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Serological and Genomic Characterization of Two Porcine Rotaviruses with Serotype G1 Specificity

MAX CIARLET AND FERDINANDO LIPRANDI*

Laboratorio Biología de Virus, Instituto Venezolano de Investigaciones Científicas, Caracas, Venezuela

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Two porcine rotavirus strains, C60 and C95, which had been previously shown to be reactive in an enzyme-linked immunosorbent assay with serotype G1-specific monoclonal antibodies, were classified as G1 by cross-neutralization tests and on the basis of the homology of the sequenced VP7 gene. This report confirms that porcine rotavirus strains with a G1 serotype occur in nature.

Group A rotaviruses constitute the viral agent most commonly associated with acute diarrheal illness in piglets (8). Two surface proteins of group A rotaviruses, VP4 and VP7, associated with P and G serotype specificity, respectively, have been shown to independently elicit neutralizing antibodies and induce protective immunity (8). Among group A rotaviruses, 14 G serotypes have been identified in different species (4, 5, 8, 20).

Rotavirus strains isolated from pigs have been assigned to G serotypes 3, 4, 5, and 11 (8); additional porcine strains with unique antigenic characteristics have been described but not fully characterized (2, 16, 18). Among these, a group of fecal samples and the respective cell-adapted strains, obtained from diarrheic piglets in Argentina, were shown to be reactive in a serotyping immunoassay with monoclonal antibodies specific for human serotype G1 or G2 (2). When studied in neutralization tests, one of these strains, C60, exhibited a reciprocal cross-reactivity with the human serotype 1 Wa strain and a one-way reactivity with the OSU strain (G5). Rotavirus strains of G1 specificity have been isolated worldwide from humans but not from other species, with the exception of one bovine isolate, strain T449, from Argentina (3). To establish in a definitive way that serotype G1 strains circulate among pigs, we retested two porcine strains, C60 and C95, by cross-neutralization and sequenced their respective genes coding for the serotype-specific glycoprotein (VP7). Both serology and sequence homology data show that the two strains have a G1 serotype specificity.

Strains C60, C91, C95, and CN117 (kindly provided by R. Bellinzoni and N. Mattion, Centro de Virología Animal, Capital Federal, Argentina) were propagated in MA104 cells in the presence of 1 μg of trypsin per ml and were further plaque purified three times before characterization. The electropherotypes of the four plaque-purified strains are shown in Fig. 1. The electropherotypes of strains C95 and C91 were identical, and strain CN117 differed from these strains in the mobility of at least gene 7, while strain C60 differed in the mobilities of at least three genes (genes 7, 9, and 11). Reference rotavirus strains Wa (G1), DS-1 (G2), Rhesus (G3), Ch-2 (G7), 69M (G8), and WI61 (G9) and their respective hyperimmune antisera (except for that for Ch-2), produced in guinea pigs, were kindly supplied by Y. Hoshino and M. Gorziglia (National Institute of Allergy and Infectious Diseases, Bethesda, Md.). Bovine rotavirus B223 (G10) was provided by D. Snodgrass (Moredun Research Institute, Edinburgh, United Kingdom). Hyperimmune antisera to strains C60 and C95 and to the other standard rotavirus strains were raised as reported elsewhere (15). Cross-neutralization tests were performed in 96-well plates by a fluorescent-focus neutralization assay essentially as described previously (13).

The results of the cross-neutralization tests between strains C60, C95, and the standard rotavirus strains representative of 9 G serotypes are shown in Table 1. Different viral serotypes were defined by a >20-fold difference in neutralization titer. Strains C60 and C95 showed a two-way neutralization reaction with G1 strain Wa and no reactivity

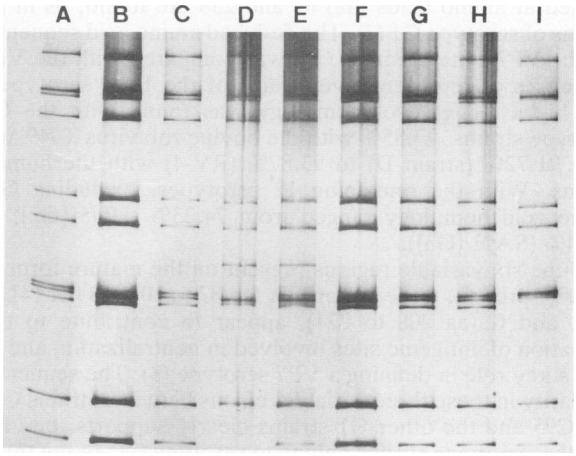

FIG. 1. Coelectrophoresis of genome RNAs of strains with G1 serotype specificity. Lanes: A, C91; B, CN117; C, C95; D, C60; E, C95 plus C60; F, C95 plus CN117; G, C95 plus C91; H, C95; I, OSU. Samples were subjected to electrophoresis in a 7% polyacrylamide gel, and genome segments were visualized by silver staining.

* Corresponding author. Mailing address: Centro de Microbiología, Instituto Venezolano de Investigaciones Científicas, Apdo. 21827, Caracas 1020-A, Venezuela. Phone: (582) 501-1377. Fax: (582) 501-1382. Electronic mail address: mciarlet@pasteur.ivic.ve.

TABLE 1. Antigenic characterization of porcine strains C60 and C95 by cross-neutralization of standard G serotype strains

Virus (serotype)	Titer[a] with antiserum to:										
	Wa	DS-1	RRV	Gottfried	OSU	NCDV	69M	WI61	A253	C60	C95
Wa (G1)	**6,400**	>100	>100	>100	>100	>100	>100	>100	>100	**3,200**	**800**
DS-1 (G2)	>100	**12,800**	>100	>100	>100	>100	>100	>100	>100	>100	>100
RRV (G3)	>100	>100	**51,200**	>100	>100	>100	>100	>100	>100	>100	>100
Gottfried (G4)	>100	>100	>100	**12,800**	>100	>100	>100	>100	>100	>100	>100
OSU (G5)	>100	>100	>100	>100	**25,600**	>100	>100	>100	400	400	400
NCDV (G6)	>100	>100	>100	>100	>100	**6,400**	>100	>100	>100	>100	>100
69M (G8)	>50	>50	>50	>50	>50	>50	**3,200**	>50	>50	>50	>50
WI61 (G9)	>100	>100	>100	>100	>100	>100	>100	**6,400**	>100	>100	>100
A253 (G11)	>100	>100	>100	>100	400	>100	>100	100	**6,400**	>100	>100
C60	**3,200**	>100	>100	>100	800	>100	>100	>100	>100	**12,800**	**6,400**
C95	**6,400**	>100	>100	>100	50	>100	>100	>100	>100	**12,800**	**6,400**
C91	**6,400**	>100	>100	>100	100	>100	>100	>100	>100	**12,800**	**6,400**
CN117	**3,200**	>100	>100	>100	100	>100	>100	>100	>100	**6,400**	**6,400**

[a] Numbers represent the reciprocal values of neutralization titers of sera with the indicated strains. Neutralization titer is defined as the highest serum dilution which inhibits >66% of foci of infection, measured by immunofluorescence. Homologous titers are given in boldface type.

with the remaining eight strains, except for a borderline reaction seen with the antiserum against C95 with strain OSU (G5). Strains C91 and CN117, for which antisera were not available, were efficiently neutralized by Wa, C60, and C95 antisera. Antisera to strains C60 and C95 did not react with either Ch-2 or B223 strains (≥32-fold difference) (data not shown). Thus, it was concluded from these results that these porcine strains belonged to serotype G1.

To confirm this classification, the nucleotide sequence of the gene encoding the VP7 of strains C60 and C95 was determined from in vitro-transcribed mRNA prepared from purified single-shelled particles as previously described (9).

A comparison of the complete deduced amino acid sequence for the gene encoding VP7 of strain C60 with that of strain C95 and with the VP7 sequences of rotavirus prototypes of various serotypes is shown in Fig. 2. The nucleotide sequences of strains C60 and C95 differed from each other at 19 positions, corresponding to a single-amino-acid change at position 284. Two potential N-linked glycosylation sites located at amino acids (aa) 69 and 238 are found, as in all strains of serotype G1 (3). The deduced amino acid sequence for the VP7 gene of strain C60 was compared with the VP7 sequences of representative strains of the 14 G serotypes. The highest degree of homology was found with the G1 serotype strains, 97.85% with the bovine rotavirus T449 and from 91.72% (strain D) to 93.87% (RV-4) with the human strains. With the remaining 12 serotypes, excluding G7, amino acid homology ranged from 74.23% (HU5 [G2]) to 83.44% (SA11 [G3]).

Of the six variable regions present on the mature form of the VP7 protein, three regions, A (aa 87 to 101), B (aa 141 to 152), and C (aa 208 to 224), appear to contribute to the formation of antigenic sites involved in neutralization and to play a key role in defining a VP7 serotype (8). The sequence similarity in these three variable regions between strains C60 and C95 and the other G1 strains clearly supports the idea that these porcine strains belong to serotype G1. In the three variable regions, the porcine strains had six amino acid substitutions compared with human rotavirus Wa, while the bovine strain has nine (the additional three being in region C). Perhaps such a difference in this region may explain why the antiserum to the bovine strain failed to neutralize the human strain Wa (3), while antisera to porcine strains neutralized strain Wa effectively.

As previously noted in comparisons of human and porcine VP7 genes of strains of serotypes G3 and G4 (12), the sequence similarity between animal and human G1 strains raises the question of the possibility of interspecies transmission of this gene (3). If this occurred, a genetic reassortment mechanism was probably involved since the four porcine strains studied had at least two genes, 4 and 6, typical of porcine strains. In fact, the VP4 of the porcine strains described in this paper appeared to be OSU-like (serotype P7) on the basis of neutralization reactivity with a hyperimmune serum raised against baculovirus-expressed OSU VP4 and of immunofluorescence reactivity with a panel of monoclonal antibodies specific to OSU VP8 (15). Likewise, these strains reacted by enzyme-linked immunosorbent assay (ELISA) with a panel of anti-VP6 monoclonal antibodies specific for porcine subgroup I rotavirus strains (14) (data not shown).

Data on the prevalence of different G serotypes among pigs are scanty. Prevalence has been studied by serotyping ELISAs incorporating serotype-specific monoclonal antibodies, but in the few studies reported, panels of monoclonal antibodies covering only a subset of the possible circulating serotypes were used (2, 17, 19). In a survey in Australia, with a serotyping ELISA that could detect G3, G4, G5, and G3/5 strains, approximately 27% of samples containing infectious rotavirus particles could not be typed, suggesting the presence of other serotypes. Although G1 porcine strains have been isolated only in Argentina, their widespread presence on at least two different farms suggests that they may be of epidemiological importance in this species (1, 2, 16).

Nucleotide sequence accession number. Sequence data reported in this report have been deposited in the GenBank data library under accession numbers L24164 (C60) and L24165 (C95).

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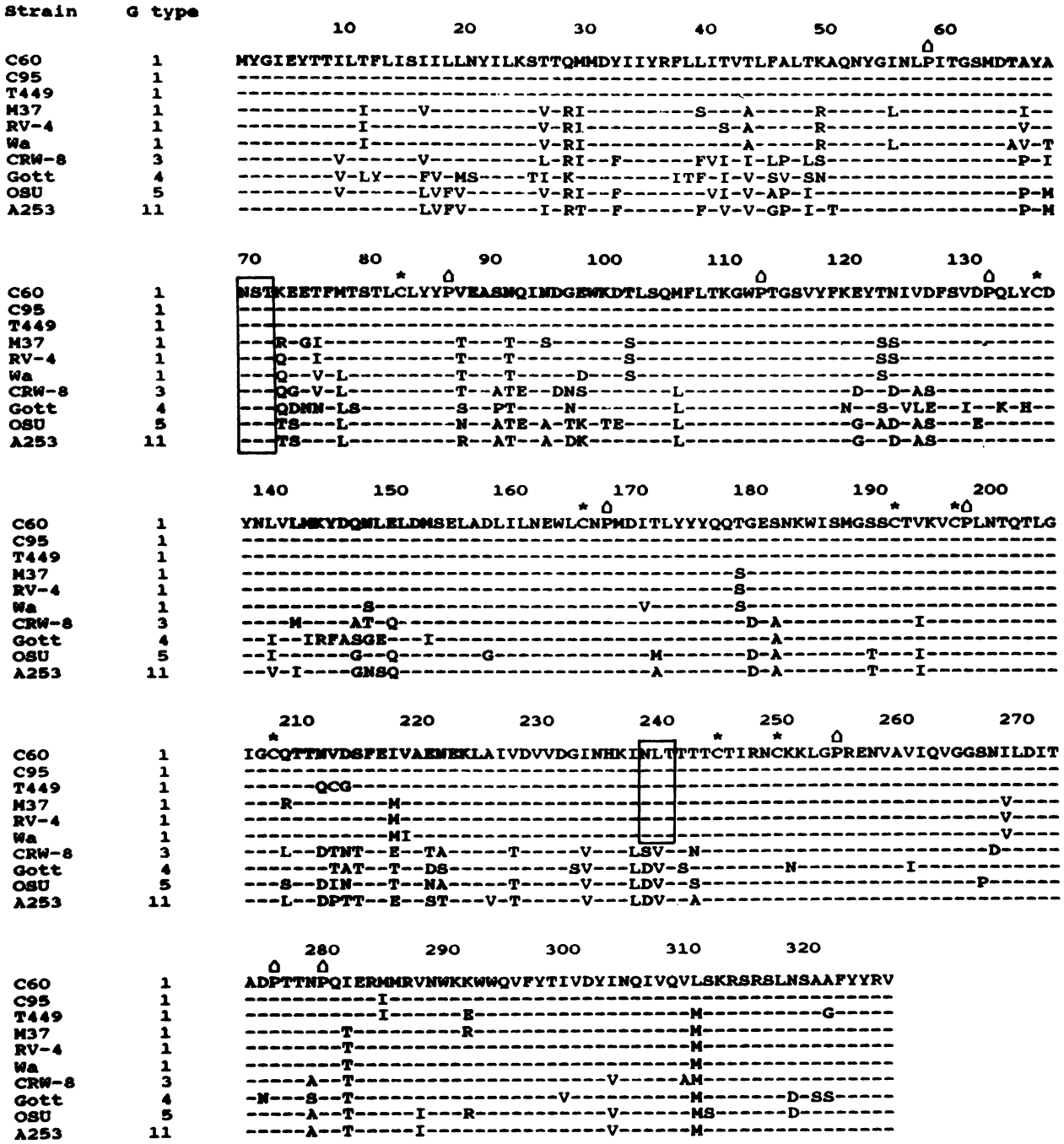

FIG. 2. Comparison of the deduced amino acid sequences for the genes encoding VP7 of porcine strains C60 and C95 with the amino acid sequences of VP7 of rotavirus strains of different G serotypes. Variable regions A, B, and C (aa 87 to 101, 141 to 152, and 208 to 221, respectively) (8) are shown in boldface type. Potential N-linked glycosylation sites are shown boxed. Conserved cysteine (*) and proline (Δ) residues in all strains are indicated. The VP7 sequences of strains T449 (3), M37 and Wa (11), RV-4 (7), CRW-8 (12), Gottfried (Gott) (10), OSU (9), and A253 (6) were previously published.

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