Porcine Rotaviruses Antigenically Related to Human Rotavirus Serotypes 1 and 2

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Fecal samples from rotavirus-infected piglets were characterized by a serotyping enzyme-linked immunosorbent assay (ELISA) by using monoclonal antibodies (MAbs) specific to human serotypes 1, 2, 3, and 4 (D. O. Matson, M. K. Estes, J. W. Burns, H. B. Greenberg, K. Taniguchi, and S. Urasawa, submitted for publication). Rotavirus in 19 of 25 specimens tested from two herds of pigs from Buenos Aires province, Argentina, were classified antigenically as follows: one serotype 1, four serotype 2, two serotype 3, and no serotype 4. Six specimens reacted with both serotype 1 and 2 MAbs, and viruses in six specimens probably belonged to other serotypes because they reacted only with a VP7 common epitope MAb. Two porcine rotavirus fecal samples found to contain both serotype 1 and 2 viruses by the MAb-based test and one found to contain a serotype 2 virus were grown in tissue culture. When plaque-purified preparations of these tissue culture-adapted viruses were analyzed in the serotyping ELISA, the C60 and C86 preparations reacted only as serotype 1 viruses, indicating that the original fecal samples, which showed multiple VP7 reactivities, were heterogeneous and apparently contained two types of viruses. Testing of plaque-purified C134 virus confirmed its serotype 2 reactivity. The MAb-based serotype designations of these viruses also were confirmed by using a neutralization immunoperoxidase focus reduction assay. This is the first report of the occurrence of serotype 1 and 2 rotaviruses in animals. The MAbs originally developed to serotype human rotaviruses can be utilized to type animal rotaviruses.

Rotaviruses are one of the major etiological agents of acute viral gastroenteritis in children and in the young of a large number of animal species (5, 7). Rotaviruses are classified into groups, and within each group they are further divided into serotypes based on their reactivities in plaque reduction or focus reduction neutralization assays using hyperimmune serum (6, 13, 27). Eleven serotypes have been identified, and viruses classified in two of these serotypes (VP7 types 3 and 4) have been isolated from both humans and animals (6). Neutralization assays evaluate antigenicity of the two outer capsid proteins, VP4 and VP7, which can both induce neutralizing antibody and segregate independently (12, 15, 17). However, in most cases the predominant reactivity with hyperimmune serum is with the glycoprotein VP7. Therefore, the serotypes established by neutralization assays represent distinct VP7 types, which has been confirmed by the use of VP7-specific monoclonal antibodies (MAbs).

Recently, enzyme-linked immunosorbent assay (ELISA) serotyping tests performed with VP7 type-specific MAbs have been developed and used to type human viruses (3, 8, 21, 25-27; D. O. Matson, M. K. Estes, J. W. Burns, H. B. Greenberg, K. Taniguchi, and S. Urasawa, submitted for publication) and to detect serotype 6 rotavirus in calves (2). We report use of the ELISA (originally developed to serotype human rotaviruses) to characterize animal rotaviruses.

We serotyped rotavirus-positive diarrheal fecal samples collected from piglets in Argentina (14), using an ELISA with MAbs specific for VP7 types 1, 2, 3, and 4 (27; Matson et al., submitted). The fecal samples were collected from 25 diarrheic piglets in 1986 or 1987, at two farms in Buenos

Aires province, Argentina. The epidemiologic patterns of illness among piglets at these farms are described elsewhere (14). The serotyping ELISA utilized purified MAbs as capture antibodies. Captured antigens in test samples were detected with hyperimmune serum prepared against the homologous serotype. The substrate was ABTS [2,2'-azino-di(3-ethylbenzothiazoline-6-sulfonate; Sigma Chemical Co., St. Louis, Mo.) and H₂O₂, and the absorbance (optical density) was determined at 414 nm (OD₄₁₄) in a micro ELISA reader (Lab System Inc., Chemetron, Argentina). A sample was considered to be positive for a determined serotype if the OD₄₁₄ was at least three times the mean OD₄₁₄ obtained with prototype virus strains which belong to other serotypes.

Typing results were obtained with 19 of 25 samples tested. One porcine fecal sample reacted as VP7 type 1, four reacted as type 2, two reacted as type 3, none reacted as type 4, and six reacted as type 1 and 2. Six samples reacted with the MAb against the common epitope on VP7 but not with any of the serotype-specific MAbs, and these probably represented other serotypes. Another six samples did not react with any of the VP7 MAbs, including the one directed to the common epitope on VP7, but they did react with subgroup MAbs to determinants on VP6. Representative results of typing viruses in fecal samples and in plaque-purified tissue culture preparations from three strains (Po/C86, Po/C60, and Po/C134) are shown in Table 1. Viruses in fecal samples reacted as VP7 type 1, VP7 type 2, and VP7 type 3; some showed reactivity with MAbs for both VP7 type 1 and type 2. These results with strains Po/C86, Po/C60, and Po/C134 were particularly surprising, because each of these three samples had previously been adapted to grow in MA104 cells and characterized by a neutralization immunoperoxidase focus

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TABLE 1. Serologic characterization of porcine rotavirus using the VP7 MAb serotyping ELISA

Virus	Known VP7 type	Origin ^a	OD ₄₁₄ with MAb specific to the following serotype:					VP7 type interpretation
			1	2	3	4	VP7 common	
Wa	1	Hu-tc	>2.00 ^b	0.07	0.07	0.05	0.50	1
DS-1	2	Hu-tc	0.08	0.28	0.06	0.04	0.10 ^c	2
SA11	3	Si-tc	0.09	0.07	0.88	0.06	0.70	3
Gottfried	4	Po-tc	0.04	0.03	0.02	0.18	0.09 ^c	4
OSU	5	Po-tc	0.09	0.08	0.09	0.04	0.40	5
C135		Po-f	0.15	0.63	0.02	0.08	0.60	2
C167		Po-f	1.90	0.86	0.09	0.04	0.78	1 and 2
C264		Po-f	0.40	0.16	0.10	0.05	0.26	1
C269		Po-f	0.44	0.30	0.05	0.02	0.74	1 and 2
C91		Po-f	1.10	0.29	0.04	0.11	0.32	1 and 2
C117		Po-f	0.18	>2.00	0.03	0.08	0.36	2
C158		Po-f	0.18	>2.00	0.03	0.07	0.32	2
C95		Po-f	0.42	0.83	0.06	0.07	0.35	1 and 2
C176		Po-f	0.12	0.18	0.40	0.10	1.80	3
C215		Po-f	0.09	0.15	1.74	0.04	1.07	3
C86		Po-f	0.43	0.27	0.04	0.02	0.46	1 and 2
C60		Po-f	>2.00	0.29	0.02	0.08	0.60	1 and 2
C134		Po-f	0.18	>2.00	0.03	0.07	0.32	2
C86		Po-tc	1.48	0.10	0.06	0.05	0.30	1
C60		Po-tc	1.80	0.13	0.12	0.08	0.50	1
C134		Po-tc	0.15	0.62	0.02	0.12	>2.00	2

^a Abbreviations: Hu, human; Si, simian; Po, porcine; Bo, bovine; ND, not done; tc, tissue culture-adapted virus; f, fecal sample.^b Numbers appearing in boldface type indicate positive reactions.^c Low reactivities of this MAb with these and other rotavirus strains have been noted.

reduction assay (IPFRA) as being related to the swine prototype serotype 5, OSU virus strain (14). However, in these previous IPFRA comparisons, only reactivity with other porcine viruses was tested. In the MAb ELISA, the fecal samples of two of these strains (Po/C60 and Po/C86) reacted as VP7 type 1 and 2, and the third fecal sample (Po/C134) reacted as a VP7 type 2 virus (Table 1). When triply plaque-purified tissue culture-adapted virus of each of these samples was analyzed in the ELISA serotyping test, Po/C60 and Po/C86 were classified as VP7 type 1 and Po/C134 was classified as VP7 type 2, respectively (Table 1). The prototype porcine OSU strain did not react with any of the VP7 MAbs in the serotyping ELISA.

The IPFRA (performed as described previously [14]) was repeated, using the Argentine porcine culture-adapted strains and the prototype rotaviruses Wa (serotype 1), DS-1 (serotype 2), SA11 (serotype 3), Gottfried (serotype 4), and OSU (serotype 5) strains (14) (Table 2). All these strains were plaque-purified three times before being analyzed by IPFRA. Neutralization titers with a 20-fold or greater difference were used as the criterion to distinguish between viruses in different serotypes (13, 18). The Po/C86 strain showed a one-way cross-reaction with the serotype 1 Wa strain and reciprocal cross-reactivity with the serotype 5 porcine OSU strain. Strain Po/C60 showed reciprocal cross-reactivity with the human serotype 1 Wa strain and a

TABLE 2. Serologic cross-reaction between porcine rotavirus from Argentina and human rotavirus serotypes 1 and 2: characterization by IPFRA

Rotavirus strain	Origin ^a	VP7 type	Reciprocal of 80% neutralization titer with hyperimmune antiserum to:									Serotype interpretation ^b
			Wa	DS-1	SA11	Gottfried ^b	OSU	M69 ^b	Po/C86	Po/C60	Po/C134	
Wa	Hu	1	102,400^c	400	ND ^d	ND	ND	ND	100	6,400	<100	1 (2W) ^e
DS-1	Hu	2	<100	25,600	ND	ND	<100	200	<100	100	400	2 (2W)
SA11	Si	3	ND	ND	25,600	ND	ND	ND	<100	<100	<100	3 (2W)
Gottfried ^f	Po	4	ND	ND	ND	3,200	100	ND	100	800	<100	4 (2W)
OSU	Po	5	<100	200	ND	<100	25,600	200	1,600	3,200	800	5 (2W)
M69 ^g	Hu	8	<100	100	ND	ND	100	25,600	<100	400	<100	8 (2W)
Po/C86	Po	NC ^g	12,800	200	<100	<100	3,200	400	12,800	102,400	25,600	1 (1W); 5 (2W)
Po/C60	Po	NC	6,400	200	<100	<100	3,200	200	6,400	102,400	3,200	1 (2W); 5 (1W)
Po/C134	Po	NC	200	1,600	<100	<100	25,600	1,600	12,800	3,200	25,600	2 (1W); 5 (1W); 8 (1W)

^a Abbreviations: Hu, human; Si, simian; Po, porcine.^b Cross-reactivities of Wa, DS-1, SA11, Gottfried, OSU, and M69 are shown from previous studies of these prototype strains.^c Numbers appearing in boldface type indicate homologous titers.^d ND, Not done.^e 2W and 1W, two-way and one-way cross-reactions with indicated type, respectively.^f Data from Mattion et al. (14).^g NC, Not previously classified.

one-way reactivity with the OSU strain. Strain Po/C134 showed a one-way cross-reactivity with the human serotype 2 DS-1 strain and one-way cross-reactivities with OSU and the serotype 8 M69 strain. Reactivities in the IPFRA with the porcine OSU strain confirmed those shown previously (14).

The fact that Po/C60 and Po/C86 in the fecal specimens reacted with MABs for both VP7 types, 1 and 2, but that after plaque purification they reacted only with type 1 (Table 1) probably indicates that these fecal samples were heterogeneous and also contained type 2 viruses that did not grow in MA104 cells. Type 2 viruses were present on the farm where C60 was isolated, since the type 2 Po/C134 viruses were isolated from the same farm 1 year later.

The serotype relationship of these porcine rotaviruses with more than one previously characterized serotype points out the importance of performing a complete serologic testing of any new potential virus serotype before assigning a serotype. In addition, these results highlight the increasing problems in classifying rotaviruses into discrete serotypes (1). Because the two outer capsid proteins VP4 and VP7 both possess neutralizing epitopes and both can segregate independently, a dual classification system must be used (9, 12).

Our present work with VP7 MABs indicates that at least some of the cross-reactivity of these porcine strains with human serotypes 1 and 2 must be located on VP7, so Po/C60 and Po/C86 should be classified as VP7 type 1 and Po/C134 should be classified as VP7 type 2. Presumably, the two-way cross-reactions detected with the IPFRA reflect common epitopes on VP4 as well, but reagents are not yet available to classify types of VP4 (6). Sequence analyses of genome segments 9 and 4 of these strains are expected to reveal regions which confirm the serologic reactivities found here (10, 11). This information should also improve our understanding of the relationship between swine and human strains.

Porcine rotaviruses sharing serotype antigenicity with human serotypes 3 and 4 (based on two-way cross-reactivities) and with serotype 8 (based on a one-way reactivity) have been described previously (13, 14, 16, 20). Ours is the first report of any animal rotaviruses with serotype 1 and 2 specificities. In this study, 11 of the 25 fecal samples tested were VP7 type 1 or 2. Taken together, these results indicate that pigs and humans share five (1, 2, 3, 4, and 8) rotavirus serotypes (only human serotype 9 has not yet been reported in pigs).

Rotaviruses from humans and pigs have several interesting similarities. For example, humans and swine are the species in which the greatest number of non-group A rotaviruses have been isolated (4, 19, 22–24). In addition, most animal rotaviruses belong to subgroup I, while human viruses containing subgroup I and II or containing I or II or neither of these subgroups have been described (12). However, the Gottfried strain isolated from a piglet belongs to subgroup II (13), and other porcine rotaviruses belonging to subgroup II or to neither subgroup I nor II have recently been described (14). Our results suggest that human and porcine rotaviruses may share evolutionary processes in nature and that porcine strains may be able to infect humans or vice versa. If epizootic transmission were common, the development of effective vaccine programs, particularly in rural settings, could be impeded. The information described in this report suggests that further characterization of animal rotaviruses using rapid MAB-based ELISAs will be useful to more fully understand the relationships between animal and human viruses.

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LITERATURE CITED

1. Albert, M. J., L. E. Unicomb, S. R. Tzipori, and R. F. Bishop. 1987. Isolation and serotyping of animal rotaviruses and antigenic comparison with human rotaviruses. *Arch. Virol.* **93**: 123–130.
2. Bellinzoni, R. C., J. O. Blackhall, N. M. Mattion, M. K. Estes, D. R. Snodgrass, J. L. La Torre, and E. A. Scodeller. 1989. Serological characterization of bovine rotaviruses isolated from dairy and beef herds in Argentina. *J. Clin. Microbiol.* **27**: 2619–2623.
3. Coulson, B. S., L. E. Unicomb, G. A. Pitson, and R. F. Bishop. 1987. Simple and specific enzyme immunoassay using monoclonal antibodies for serotyping human rotaviruses. *J. Clin. Microbiol.* **25**:509–515.
4. Dimitrov, D. H., M. K. Estes, S. M. Rangelova, L. M. Shindarov, J. L. Melnick, and D. Y. Graham. 1983. Detection of antigenically distinct rotaviruses from infants. *Infect. Immun.* **41**:523–526.
5. Estes, M. K. 1990. Rotaviruses and their replication, p. 1329–1352. *In* B. N. Fields (ed.), *Virology*, 2nd ed. Raven Press, New York.
6. Estes, M. K., and J. Cohen. 1989. Rotavirus gene structure and function. *Microbiol. Rev.* **53**:410–449.
7. Flewett, T. H., and G. N. Woode. 1978. The rotaviruses. *Arch. Virol.* **57**:1–23.
8. Gerna, G., A. Sarasini, A. di Matteo, M. Parea, P. Orsolini, and M. Battaglia. 1988. Identification of two subtypes of serotype 4 human rotavirus by using VP7-specific neutralizing monoclonal antibodies. *J. Clin. Microbiol.* **26**:1388–1392.
9. Graham, D. Y., and M. K. Estes. 1985. Proposed working serologic classification system for rotaviruses. *Ann. Inst. Pasteur* **136**:5–12.
10. Green, K. Y., Y. Hoshino, and N. Ikegami. 1989. Sequence analysis of the gene encoding serotype-specific glycoprotein (VP7) of two new human rotavirus serotypes. *Virology* **168**: 429–433.
11. Green, K. Y., J. F. Sears, K. Taniguchi, K. Midthun, Y. Hoshino, M. Gorziglia, K. Nishikawa, S. Urasawa, A. Z. Kapikian, R. M. Chanock, and J. Flores. 1988. Prediction of human rotavirus serotype by nucleotide sequence analysis of the VP7 protein gene. *J. Virol.* **62**:1819–1823.
12. Hoshino, Y., M. M. Sereno, K. Midthun, J. Flores, A. Z. Kapikian, and R. M. 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. Flores, and A. Z. Kapikian. 1984. Serotypic similarity and diversity of rotaviruses of mammalian and avian origin as studied by plaque-reduction neutralization. *J. Infect. Dis.* **149**:694–702.
14. Mattion, N. M., R. C. Bellinzoni, J. O. Blackhall, J. L. La Torre, and E. A. Scodeller. 1989. Antigenic characterization of swine rotaviruses in Argentina. *J. Clin. Microbiol.* **27**:795–798.
15. Matsui, S. M., E. Mackow, and H. B. Greenberg. 1989. Molecular determinants of rotavirus neutralization and protection. *Adv. Virus Res.* **36**:181–214.
16. 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.
17. Ofit, P. A., and G. Blavat. 1986. Identification of the two rotavirus genes determining neutralization specificities. *J. Virol.* **57**:376–378.

18. Ojeh, C. K., D. R. Snodgrass, and A. J. Herring. 1984. Evidence for serotypic variation among bovine rotaviruses. *Arch. Virol.* **79**:161-171.
19. Pedley, S., J. C. Bridger, J. F. Brown, and M. A. McCrae. 1983. Molecular characterization of rotaviruses with distinct group antigens. *J. Gen. Virol.* **64**:2093-2101.
20. Pongsuwanne, Y., K. Taniguchi, M. Choonthanom, M. Chiwakul, T. Susansook, S. Saguanwongse, C. Yayavas, and S. Urasawa. 1989. Subgroup and serotype distributions of human, bovine and porcine rotavirus in Thailand. *J. Clin. Microbiol.* **27**:1956-1960.
21. Shaw, R. D., D. L. Stoner-Ma, M. K. Estes, and H. B. Greenberg. 1985. Specific enzyme-linked immunoassay for rotavirus serotypes 1 and 3. *J. Clin. Microbiol.* **22**:286-291.
22. Sigolo de San Juan, C., R. C. Bellinzoni, N. Mattion, J. 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. Snodgrass, D. R., A. J. Herring, I. Campbell, J. M. Inglis, and F. D. Hargreaves. 1984. Comparison of atypical rotaviruses from calves, piglets, lambs, and man. *J. Gen. Virol.* **65**:909-914.
24. Sorrentino, A., E. A. Scodeller, R. Bellinzoni, G. Muchinick, and J. L. La Torre. 1986. Detection of an atypical rotavirus associated with diarrhoea in Chaco, Argentina. *Trans. R. Soc. Trop. Med. Hyg.* **80**:120-122.
25. Taniguchi, K., S. Urasawa, and T. Urasawa. 1985. Preparation and characterization of neutralizing monoclonal antibodies with different reactivity patterns to human rotaviruses. *J. Gen. Virol.* **66**:1045-1053.
26. Urasawa, S., T. Urasawa, K. Taniguchi, Y. Morita, N. Sakurada, Y. Saeki, O. Morita, and S. Hasegawa. 1988. Validity of an enzyme-linked immunosorbent assay with serotype specific monoclonal antibodies for serotyping human rotavirus in stool specimens. *Microbiol. Immunol.* **32**:699-708.
27. Urasawa, S., T. Urasawa, K. Taniguchi, F. Wakasugi, N. Kobayashi, S. Chiba, N. Sakurada, S. Morita, O. Morita, M. Tokieda, T. Kawamoto, K. Minekawa, and M. Ohseto. 1989. Survey of human rotavirus serotypes in different locales in Japan by enzyme-linked immunosorbent assay with monoclonal antibodies. *J. Infect. Dis.* **160**:44-51.