Detection of a Porcine-Like Rotavirus in a Child with Enteritis in Italy

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During a 1-year rotavirus surveillance of children hospitalized with acute gastroenteritis in Brescia Hospital, Italy, a chimerical rotavirus strain, G3P[6], was detected, displaying the VP7 and VP4 genes of porcine origin and the NSP4 and VP6 genes of human origin. The reassortant nature of the virus rules out a direct zoonotic event.

Group A rotaviruses, members of the Reoviridae family, are regarded as the single most important cause of severe acute gastroenteritis in infants and young children in both developed and developing countries (37). Rotaviruses possess 11 double-stranded RNA segments surrounded by three protein layers. The outer capsid proteins VP7 and VP4 carry independent neutralization and protective antigens (12). Thus far, 19 VP7 (G type) and 27 VP4 (P type) rotavirus genotypes have been described (27, 31, 40). Global epidemiological surveys have revealed that five G types (G1 to G4 and G9) and two P types (P[4] and P[8]) represent more than 90% of rotavirus strains of clinical importance globally (14, 39). Because serotype-specific protection appears to play an important role against rotavirus disease (19), monovalent or polyvalent human vaccines that target the G and/or P types of epidemiological importance have been developed (9, 35). The study of G and P type distributions is important to monitor the dynamics of rotavirus strain replacement and the emergence of novel strains and to better understand the efficacy of rotavirus vaccines.

Out of 775 stool samples collected from children under 5 years of age hospitalized with acute diarrhea in Brescia Hospital, Italy, during 2006, rotavirus was detected by an immune enzymatic assay in 192 (24.7%) samples. A subset of 83 rotavirus-positive samples was characterized to determine the G and P types by reverse transcription-PCR genotyping with multiple sets of primers as described previously (13, 18). For the sequence analyses, the VP7 gene was amplified with primer Beg9/End9deg (18, 28). The VP8* subunit of the VP4, the connecting peptide, and the N terminus of the VP5* subunit of VP4 (about 880 bp) were reverse transcribed and amplified with primer pair Con2-Con3 (13). The nearly full-length NSP4 gene was amplified with primer pair 10Beg16-10End722 (24). The VP6 genogroup, predictive of the VP6 subgroup, was determined by amplification and direct sequencing of a 379-bp fragment, spanning from nucleotides encoding amino acids 241 to 367 of the VP6, by using primer pair VP6F–VP6R (20). The sequences were analyzed using the Web-based programs BLAST (http://www.ncbi.nlm.nih.gov) and FASTA (http://www.ebi.ac.uk/fasta33). Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 2.1 (23).

Among the rotavirus-positive samples, the predominant G/P combination found was G1P[8] (51.8%), followed by G9P[8] (27.7%), G4P[8] (3.6%), G2P[4] (2.4%), and G3P[8] (1.2%). Infection with multiple rotavirus strains was diagnosed in 12% of the samples. The rotavirus strain detected in one sample, 128/07-34, was characterized as G3P[6]. The identification of a human G3P[6] strain is of interest because the majority of the human G3 strains are associated with P[8] and the G3P[6] combination is uncommon.

In terms of the VP7 protein, the human rotavirus strain 128/07-34 displayed the highest amino acid identity (97.4%) to porcine rotaviruses of serotype G3, while the amino acid identity to human G3 viruses was ≤95.8%. Phylogenetic analysis revealed that the strain 128/07-34 clustered with porcine G3 strains, while common human G3P[8] viruses formed a homogeneous group (Fig. 1A). When comparing the VP7 antigenic regions A, B, C, and F (7, 11, 21, 34) in detail (Fig. 2), the human strain 128/07-34 displayed several changes with respect to the reference human rotavirus G3 strain YO, 92E→Q, 98E→K (region A), V218→I and 222→E (region C), and N238→S (region F). The change 92E→Q was shared by several porcine G3 strains, while the change V218→I was found only in the VP7 protein of G3 porcine strains 4F and 4S and in some G3P[9] human strains. Interestingly, the change N238→S in region F was conserved in the majority of the porcine strains analyzed. This change disrupts a potential glycosylation site (NV[T]→SV[T]) that is present in the majority of human G3 strains, whether in combination with P[8] or P[9].

The NSP4 protein of human strain 128/07-34 was 175 amino acids in length and displayed the highest amino acid identity (98.9%) to a Slovenian human rotavirus strain, SI-MB7 (NSP4 B, Wa-like), while amino acid identity to the porcine strains ranged from 92% to 95%. Phylogenetic analysis revealed that the NSP4 of strain 128/07-34 clustered with NSP4 B human viruses (Fig. 1B). In addition, in a sequence com-

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FIG. 1. Phylogenetic trees based on VP7 (A), NSP4 (B), VP8* (C), and VP6 (D). The various lineages (A, C, and D) and genotypes (B) are indicated. hu, human; po, porcine; fe, feline; ca, canine; la, lapine; si, simian; bo, bovine; eq, equine.
parison with a selection of VP6 sequences of human and animal viruses, strain 128/07-34 clearly clustered with SGII human strains (Fig. 1D).

The VP8* subunit of the human strain 128/07-34 displayed the highest amino acid identity (93.4 to 93.9%) to porcine P[6]-I strains (221/04-7, 221/04-13, and 134/04-8) identified in Italy within lineage I (P[6]-I) (26). In the phylogenetic analysis of VP8* (Fig. 1C), strain 128/07-34 segregated with porcine P[6]-I strains, while human P[6] strains, M37-like, formed a tightly homogeneous group. Similarly, other P[6] human strains segregated outside the M37-like group and appeared intermingled with porcine P[6]-I strains, such as in the case of the G5P[6] human strains LL4260, LL3354, LL36755, KH228, and KH210, identified in China and Vietnam (1, 10); the Vietnamese strains VN846/2003, VN592/2003, and VN904/2003 (33); and the Brazilian strain NB150/BR/97 (29).

These findings strongly suggest that the diversity of human P[6] rotaviruses has been generated by multiple interspecies transmissions (26). The P[6] VP4 specificity (M37-like) is the third-most common VP4 genotype. Genotype P[6] is infre-
FIG. 1—Continued.
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quent (0.3 to 1.5%) in infections in humans on the European and Australian continents, while it is found in 3 to 4% of the rotavirus infections on the American continent, 7% of those in Asia, and 29.4% of those in Africa (39). Genotype P[6] has been also identified in piglets (4). Antigenic differences have been observed between the porcine P[6] strain Gottfried and the M37-like human P[6] strains; consequently, they have been classified antigenically as distinct P subtypes of serotype P2, P2B (strain Gottfried), and P2A (M37-like viruses) (16, 25, 32, 38). In addition, an atypical G1 strain, AU19, has been proposed as a possible new subtype, P2C (32). Based on sequence comparison and phylogenetic inference, the three P[6] prototype strains M37, Gottfried, and AU19 have been assigned to three distinct P[6] genetic lineages, I, II, and III, respectively (32). Analyses of G4P[6] strains, detected sporadically but continually in Hungary, have identified two novel distinct P[6] genetic lineages, IV and V, represented by BP1198/98-like strains and BP720/93-like strains, respectively (3). In addition, analyses of porcine rotavirus strains in Japan and Italy have identified P[6] strains that resemble human P[6] viruses of lineage I (M37-like), III (AU-19-like), and V (BP720/93-like) (26, 41).

In conclusion, analyses of VP7, the VP8* subunit of VP4, the VP6 fragment predictive of subgroup specificity, and the NSP4 protein of the human rotavirus strain 128/07-34 revealed that the genes encoding the outer capsid proteins were likely acquired from a porcine virus, while the genes encoding the VP6 and NSP4 proteins were derived from human viruses. There is evidence that heterologous rotaviruses of porcine origin or natural porcine-human reassortants may have occurred and spread successfully throughout human populations in several instances, such as G5 rotaviruses in Latin America (2, 5, 6, 8, 17, 30) and G9P[19] viruses in Southeast Asia in the late 1980s (15, 22, 36, 42, 43).

The findings of this study reinforce the hypothesis that there is a dynamic interaction between rotaviruses of humans and animals and that reassortment can result in the introduction of animal gene alleles in human rotaviruses. Simultaneous surveillance of animal and human rotavirus infections is therefore paramount for the understanding of the evolution of these viruses.

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