil, Ohio State University, Columbus, Ohio), CC86, CN86, C60 and C134 local strains (17), and the human strain 69M (9) (kindly provided by Mary Estes, Baylor College of Medicine, Houston, Tex.). Hyperimmune antisera were prepared in seronegative guinea pigs by intramuscularly injecting 10 μg of the corresponding purified virus emulsified with an equal volume of Freund complete adjuvant. Two more injections were applied subsequently with the same quantity of virus but mixed with Freund incomplete adjuvant. Sera were collected 1 week after the last inoculation. The neutralization assays were carried out as described by Gerna et al. (7). Briefly, 50 μl of virus, diluted with medium to give 200 focus-forming units per well, was mixed with 50 μl of twofold serial dilutions of the antisera to be tested. Mixtures were incubated for 90 min at 37°C and inoculated on top of confluent monolayers of MA104 cells growing in 96-well microdilution plates. The plates were incubated for 14 to 16 h. After fixation with acetone, cells were stained by the immunoperoxidase technique as described previously (17). The neutralizing titer of each serum was expressed as the reciprocal of the highest dilution that gave 80% reduction in the number of the stained cells as compared with control wells.

Fecal specimens and culture-adapted rotavirus strains were subgrouped by ELISA with specific monoclonal antibodies for subgroups I and II (8) (kindly provided by Mary Estes). A monoclonal antibody for a common epitope on VP6 was included in the test. Subgroup designation was made as described previously (8).

As shown in Table 1, 60 samples (38.4 per cent) were positive by polyacrylamide gel electrophoresis (PAGE); 5 of these samples were negative by ELISA and displayed atypical electropherotypes. Figure 1B, C, and D shows the electropherotypes of three of these viruses. On the basis of the characteristic distribution of the RNA genomic segments, these viruses can be tentatively classified either as group B (lanes B and D) or as group C (lane C). However, these samples have not yet been tested serologically with antisera against these groups. It should be pointed out that the putative group C viruses were isolated from several 4- to 6-day-old, diarrheic piglets within the same herd.

In two of the herds from the province of Buenos Aires, viruses displaying unusual electropherotypes were isolated. These samples were ELISA positive and showed two types of genomic rearrangements, which in both cases were generated by an unusual migration of genomic segment 11 (1, 17). Figure 2 shows both types of patterns compared with the prototype strains OSU and Gottfried and with the human supershort strain 69M (9). The origins of strains CN86, CC86, CC117, CN117, and C60 have already been described (17). Strain C60 was isolated in 1985, whereas strains C134 and C158 were isolated from the same farm in 1986 and 1987, respectively. As can be seen in Fig. 2C, all the segments of these three strains showed similar electrophoretic mobility, except in the region of the triplet corresponding to genomic segments 7, 8, and 9, where the main immunogenic determinants are codified.

From the 55 group A-positive samples, 10 were adapted to grow in MA104 cells. Six of these samples (CC117, C60, C134, C135, C158, and CC86) belonged to the rearranged group. The antigenic relationship between these adapted strains and the swine standard strains OSU (serotype 5) and Gottfried (serotype 4) was determined. The human strain 69M, which has a supershort electropherotype similar to those of strains CC86 and CC117, was also included for comparison. Results are shown in Table 2. Homologous titers are in boldface. Different serotypes were defined when a 20-fold or greater difference between homologous and heterologous titers was obtained, using antisera and viruses in a two-way reaction (28). As can be seen, all strains were antigenically distinct from serotype 4. Two strains, CN86 and CC86, showed a two-way reaction with strain OSU, and they could be considered to belong to serotype 5. Strains C60 and C134 showed only a one-way reaction with strain OSU, although they were neutralized with OSU antiserum to very different titers. These strains were found to belong to a different serotype by the inverse reaction; i.e., serum to C60 neutralized strains C134 and C158 (which were isolated from the same farm in two successive years) only to a minor extent (less than 20-fold). Strains CN117 and CC117 had at least a one-way reaction with serotype 5 since both were neutralized by OSU, CC86, and CN86 antisera. Due to the lack of the corresponding sera, the inverse reaction was not performed. These strains were also neutralized in a one-way reaction by sera against strains C60 and C134.

On the other hand, antiserum against human strain 69M neutralized two porcine strains, C134 and CC117, both of which had rearranged genomes, but strain 69M was not neutralized by any of the porcine antisera tested. Finally, none of the porcine antisera was able to neutralize Gottfried strain (serotype 4) or the bovine strain T5 (serotype 6).

From 32 samples tested, 13 were defined as subgroup I and

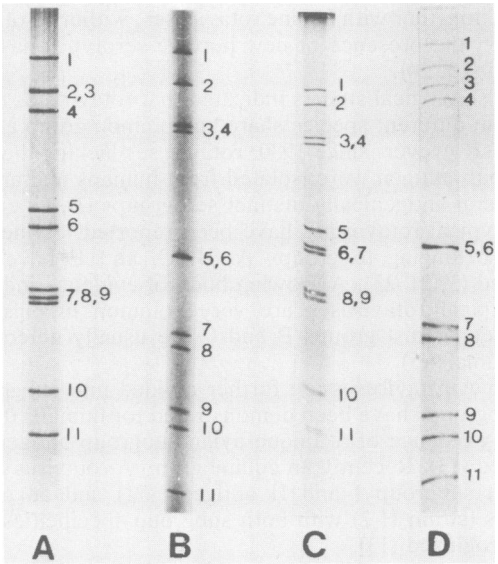

FIG. 1. Electropherotypes of rotaviruses extracted from feces (10% acrylamide gel). Lanes B to D, rotaviruses exhibiting atypical electropherotypes. Lanes A, B, C, and D correspond to strains CN86, C70, C155, and C133, respectively.

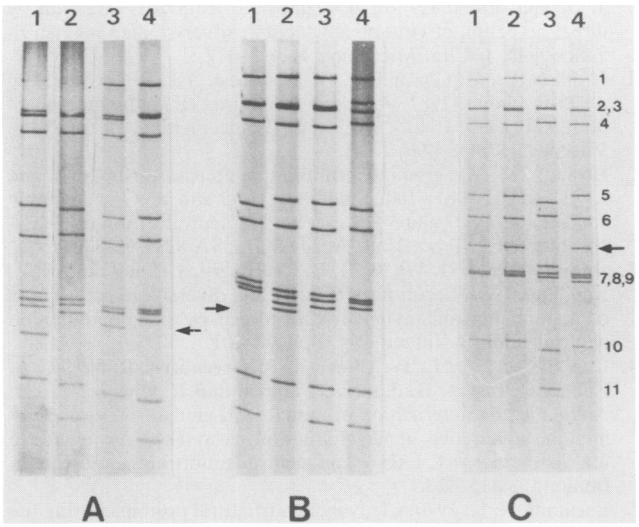

FIG. 2. Electropherotypes of culture-adapted rotavirus strains with normal (N) and rearranged (R) genomes. Samples were analyzed in three different 10% polyacrylamide gels. (A) Lanes: 1, human strain 69M; 2, human strain Wa; 3, porcine strain CC117 (R); 4, porcine strain CN117 (N). (B) Lanes: 1, strain OSU; 2, strain CC86 (R); 3, strain CN86 (N); 4, Gottfried strain. (C) Porcine rotaviruses with rearranged genomes detected in the same farm in successive years. Lanes: 1, strain C134 (R) (isolated in 1986); 2, strain C158 (R) (isolated in 1987); 3, strain OSU; 4, strain C60 (R) (isolated in 1985). Arrows indicate positions of rearranged genome segments.

2 were defined as subgroup II. Subgroup I specificity was found in samples displaying both long and short electropherotypes, whereas subgroup II specificity was found only in samples with long electropherotypes. Two samples had both specificities, and 15 samples had neither subgroup I nor subgroup II specificity, although they were clearly positive with monoclonal antibodies directed to common epitopes on VP6. In this latter case all of them had long electropherotypes, with the exception of sample CC117, a culture-adapted strain which displayed a supershort electropherotype. Except for CC117, all the other tissue culture-adapted strains belonged to subgroup I.

There are no previous reports of epidemiological or antigenic characterization of swine rotaviruses in Argentina. The present study suggests a high incidence of rotaviral infections in 1- to 45-day-old diarrheic piglets. Many different electrophoretic patterns of genomic RNA, including atypical electropherotypes, presumably corresponding to three different antigenic groups (A, B, and C), were detected. In addition, several group A rotaviruses displaying unusual distributions of genome segments were found. It was later shown that these strains have undergone genomic rearrangements of their genomic RNA segment 11 (1, 17).

In this study, we have serotyped those strains that were adapted to grow in cell cultures. The results showed a complex cross-neutralization relationship and the existence, besides serotype 5, of at least two new porcine serotypes (strains C60 and C134). None of the strains could be classified within serotype 4. We suggest, as has been proposed previously (13), that a precise interpretation of our results needs a new system of rotaviral classification indicating distinct serotypic specificities encoded by different genes for each isolate of rotavirus. It has been reported that in vitro neutralization is mediated by at least two gene products, VP3 and VP7 (12). It is likely that VP3 or VP7 of our local strains might contain neutralizing domains not found in those of serotypes 4 and 5. It is also important to mention that it may be possible that in the samples adapted to grow in tissue culture we could have selected only strains related to serotype 5. It has been reported that strains belonging to serotype 4 are more difficult to grow in cell cultures (4). In this regard, it will be important to develop methods for the determination of serotypes directly from the feces so that a larger quantity of samples can be screened and all representative serotypes can be detected.

In this report we have also described two local swine rotavirus strains with a one-way relationship with the human strain 69M, which was recently reported as a new human serotype (16). Antigenic relationships between human and swine strains have been published. Gottfried strain belongs to human serotype 4 (13), and recently a strain belonging to human serotype 3 was reported (19).

Another interesting observation was the change of serotype of strains C60, C134, and C158, which were isolated from the same farm in three successive years. Although it cannot be ruled out that C134 and C158 strains could have been present but undetected at the time of the first survey, it is tempting to speculate that these two strains originated from strain C60. If this is the case, the degree of antigenic

TABLE 2. Antigenic characterization of swine rotaviruses in neutralization assays

Rotavirus strain	Neutralizing titer[a] with hyperimmune antisera to:						
	OSU	Gottfried	CC86	CN86	C60	C134	69M[b]
OSU	**>25,600**	<50	3,200	25,600	3,200	800	200
Gottfried	100	**3,200**	100	100	800	10	
CC86	1,600	<50	**51,200**	1,600	51,200	12,800	400
CN86	3,200	10	102,400	**12,800**	>102,400	25,600	400
C60	1,600	10	25,600	1,600	**>102,400**	3,200	200
C134	25,600	10	12,800	12,800	3,200	**51,200**	1,600
C158	1,600				3,200	6,400	
CN117	3,200	10	51,200	1,600	102,400	6,400	200
CC117	12,800	<50	12,800	12,800	6,400	25,600	3,200
C135	6,400	10	6,400		6,400	12,800	
C91	3,200	10	51,200	800		12,800	
69M[b]	100	100	400	10	400	10	**25,600**
T5[c]	100	200	200	100	400	400	

[a] Homologous titers are in boldface type.
[b] Human strain (9).
[c] Bovine strain belonging to serotype 6.

variation detected after a year of field evolution is noteworthy. This could be the result of a reassortment event or a genetic drift in the genomic regions of VP7 coding for some neutralizing epitopes. In agreement with both hypotheses is the fact that the only differences detected among the three strains by PAGE are located in the triplet constituted by genomic segments 7, 8, and 9, where the gene coding for VP7 is located (see Fig. 2C).

Finally, this paper reports new data about subgrouping of swine rotaviruses in Argentina. Most culture-adapted strains belonged to subgroup I, and they displayed either short or long electropherotypes. It is especially important to point out that strain CC117, which belongs neither to subgroup I nor to subgroup II, was adapted to grow in tissue culture and cloned, so we could unequivocally confirm its subgroup specificity (non-I/II) first obtained with the fecal specimen. We have detected several samples with this characteristic antigenic subgroup specificity and displaying either short or long electropherotypes in different swine farms located at considerable distances from each other. Hoshino et al. (13) had reported an equine strain, H-2, with non-I/II specificity, but up to the present this is the first report of this type of specificity in swine strains. Two porcine samples eliciting both subgroup I and II specificities were also detected in this survey. A similar finding was reported by Hoshino et al. (11) with the equine rotavirus strain F1-14. At present we are in the process of adapting these strains to grow in cell cultures for further unequivocal characterization of their subgroup specificities.

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LITERATURE CITED

1. **Bellinzoni, R. C., N. M. Mattion, O. R. Burrone, S. A. González, J. L. La Torre, and E. A. Scodeller.** 1987. Isolation of group A swine rotaviruses displaying atypical electropherotypes. J. Clin. Microbiol. **25**:952–954.
2. **Bohl, E. H., E. M. Kohler, L. J. Saif, R. F. Cross, A. G. Agnes, and K. W. Theil.** 1978. Rotaviruses as a cause of diarrhea in pigs. J. Am. Vet. Med. Assoc. **172**:458–463.
3. **Bohl, E. H., L. J. Saif, K. W. Theil, A. G. Agnes, and F. Robert.** 1982. Porcine pararotavirus: detection, differentiation from rotavirus, and pathogenesis in gnotobiotic pigs. J. Clin. Microbiol. **15**:312–319.
4. **Bohl, E. H., K. W. Theil, and L. J. Saif.** 1984. Isolation and serotype of porcine rotaviruses and antigenic comparison with other rotaviruses. J. Clin. Microbiol. **19**:105–111.
5. **Bridger, J. C., and J. F. Brown.** 1985. Prevalence of antibody to typical and atypical rotaviruses in pigs. Vet. Rec. **116**:50.
6. **Flewet, T. H., and G. N. Woode.** 1978. The rotaviruses. Brief review. Arch. Virol. **57**:1–23.
7. **Gerna, G., M. Battaglia, G. Milenesi, N. Passarani, E. Percivalle, and E. Cattaneo.** 1984. Serotyping of cell culture-adapted subgroup 2 human rotavirus strains by neutralization. Infect. Immun. **43**:722–729.
8. **Greenberg, H. B., V. McAuliffe, J. Valdesuso, R. Wyatt, J. Flores, A. Kalica, Y. Hoshino, and N. Singh.** 1983. Serological analysis of the subgroup protein of rotavirus using monoclonal antibodies. Infect. Immun. **39**:91–99.
9. **Hasegawa, A., S. Inouye, S. Matsuno, K. Yamaoka, R. Eko, and W. Suharyono.** 1984. Isolation of human rotaviruses with a distinct RNA electrophoretic pattern from Indonesia. Microbiol. Immunol. **28**:719–722.
10. **Herring, A. J., N. F. Inglis, C. K. Ojeh, D. R. Snodgrass, and J. D. Menzies.** 1982. Rapid diagnosis of rotavirus infection by direct detection of viral nucleic acid in silver-stained polyacrylamide gels. J. Clin. Microbiol. **16**:473–477.
11. **Hoshino, Y., M. Gorziglia, J. Valdesuso, J. Askaa, R. Glass, and A. Z. Kapikian.** 1987. An equine rotavirus (F1-14) which bears both subgroup I and subgroup II specificities on its VP6. Virology **157**:488–496.
12. **Hoshino, Y., M. Sereno, K. Midthun, J. Flores, A. Kapikian, and R. Chanock.** 1985. Independent segregation of two antigenic specificities (VP3 and VP7) involved in neutralization of rotavirus infectivity. Proc. Natl. Acad. Sci. USA **82**:8701–8704.
13. **Hoshino, Y., R. G. Wyatt, H. B. Greenberg, J. Jones, and A. Z. Kapikian.** 1984. Serotypic similarity and diversity of rotaviruses of mammalian and avian origin studied by plaque reduction neutralization. J. Infect. Dis. **149**:694–701.
14. **Kapikian, A. Z., L. W. Cline, H. B. Greenberg, R. Wyatt, A. Kalica, C. Banks, H. James, J. Flores, and R. Chanock.** 1981. Antigenic characterization of human and animal rotaviruses by immune adherence hemagglutination assay (IAHA): evidence for distinctness of IAHA and neutralization antigens. Infect. Immun. **33**:415–426.
15. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
16. **Matsuno, S., A. Hasegawa, A. Mukoyama, and S. Inouye.** 1985. A candidate for a new serotype of human rotavirus. J. Virol. **54**:623–624.
17. **Mattion, N. M., S. A. González, O. R. Burrone, R. C. Bellinzoni, J. L. La Torre, and E. A. Scodeller.** 1988. Rearrangement of genomic segment 11 in two swine rotavirus strains. J. Gen. Virol. **69**:695–698.
18. **McNulty, M. S., W. L. Curran, and J. B. McFerran.** 1976. A reovirus-like agent (rotavirus) associated with diarrhea in neonatal pigs. Vet. Microbiol. **1**:55–56.
19. **Nagesha, H. S., and I. H. Holmes.** 1988. New porcine rotavirus serotype antigenically related to human rotavirus serotype 3. J. Clin. Microbiol. **26**:171–174.
20. **Paul, P. S., Y. S. Lyoo, J. J. Andrews, and H. T. Hill.** 1988. Isolation of two new serotypes of porcine rotavirus from pigs with diarrhea. Arch. Virol. **100**:139–143.
21. **Pedley, S., J. C. Bridger, D. Chasey, and M. A. McCrae.** 1986. Definition of two new groups of atypical rotaviruses. J. Gen. Virol. **67**:131–137.
22. **Sigolo de San Juan, C., R. C. Bellinzoni, N. M. Mattion, J. L. La Torre, and E. A. Scodeller.** 1986. Incidence of group A and atypical rotaviruses in Brazilian pig herds. Res. Vet. Sci. **41**:270–272.
23. **Theil, K., and L. J. Saif.** 1985. In vitro detection of porcine rotavirus-like virus (group B rotavirus) and its antibody. J. Clin. Microbiol. **21**:844–846.
24. **Thouless, M. E., A. S. Bryden, T. H. Flewett, G. N. Woode, J. C. Bridger, D. R. Snodgrass, and J. A. Herring.** 1977. Serological relationship between rotaviruses from different species as studied by complement fixation and neutralization. Arch. Virol. **53**:287–294.
25. **Utrera, V., R. M. de Ilja, M. Gorziglia, and J. Esparza.** 1984. Epidemiological aspects of porcine rotavirus infection in Venezuela. Res. Vet. Sci. **36**:310–315.
26. **Woode, G. N., J. Bridger, G. A. Hall, J. M. Jones, and G. Jackson.** 1976. The isolation of reovirus-like agents (rotaviruses) from acute gastroenteritis of piglets. J. Med. Microbiol. **9**: 203–209.
27. **Woode, G. N., J. C. Bridger, J. M. Jones, T. H. Flewett, A. S. Bryden, H. A. Davies, and G. B. White.** 1976. Morphological and antigenic relationship between viruses (rotaviruses) from acute gastroenteritis of children, calves, piglets, mice, and foals. Infect. Immun. **14**:804–810.
28. **Wyatt, R., H. B. Greenberg, W. D. James, A. Pittman, A. R. Kalica, J. Flores, R. M. Chanock, and A. Z. Kapikian.** 1982. Definition of human rotavirus serotypes by plaque reduction assay. Infect. Immun. **37**:110–115.