Available online at www.sciencedirect.com

SCIENCE DIRECT

Virology 344 (2006) 509 – 519

VIROLOGY

www.elsevier.com/locate/yviro

Relationships among porcine and human P[6] rotaviruses: Evidence that the different human P[6] lineages have originated from multiple interspecies transmission events

Vito Martella [a,*], Krisztián Bányai [b], Max Ciarlet [c], Miren Iturriza-Gómara [d], Eleonora Lorusso [a], Simona De Grazia [e], Serenella Arista [e], Nicola Decaro [a], Gabriella Elia [a], Alessandra Cavalli [a], Marialaura Corrente [a], Antonio Lavazza [f], Rafael Baselga [g], Canio Buonavoglia [a]

[a] *Dipartimento di Sanità e Benessere Animale, Facoltà di Medicina Veterinaria di Bari, S.p. per Casamassima km 3, 70010 Valenzano, Bari, Italy*
[b] *Regional Laboratory of Virology, Baranya County Institute of State Public Health Service, Hungary*
[c] *Department of Biologics-Clinical Research, Merck and Co., Inc., Blue Bell, PA 19422, USA*
[d] *Enteric Virus Unit, Virus Reference Department, Centre for Infections, Health Protection Agency, London, UK*
[e] *Dipartimento di Igiene e Microbiologia, Università di Palermo, Italy*
[f] *Istituto Zooprofilattico Sperimentale di Lombardia/Emilia Romagna, Brescia, Italy*
[g] *Exopol, Saragoza, Spain*

Received 22 March 2005; returned to author for revision 30 June 2005; accepted 21 August 2005
Available online 27 September 2005

Abstract

Porcine rotavirus strains (PoRVs) bearing human-like VP4 P[6] gene alleles were identified. Genetic characterization with either PCR genotyping or sequence analysis allowed to determine the VP7 specificity of the PoRVs as G3, G4, G5 and G9, and the VP6 as genogroup I, that is predictive of a subgroup I specificity. Sequence analysis of the VP8* trypsin-cleavage product of VP4 allowed PoRVs to be characterized further into genetic lineages within the P[6] genotype. Unexpectedly, the strains displayed significantly higher similarity (up to 94.6% and 92.5% at aa and nt level, respectively) to human M37-like P[6] strains (lineage I), serologically classifiable as P2A, or to the atypical Hungarian P[6] human strains (HRVs), designated as lineage V (up to 97.0% aa and 96.1% nt), than to the porcine P[6] strain Gottfried, lineage II (<85.1% aa and 82.2 nt), which is serologically classified as P2B. Interestingly, no P[6] PoRV resembling the original prototype porcine strain, Gottfried, was detected, while Japanase P[6] PoRV clustered with the atypical Japanase G1 human strain AU19. By analysis of the 10th and 11th genome segments, all the strains revealed a NSP4B genogroup (Wa-like) and a NSP5/6 gene of porcine origin. These findings strongly suggest interspecies transmission of rotavirus strains and/or genes, and may indicate the occurrence of at least 3 separate rotavirus transmission events between pigs and humans, providing convincing evidence that evolution of human rotaviruses is tightly intermingled with the evolution of animal rotaviruses.

Keywords: VP4; P[6]; Genotyping; Porcine rotaviruses

Rotaviruses are the major etiologic agents of infantile diarrhea worldwide. Rotaviruses are included in the Reoviridae family and possess a triple-layered capsid enclosing 11 segments of double-stranded RNA. The two outer capsid proteins, VP7 and VP4, elicit neutralizing antibody responses and define the G (VP7) and P (VP4) types (Estes, 2001; Hoshino et al., 2002). Thus far, based on both antigenic and genetic differences, 15 G types and 25 P genotypes have been established. Due to the lack of serological reagents, only 14 P serotypes and 3 P subtypes have been defined (Estes, 2001; Rao et al., 2000; Hoshino et al., 2002; Martella et al., 2003; Liprandi et al., 2003; McNeal et al., 2005; Rahman et al., 2005). Epidemiological studies have demonstrated that five rotavirus G serotypes (G1, G2, G3, G4 and G9) and two P serotypes (P1A[8] and P1B[4]) are the most frequent VP7 and VP4 types associated with human rotavirus (HRV) infection globally, and thus, are the targets for vaccine development (Hoshino and Kapikian, 2000; Hoshino et al., 2002; Kapikian et al., 2001; Santos and Hoshino, 2005).

* Corresponding author. Fax: +39 80 4679843.
E-mail address: v.martella@veterinaria.uniba.it (V. Martella).

0042-6822/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.virol.2005.08.029

Serotype P2A[6] HRV strains have been often associated with neonatal infections and initially were believed to render avirulent or less virulent neonatal (nursery) strains (Hoshino et al., 1985; Flores et al., 1986; Gorziglia et al., 1986, 1988). Two of such P[6] HRV strains, M37 and RV-3, were proposed as candidate HRV vaccines and clinical evaluation with RV-3 is still in phases I–II (Flores et al., 1990; Midthun et al., 1991; Pérez-Schael et al., 1994; Vesikari et al., 1991; Barnes et al., 1997, 2002). However, several surveys have revealed that P[6] HRV strains may also be associated with symptomatic infection in children and there is a high frequency of detection of P[6] HRVs in Africa (Timenetsky et al., 1994; Santos et al., 1994a; Steele et al., 1995; Ramachandran et al., 1996; Adah et al., 1997; Cunliffe et al., 1999). Nevertheless, there is only little genetic/antigenic difference between the VP8* of the VP4 of asymptomatic and symptomatic P[6] strains (Santos et al., 1994b; Kirkwood et al., 1996; Pager et al., 2000; Hoshino et al., 2003).

The porcine rotavirus (PoRV) strain Gottfried was originally isolated from the intestinal content of a suckling pig with diarrhea (Bohl et al., 1984). Subsequently, the VP4 of the Gottfried strain was shown to be genetically similar to the VP4s of the HRV P[6] strains, though being antigenically distinguishable as a distinct subtype, P2B, whereas the HRV strains were classified as P2A (Gorziglia et al., 1990; Nakagomi et al., 1999; Li and Gorziglia, 1993; Santos et al., 1994b). In addition, an atypical G1 HRV, AU19, has been proposed as a possible new subtype, P2C[6], because of its relatively low sequence similarity in the VP8* subunit of VP4 and because of its poor cross-reactivity with P2A[6] HRV strains in reciprocal neutralization assays (Nakagomi et al., 1999). Based on sequence comparison and phylogenetic inference, the three prototype strains M37, Gottfried and AU19 have been assigned to three distinct P[6] genetic lineages, namely I, II and III (Nakagomi et al., 1999). More recently, P[6] human strains belonging to two novel separate genetic lineages, BP1198/98-like (IV) and BP720/93-like (V), have been identified in Hungary (Bányai et al., 2004), providing evidence for genetic heterogeneity within the P[6] VP4 allele and raising some interrogatives on the origin of the various human P[6] lineages. Also, two P[6] PoRV that genetically resemble (89% nt and 90% aa identity in the VP8* portion of VP4) the VP4 of the P[6]-III HRV strain, AU-19, have been described in Japan (Teodoroff et al., 2005).

Animal rotaviruses are regarded as a potential reservoir for genetic/antigenic diversity of HRVs and the potential of such transmission has been reported in several studies (Nakagomi and Nakagomi, 1993, 2002; Iturriza-Gómara et al., 2001, 2004; Das et al., 1993). Thus, the study of animal rotaviruses is a key step to acquire a more in-depth understanding of rotavirus evolution and ecology. To investigate the genetic heterogeneity of the P[6] lineages and the relationships between the animal and HRV P[6] strains, we analyzed the VP8* sequence of porcine P[6] PoRV strains identified in Spain and Italy and compared them to those derived from HRV strains.

Results

Genetic characterization

Twelve PoRVs (3 from Spain, 9 from Italy) were identified to be of P[6] genotype. Table 1 shows the country of origin, e-type, VP6 genogroup, G type, the lineage of the P[6] genotype of the P[6] PoRV strains and the reactivity to the various P[6]-specific primers used. All the P[6] PoRV strains were identified with primer SP6, while recognition by the other P[6]-specific primers varied (Table 1). Six of the P[6] PoRV strains were identified with all 3 primers sets, while 4 of the P[6] PoRV strains (221/04-13, ES51/02, 221/04-19 and 221/04-20) were identified with the M37-like primer 3T-1 (pool H) and the Gottfried-like primer pGott (pool A2). The additional two P[6] PoRV strains (221/04-21 and 134/04-7) were identified with only the Gottfried-like SP6 primer (pool A).

*Sequence and phylogenetic analysis of the VP8**

The sequence of the VP8* cleavage product of VP4 of all 12 PoRVs identified in Italy and Spain was determined. All the

Table 1
Results of the genetic characterization of the P[6] PoRV strains

PoRV strain	Country of origin	E-type	VP6 genogroup	NSP4 genogroup	G type	P genotype and lineage	3T-1 (Gentsch et al., 1992)	pGott (Gouvea et al., 1994a, 1994b)	SP6 (Winiarczyk et al., 2002)
221/04-7	Italy	L	I	B	Nd	P[6]-Ib	+	+	+
221/04-13	Italy	Nd	I	B	3	P[6]-Ib	±	–	+
134/04-8	Italy	Nd	I	B	Nd	P[6]-Ib	+	+	+
134/04-10	Italy	L	I	B	3	P[6]-Ic	+	+	+
134/04-11	Italy	L	I	B	3	P[6]-Ic	+	+	+
ES51/02	Spain	Nd	I	B	5	P[6]-Id	+	–	+
ES51/03	Spain	Nd	I	B	4	P[6]-Id	+	+	+
ES51/04	Spain	L	I	B	4	P[6]-Id	+	+	+
221/04-19	Italy	Nd	I	B	5	P[6]-V	±	–	+
221/04-20	Italy	Nd	I	B	4	P[6]-V	±	–	+
221/04-21	Italy	Nd	I	B	9	P[6]-V	–	–	+
134/04-7	Italy	L	I	B	4	P[6]-V	–	–	+

Negative (–), borderline (±), positive (+).
Nd: not determinable or not typeable.

PoRVs were confirmed to be P[6] by sequence analysis, but a high genetic heterogeneity was observed. Direct inspection of the primer binding sites in the VP8* nucleotide sequences of the Italian and Spanish PoRV and several P[6] strains failed to reveal a clear explanation of the different pattern of recognition by the various P[6]-specific primers used in our study (Fig. 1). Borderline or no reactivity could be attributable to the accumulation of point mutations in the sequence derived from the HRV strains or to the quality of the samples under the PCR conditions used.

The deduced amino acid sequences of the VP8* cleavage product of VP4 of all 12 P[6] Italian and Spanish PoRVs were aligned with those of P[6] HRV strains and with those of the prototypes of all the 5 existing P[6] lineages recognized thus far (Fig. 2). Analysis of the inferred amino acid sequences revealed that the potential cleavage sites (arginines 231, 241 and 247) and the highly conserved prolines at residues 68, 71, 225 and 226 were maintained in all 12 P[6] Italian and Spanish strains (Arias et al., 1996; Estes, 2001) (Fig. 2). Several aa residues were conserved within the P[6]-I human and animal strains but differed from the prototype P[6]-II PoRV Gottfried. Synapomorphisms (shared-derived amino acid substitutions) were observable among the P[6]-I strains, i.e. 105-L, 108-V, 134-T, 196-V, 203-E. Also, a number of synapomorphisms were clearly observable within the P[6]-V human and animal strains, i.e. 90-K, 92-I, 112-T, 130-I, 135-S, the aa triplet PDI at positions 194–196. In addition, several residues were highly conserved only within the P[6]-I HRV strains, i.e. 91-V, 101-I, 147-V and the aa triplet YNS at residues 170–172 and 202-V.

A distance matrix of nucleotide and amino acid comparison was elaborated (Table 2), and phylogenetic analysis of the PoRV strains allowed the clarification of the relationships between the PoRVs identified in this study and the established human and animal P[6] prototype strains. On the basis of the inferred phylogeny, the PoRVs analyzed in this study were clustered into two distinct groups (Fig. 3). The first group included the PoRVs 221/04-7, 221/04-13, 134/4-8, 134/04-10, 134/04-11 and ES51/02, ES51/03, ES51/04. All such strains were clustered with the M37-like (I) genetic lineage (bootstrap value 100%). Within the P[6]-I lineage, identity of PoRVs to HRV ranged from 85.8 to 94.6% aa and from 86.0 to 92.5% nt. However, since all the M37-like HRV strains maintained a clear distinction from the PoRV strains, the M37-like HRV was considered to represent a P[6] sublineage, P[6]-Ia (bootstrap value 100%). In addition, based on the tree topology, three distinct P[6] porcine sublineages, Ib, Ic and Id, were clearly evident, with the P[6]-Ib PoRVs being more tightly related to the P[6]-Ia HRVs (up to 94.6% aa and 92.5% nt). The second group of PoRVs included strains 221/04-19, 221/04-20, 221/04-21 and 134/04-7. All such strains clustered with the Hungarian human strains, BP720/93-like, of lineage V (bootstrap value 100%). Within this group, a tight sequence identity was observed between the PoRVs and the HRVs (ranging from 93.2 to 97.0% aa and from 91.8 to 96.1% nt). Also, two recently described Japanese P[6] PoRVs, JP3-6 and JP29-6, clustered with the prototype HRV strain AU-19 (P[6] lineage III). Identities of the PoRVs JP3-6 and JP29-6 to strain AU19 were 89.2% nt and 89.2% aa. Noteworthy, none of the P[6] strains analyzed during this study was found to resemble the original P[6]-II prototype, the porcine strain Gottfried. Identity of the P[6] Italian PoRVs to strain Gottfried ranged from 82.8 to 85.1% aa and from 80.7 to 82.2% nt.

Rotavirus strains exhibiting a VP4 aa identity of 89% or greater belong to the same P serotype or subtype (Gorziglia et al., 1990). Since the greatest VP4 sequence divergence is observed in the hypervariable region (region B, spanning aa 92 to 192) of the VP4 trypsin-cleavage product VP8*, which almost always correlates with VP4 serotype and subtype specificities (Larralde and Gorziglia, 1992; Larralde et al., 1991), it is possible to predict that the PoRV strains that belong to the P[6]-I lineage may be antigenically similar to the P[6] M37-like HRV strains, that belong to the P2A serotype. Conversely, the PoRV and HRV strains belonging to P[6] lineage V are likely to be antigenically distinct from those belonging to the P2A[6]-I, P2B[6]-II and the P2C[6]-III lineages, and could represent a novel P2[6] subtype.

Sequence and phylogenetic analysis of the NSP4 and NSP5/6 genes

To ascertain the exact nature of the P[6]-I and P[6]-V PoRV strains, the sequences of the NSP4 and NSP5/6 genes were determined and the complete deduced amino acid sequences were obtained (data not shown). The NSP4 amino acid sequences of all the PoRV strains analyzed in this study closely segregated with those of other PoRVs within NSP4 genogroup B (Wa-like), that includes both PoRV and HuRV strains (Fig. 3A). Likewise, the NSP5/6 amino acid sequences of all the PoRVs revealed a close relationship with PoRVs, and to a lesser extent to those of human origin (Fig. 3B). Therefore, the NSP4 and NSP5/6 sequence analyses of the P[6]-I and P[6]-V PoRV strains described in this study confirm the porcine origin of the strains.

Discussion

In this study, we analyzed P[6] PoRVs from distinct geographical areas in Southern Europe, Spain and Italy. The P[6] specificity was found to be associated with a variety of G types (G3, G4, G5 and G9), with genogroup I VP6, with NSP4B (Wa-like) genogroup and with long electropherotype where applicable. Sequence analyses of the NSP4 and the NSP5/6 genes confirmed that all the P[6] PoRV strains analyzed in this study are of porcine origin (Fig. 4).

Most of these genes constellations are commonly identified in pigs (Gouvea et al., 1994a, 1994b; Rácz et al., 2000; Winiarczyk et al., 2002; Ciarlet et al., 1995; Berreiros et al., 2003; Teodoroff et al., 2005). PoRVs with P[6] (Gottfried-like) VP4 specificity are widespread in porcine herds worldwide, as predicted by either PCR genotyping or nucleic

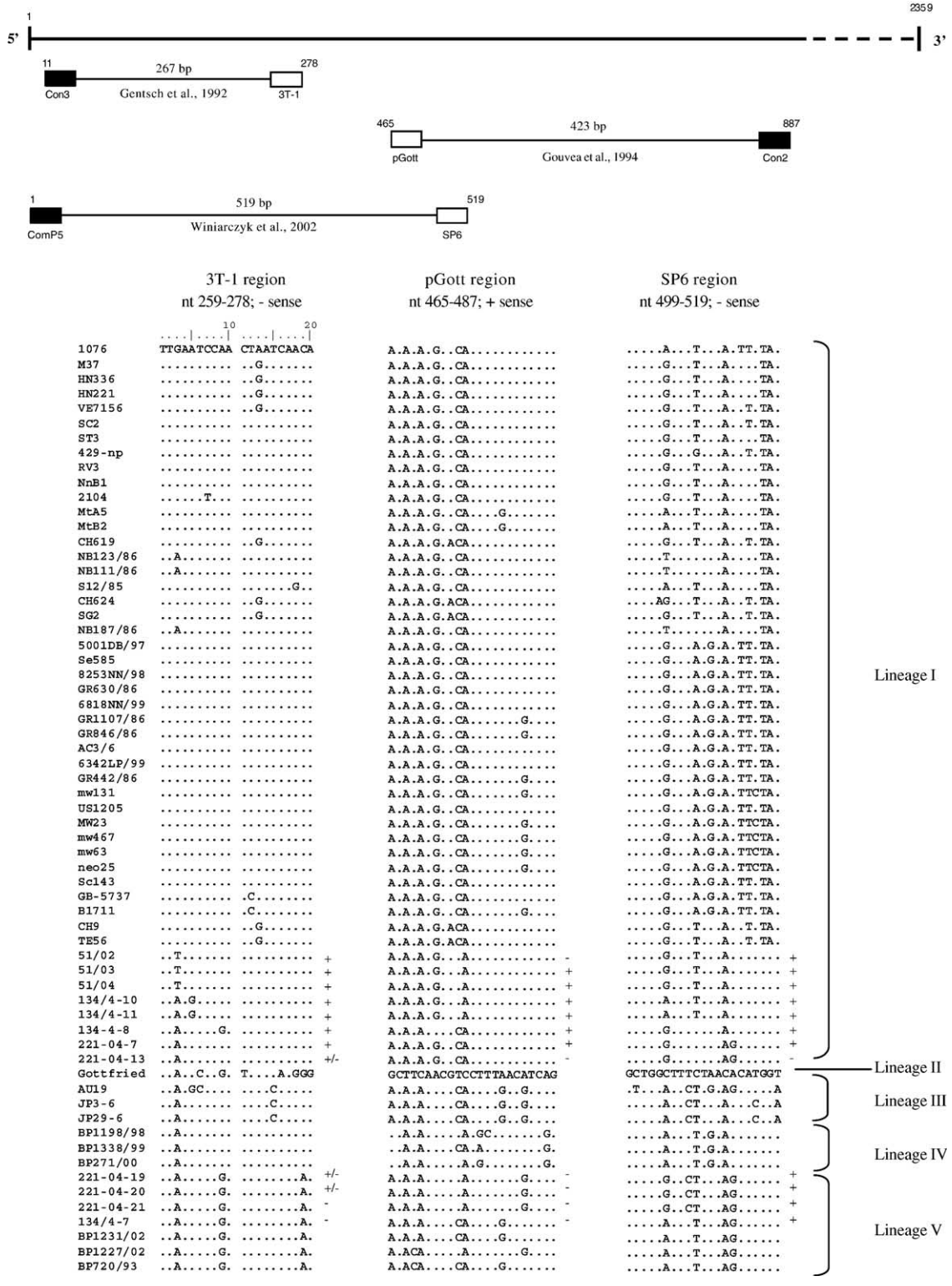

Fig. 1. Alignment (5′ to 3′) of the nucleotide sequence in the VP8* portion of the VP4 of several P[6] HRV strains and the PoRV strains identified in this study showing the binding sites of the three separate pools of P[6]-type-specific primers used in this study: pool H, including the P[6] (M37-like)-primer, 3T-1 (Gentsch et al., 1992); pool A, including the P[6] (Gottfried-like)-primer, pGott (Gouvea et al., 1994b); pool A2, including the P[6] (Gottfried-like)-primer, SP6 (Winiarczyk et al., 2002). The sequences in the left column are in consensus to HRV strain 1076. The sequences in the central and right columns are in consensus to PoRV strain Gottfried.

acid hybridization (Zaberezhny et al., 1994; Winiarczyk et al., 2002; Gouvea et al., 1994b; Rácz et al., 2000). However, most epidemiological studies on PoRVs sero/genotypes relied exclusively on specific reaction with individual P[6] primers/probes and until now, only the prototype P[6] PoRV strain Gottfried and two Japanese P[6] strains were analyzed in

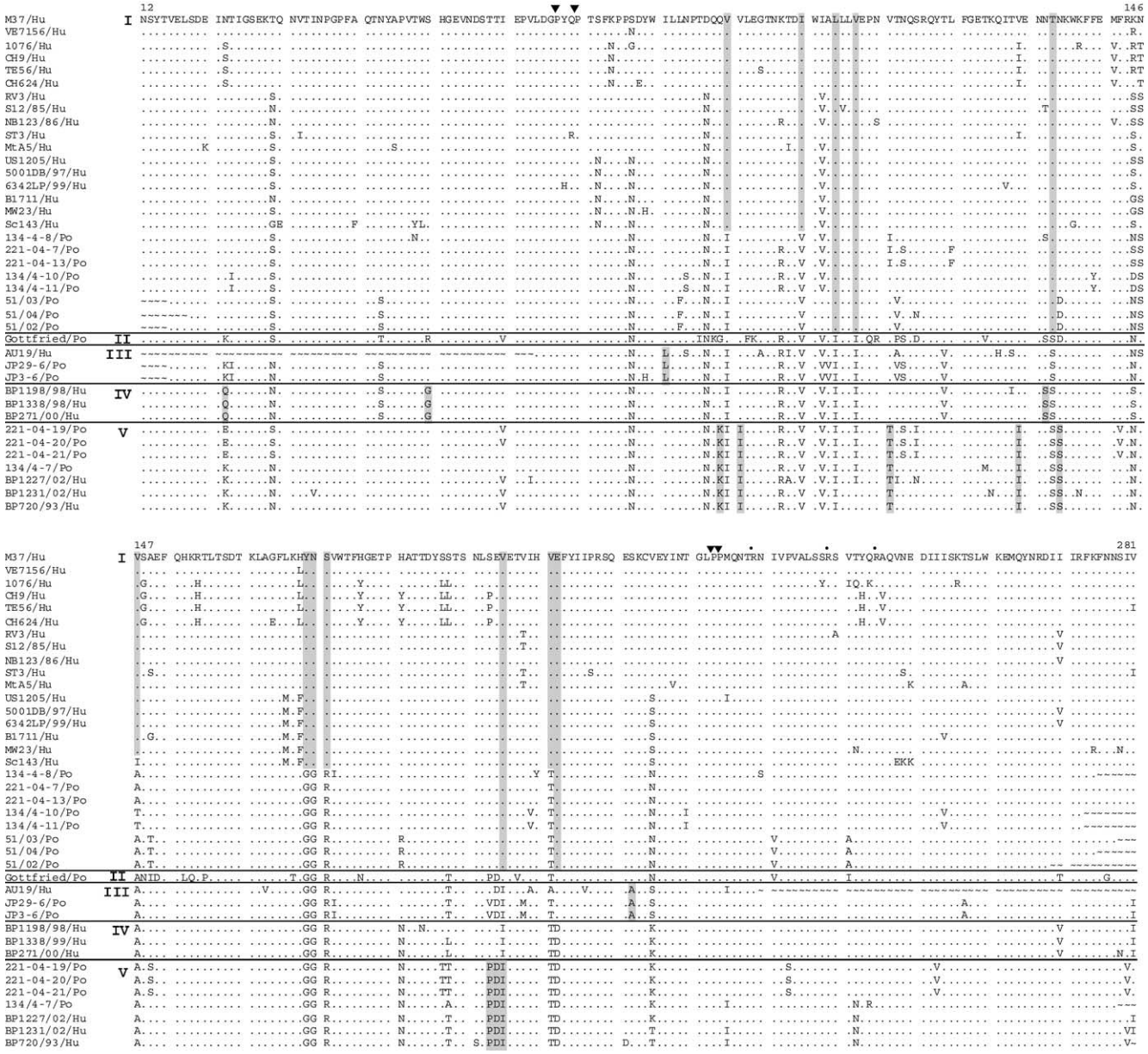

Fig. 2. Amino acid alignment of the PoRV P[6] strains identified in this study with representative HRV P[6] strains and the two Japanese PoRV P[6] strains. The highly conserved prolines (▼) and the trypsin-cleavage sites (●) are shown. Synapomorphisms are shaded in gray. The P6 lineages I to V are indicated.

detail (Gorziglia et al., 1990; Li and Gorziglia, 1993; Teodoroff et al., 2005).

The VP4 P[6] specificity of the Italian and Spanish PoRVs was initially predicted by PCR genotyping using the primer pGott (Gouvea et al., 1994b). Only 50% of the samples could be typed and 6 additional PoRV strains remained untypeable. When using primer SP6, designed to recognize strain Gottfried but targeting a different nucleotide region (Winiarczyk et al., 2002), the untypeable PoRVs were all characterized as P[6]. Subsequent sequence analysis unexpectedly revealed that all the P[6] strains identified were more similar to human P[6] strains rather than to the prototype porcine P[6] strain Gottfried. Eight of the PoRVs analyzed in this study clustered within the P[6] lineage I, which also includes the HRV prototype strain M37 and the majority of the P[6] HRV strains recognized to date from different parts of the world. The other four PoRVs (221/04-19, 221/04-20, 221/04-21 and 134/04-7), were clustered within the P[6]-V lineage, which includes the G4 HRV strains BP1231/02, PB1227/02 and BP720/93 identified in Hungary (Bányai et al., 2004).

Given the homology of the porcine P[6] sequences to the sequences derived from the human P[6] strains, all the PoRVs were retested with primer 3T-1 (Gentsch et al., 1992), designed to recognize the HRV strain 1076. Most of the strains within P[6] lineage I were recognized by primer 3T-1, while strains within lineage V were recognized weakly or they were not recognized at all. The accumulation of point mutations in HRV strains may have resulted in borderline or no reactivity, particularly for those strains within lineage V.

Table 2
VP8* nucleotide (right side) and amino acid (left side) comparison of P[6] PoRV and HRV strains

	HN336/ Hu/I	M37/ Hu/I	US1205/ Hu/I	RV3/ Hu/I	S12/ 85/ Hu/I	ST3/ Hu/I	MW23/ Hu/I	1076/ Hu/I	VE7156/ Hu/I	5001DB/ 97/Hu/I	B1711/ Hu/I	CH9/ Hu/I	TE56/ Hu/I	CH624/ Hu/I	51/03/ Po/I	51/04/ Po/I
HN336/Hu/I	–	0.0	4.5	2.9	3.9	2.7	4.7	5.4	1.5	4.2	5.6	3.8	4.0	3.9	10.0	10.5
M37/Hu/I	0.0	–	4.5	2.9	3.9	2.7	4.7	5.4	1.5	4.2	5.6	3.8	4.0	3.9	10.0	10.5
US1205/Hu/I	4.1	4.1	–	4.4	4.8	4.9	2.3	8.1	4.5	0.6	3.3	6.5	6.7	6.9	10.6	11.1
RV3/Hu/I	3.0	3.0	3.3	–	2.1	2.5	4.3	6.5	3.2	4.0	5.6	5.1	5.4	5.5	9.9	10.2
S12/85/Hu/I	3.3	3.3	4.1	1.5	–	3.6	5.1	7.5	4.2	4.4	6.1	6.1	6.4	6.5	10.1	10.5
ST3/Hu/I	4.1	4.1	5.9	4.1	4.8	–	4.8	6.4	3.2	4.5	5.9	5.0	5.0	5.4	9.9	10.2
MW23/Hu/I	5.2	5.2	2.2	4.8	5.5	7.4	–	7.7	4.4	2.2	3.1	6.4	6.6	6.7	10.8	11.1
1076/Hu/I	7.1	7.1	9.7	9.3	9.7	10.0	10.4	–	5.4	7.9	8.8	3.4	3.7	3.8	13.1	13.5
VE7156/Hu/I	1.1	1.1	3.7	3.7	4.1	4.8	4.8	6.3	–	4.2	5.6	3.8	4.0	4.2	10.0	10.3
5001DB/97Hu/I	3.7	3.7	1.1	3.0	3.7	5.5	2.6	9.7	3.3	–	3.2	6.3	6.5	6.6	10.5	11.0
B1711/Hu/I	4.4	4.4	1.8	4.4	4.4	6.6	2.6	10.0	4.1	2.2	–	7.6	7.8	8.0	10.3	10.7
CH9/Hu/I	5.9	5.9	8.9	8.1	8.5	8.9	10.0	4.1	5.5	8.9	9.2	–	0.5	0.4	12.1	12.4
TE56/Hu/I	6.6	6.6	9.6	8.9	9.2	8.9	10.7	4.8	6.3	9.6	10.0	0.7	–	0.9	12.2	12.5
CH624/Hu/I	6.3	6.3	9.6	8.9	9.2	9.6	10.7	5.2	6.6	9.6	10.0	1.1	1.8	–	12.2	12.5
ES51/03/Po/I	8.2	8.2	8.6	8.6	9.4	9.8	9.4	14.2	8.2	9.0	9.0	13.3	13.7	14.1	–	0.3
ES51/04/Po/I	8.6	8.6	8.9	8.9	9.7	10.1	9.7	14.5	8.6	9.3	9.3	13.6	14.0	14.4	0.4	–
134-4-8/Po/I	7.6	7.6	7.2	7.2	7.6	9.5	8.0	13.8	7.6	7.6	8.0	12.9	13.3	13.7	5.9	6.3
221-04-13/Po/I	6.5	6.5	5.7	5.7	6.5	8.4	7.3	12.7	6.5	6.1	6.9	11.9	12.6	12.6	5.1	5.5
221-04-7/Po/I	6.2	6.2	5.4	5.4	6.2	8.1	6.9	12.4	6.2	5.8	6.5	11.5	12.3	12.3	4.8	5.1
134/4-10/Po/I	8.0	8.0	7.6	7.6	8.4	9.9	8.0	14.2	8.0	8.0	7.6	13.4	13.7	14.1	6.7	7.5
134/4-11/I/Po/I	8.0	8.0	7.6	7.6	8.4	9.9	8.0	14.2	8.0	8.0	7.6	13.4	13.7	14.1	6.7	7.5
BP1198/98/Hu/IV	10.7	10.7	11.1	10.4	10.0	12.2	12.6	16.8	10.7	10.0	11.5	15.6	15.6	16.3	9.8	10.2
BP1338/99/Hu/IV	10.3	10.3	10.7	10.0	9.6	11.8	12.2	15.6	10.3	9.6	11.1	14.4	14.4	15.1	9.4	9.7
BP271/00/Hu/IV	10.7	10.7	11.1	10.6	10.0	12.2	11.8	16.0	10.7	10.0	11.4	14.8	14.8	15.5	9.4	9.7
221-04-19/Po/V	14.4	14.4	14.8	14.8	15.6	15.2	16.0	18.8	14.4	14.4	15.2	17.1	17.9	17.9	12.5	12.5
221-04-20/Po/V	14.3	14.3	14.7	14.7	15.5	15.1	15.8	19.0	14.3	14.3	15.1	17.0	17.7	17.7	12.5	12.5
221-04-21/Po/V	14.0	14.0	14.4	14.4	15.2	14.8	15.5	18.7	14.0	14.0	14.8	16.7	17.4	17.4	12.1	12.1
134/4-7/Po/V	12.4	12.4	12.4	13.1	13.1	13.9	13.1	16.6	12.4	12.7	13.1	15.4	15.7	16.1	11.7	12.1
BP1227/02/Hu/V	13.3	13.3	14.0	14.0	14.0	14.4	14.4	17.8	13.3	13.7	14.0	16.2	16.2	17.0	12.1	11.7
BP1231/02/Hu/V	13.3	13.3	13.3	14.0	14.0	14.4	14.4	17.5	13.3	13.7	14.0	16.2	16.2	17.0	12.1	12.5
BP720/93/Hu/V	12.6	12.6	12.6	13.4	13.4	14.1	13.8	17.2	12.6	13.0	13.4	15.6	16.0	16.4	11.7	12.1
AU19/Hu/III	19.9	19.9	18.1	18.7	19.9	22.3	19.3	25.6	19.9	19.3	19.3	25.9	25.9	27.1	16.3	16.9
JP3-6/Hu/III	13.2	13.2	13.5	13.5	13.5	14.7	13.9	18.9	13.2	13.2	13.5	17.7	17.7	18.4	10.9	11.7
Gottfried/Po/II	18.1	18.1	18.5	18.1	18.5	19.9	19.6	22.3	18.1	17.7	18.8	21.4	22.1	22.1	15.2	15.2

Sequence variation is expressed in %.

The identification of similar point mutations in the HRV strains implies that for efficient typing of all P[6] strains the primer sequence needs to be modified to include the substitution identified, particularly those closest to the 3′ end as these could significantly reduce the typing ability for strains within this genotype. Also, the recognition of most P[6]-I PoRVs by the 3T-1 primer must be taken with caution. Previously, Rácz et al. (2000) identified P[6] PoRV strains that were detected with primer 3T-1 or with both primer 3T-1 and pGott and these findings were interpreted as possible infections with human rotaviruses either alone or mixed with animal strains. In the view of the present findings, it is likely that both the M37-like P[6] and the double-reactive P[6] PoRV strains identified in Brazil may have been related to the P[6]-I PoRVs identified in this study.

An interesting and novel observation was evident from the molecular analysis of the P[6] animal and human strains. Of the five P6 lineages identified thus far (Bányai et al., 2004), at least three P[6] lineages, I, III and V, are shared by both human and porcine strains. Eight Italian P[6] PoRVs were found to be genetically more closely related to the human P[6]-I strains (up to 94.6% aa identity), rather than to the prototype strain Gottfried, P[6]-II (less than 85.1% aa identity). However, all the HRV P[6]-I strains displayed a clear monophyletic origin, strongly supported by statistical analysis (bootstrap value 100%) and by a number of synapomorphisms. Such findings are consistent with a unique bottle-neck event leading to the onset and spread of HRV P[6]-I RVs. Whether the P[6]-I VP4 gene allele has been introduced into HRVs by genetic exchange with a P[6]-I PoRV is not certain. However, this hypothesis is extremely intriguing, as such gene allele has reached, over time, a prominent diffusion among human rotaviruses and now it is regarded as one of the most common human P types (Santos and Hoshino, 2005).

Four PoRVs were clustered within the P[6]-V lineage, along with human G4 HRV Hungarian strains. In particular, strain 134/04-7 exhibited up to 96.7% aa sequence identity to the Hungarian strains. This is strongly suggestive of a common evolutionary origin of the P[6]-V HRV and PoRVs and may represent a recent interspecies transmission event, between pigs and humans, that occurred independently from the event leading to the onset of the P[6]-I (M37-like) human strains. The fact that the three unusual P[6]-V,G4 Hungarian strains were detected between 1993 and 2002 (therefore over a 10-year period) may suggest that such strains are able to persist in the human host, i.e. that they may be successfully transmitted among humans.

134-4-8/ Po/I	221-04-13/ Po/I	221-04-7/ Po/I	134/ 4-10/ Po/I	134/ 4-11/ Po/I	BP1198/ 98/Hu/IV	BP1338/ 99/Hu/IV	BP271/ 00/Hu/IV	221-04-19/ Po/V	221-04-20/ Po/V	221-04-21/ Po/V	134/4-7/ Po/V	BP1227/ 02/Hu/V	BP1231/ 02/Hu/V	BP720/ 93/Hu/V	AU19/ Hu/III	JP3-6/ Po/III	Gottfired/ Po/II
9.8	8.1	8.0	11.0	11.0	15.5	15.2	15.1	15.2	15.2	15.1	15.5	16.1	15.7	15.6	20.2	17.5	19.6
9.8	8.1	8.0	11.0	11.0	15.5	15.2	15.1	15.2	15.2	15.1	15.5	16.1	15.7	15.6	20.2	17.5	19.6
10.6	8.1	8.0	11.2	11.1	14.8	14.6	14.5	14.8	14.8	14.8	15.9	16.3	15.7	15.6	19.6	17.5	18.8
9.3	7.6	7.5	11.5	11.5	14.8	14.6	14.5	15.8	15.8	15.8	16.2	16.8	16.4	16.3	20.4	17.6	19.7
9.7	7.9	7.8	11.9	11.9	15.1	15.0	14.8	16.2	16.2	16.1	15.9	16.5	16.2	16.0	20.8	17.6	19.2
9.7	7.9	7.8	11.9	11.9	15.1	15.1	15.0	15.0	14.9	14.9	15.7	15.9	15.6	15.4	21.0	17.7	20.5
10.6	8.5	8.4	11.3	11.3	15.2	15.0	14.6	15.2	15.2	15.1	16.3	16.8	16.0	16.0	20.4	17.9	20.0
12.4	11.2	11.1	14.0	14.0	17.6	17.1	17.0	18.4	18.4	18.3	17.3	18.4	17.8	17.9	22.4	20.8	21.5
10.0	8.2	8.1	11.7	11.6	14.8	14.6	14.5	15.6	15.6	15.5	15.7	16.4	16.1	15.9	20.4	17.4	19.9
10.5	8.0	7.9	11.0	11.0	14.5	14.2	14.1	14.7	14.7	14.6	15.8	15.9	15.6	15.4	19.8	17.4	18.6
11.5	9.0	8.9	11.2	11.1	15.3	15.1	15.0	16.0	15.9	15.9	16.9	17.0	16.4	16.5	20.0	18.2	19.6
11.5	10.0	9.9	13.5	13.4	16.6	16.1	15.9	16.9	16.8	16.8	16.8	17.5	16.9	17.0	22.6	19.5	21.2
11.6	10.3	10.2	13.6	13.5	16.3	15.8	15.7	17.4	17.3	17.3	16.9	17.3	16.8	17.0	22.8	19.8	21.2
11.6	10.4	10.3	13.6	13.5	16.9	16.4	16.3	17.0	16.9	16.9	16.9	17.6	17.1	17.2	22.8	19.6	21.2
8.8	7.5	7.5	10.7	10.7	13.9	13.6	13.5	14.9	14.9	14.7	14.7	15.6	15.4	15.1	19.4	15.8	18.2
9.1	7.8	7.8	11.1	11.1	14.2	14.0	13.8	15.3	15.3	15.0	15.3	15.9	15.7	15.4	19.8	16.3	18.1
–	4.6	4.5	10.6	10.5	13.7	13.6	13.6	14.2	14.2	14.2	13.8	14.6	14.6	14.4	18.6	15.4	18.6
3.6	–	0.0	9.1	9.1	12.7	12.7	12.9	13.4	13.3	13.2	12.5	14.1	13.1	12.9	19.0	14.9	18.7
3.2	0.0	–	9.2	9.2	12.7	12.7	13.0	13.4	13.3	13.2	12.6	14.2	13.2	13.1	19.0	15.1	18.8
5.7	4.0	4.0	–	0.0	14.7	15.0	14.7	14.6	14.6	14.5	13.7	14.3	14.1	14.3	19.4	16.6	17.9
5.7	4.0	4.0	0.0	–	14.7	14.9	14.7	14.5	14.5	14.5	13.7	14.3	14.1	14.3	19.4	16.6	17.8
8.8	7.7	7.7	9.6	9.6	–	1.8	1.8	14.0	13.9	13.8	13.7	14.6	14.3	14.0	17.6	14.6	19.4
8.4	7.3	7.3	9.2	9.2	1.1	–	1.0	14.0	13.9	13.7	13.4	14.1	14.5	14.0	17.4	14.5	19.6
8.4	7.7	7.7	9.2	9.2	1.5	0.4	–	13.7	13.7	13.5	13.4	14.2	14.4	14.1	17.4	14.6	19.1
12.1	10.0	10.1	12.9	12.9	10.7	9.9	10.3	–	0.0	0.1	8.2	7.9	7.8	7.3	19.0	16.5	19.1
12.1	10.0	10.0	12.9	12.9	10.6	9.8	10.2	0.0	–	0.1	8.2	7.9	7.8	7.3	19.0	16.5	19.1
11.7	9.6	9.6	12.5	12.5	10.3	9.5	9.8	0.4	0.4	–	7.9	8.1	8.0	7.5	19.0	16.3	19.3
10.6	9.3	9.4	11.5	11.5	7.9	7.1	7.1	5.8	5.7	5.4	–	4.6	4.7	3.9	18.8	15.7	19.2
11.4	10.3	10.4	12.2	12.2	8.5	7.7	8.1	5.7	5.7	6.1	3.4	–	5.0	4.1	19.2	15.2	18.3
11.0	10.3	10.4	11.8	11.8	9.6	8.9	9.2	6.5	6.4	6.8	3.4	4.1	–	1.7	20.2	16.3	19.5
10.6	9.7	9.7	11.5	11.5	9.3	8.6	8.9	5.7	5.7	6.1	3.0	3.7	1.9	–	19.6	15.9	19.4
15.7	14.6	14.6	14.5	14.5	13.3	12.7	12.7	17.5	17.5	17.5	15.1	15.1	15.7	15.7	–	10.8	24.8
10.8	9.6	9.7	11.2	11.2	8.7	7.9	8.3	11.8	11.7	11.4	9.9	9.8	10.9	10.6	10.8	–	20.6
16.0	14.9	15.0	17.2	17.2	15.6	15.1	15.5	15.6	15.5	15.9	15.7	15.1	16.6	16.0	24.1	16.2	–

An additional P[6] lineage, AU-19-like (lineage III), was found to be shared by both human and porcine strains. Analysis of PoRVs recently detected in Japan (Teodoroff et al., 2005) identified two P[6],G9 strains, JP3-6 and JP29-6, more closely related in the VP8* (89% nt and 90% aa) to the prototype P[6]-III strain AU19, isolated in Japan in 1997, rather than to the porcine strain Gottfried. Although a single human strain has been identified in this lineage, this is suggestive of an additional meeting point, during evolution, of human and animal rotaviruses. Additional sequence analysis of either typeable or non-typeable P[6] human strains could help clarify the epidemiological relevance in humans of the minor P[6] lineages III, IV and V identified. Finally, no P[6]-II, Gottfried-like, strain was detected during our surveys in Spain and Italy. It is possible that such lineage is rare in the field, or at least in Southern Europe. A more extensive epidemiological survey could help assess the relative frequency of the P2B[6]-II strains in the European continent.

Evidence for genetic reassortment between human and animal rotaviruses has been collected in more occasions and, in particular, two animal species in particular, cows and pigs, appear to contribute often to the antigenic/genetic diversity found in human rotaviruses (Bányai et al., 2003; Iturriza-Gómara et al., 2003, 2004; Martella et al., 2005; Nakagomi et al., 1994; Santos and Hoshino, 2005), presumably because of the close interactions between humans and these animals driven either by cultural and/or economic forces. 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 stable introduction and successful spread of animal gene alleles in HRVs. Accordingly, animal rotaviruses, similar to what observed for other segmented-RNA viruses such as influenza viruses (Webster, 2002), might act as a frequent, vast source/reservoir of gene alleles for HRVs. Simultaneous surveillance of animal and human rotavirus infections is therefore paramount for the understanding of the evolution of these viruses.

Material and methods

Origin of the virus strains

Rotavirus-positive samples were collected from nursing and weaning piglets in outbreaks of diarrhea that occurred in different swine herds from Spain and Italy. Rotaviruses were identified in Spain (32 samples) during 2001–2003, and in the Umbria, Lombardia and Emilia Romagna regions, Italy (25 samples) in 2003–2004. The samples were collected directly from the rectum of animals affected with symptoms of enteritis. Rotavirus antigens were detected in the rectal swabs by an indirect immunoperoxidase technique, by electron microscopy

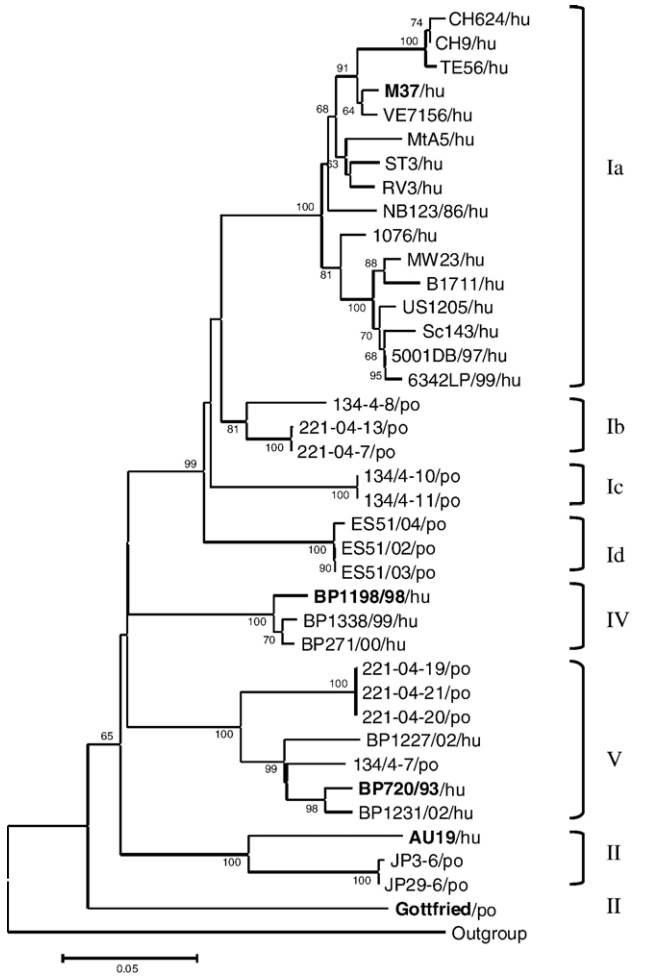

Fig. 3. Bootstrapping consensus tree constructed with the neighbor-joining method, using the VP8* genes of a selection of P[6] HRV and PoRV strains available through GenBank and the 12 PoRV strains identified in Italy and Spain in this study. The branches of the tree are drawn to scale. The tree was rooted using the human P[19] strain Mc345 as outgroup.

or a by commercial immunoenzymatic assay specific for group A rotaviruses (Rotascreen Dipstick, Microgen Bioproducts, Camberley, UK).

Extraction of virus dsRNA and prediction of the G and P by PCR genotyping and analysis of the VP6

Rotavirus-positive specimens were processed for dsRNA extraction using the guanidine isothiocyanate/Glass Milk method as described previously (Gentsch et al., 1992). G and P specificities were initially identified by genotyping techniques based on reverse transcription (RT)-PCR according to methods published elsewhere, using different sets of G- and P-type-specific primers (Gouvea et al., 1990, 1994a, 1994b; Gentsch et al., 1992; Das et al., 1994; Winiarczyk et al., 2002). For VP4 genotyping, three separate pools of P-type-specific primers were used, each including distinct P[6]-specific primers: pool H, including the P[6] (M37-like)-primer, 3T-1 (Gentsch et al., 1992); pool A, including the P[6] (Gottfried-like)-primer, pGott (Gouvea et al., 1994b); pool A2, including the P[6] (Gottfried-like)-primer, SP6 (Winiarczyk et al., 2002). Primer 3T-1 was originally designed on the sequence of strain 1076 to recognize M37-like P2A[6] HRV strains (Gentsch et al., 1992), while primers pGott and SP6 were designed on the sequence of strain Gottfried, to recognize Gottfried-like P2B[6] PoRV strains (Gouvea et al., 1994b; Winiarczyk et al., 2002).

Twelve PoRV strains (listed in Table 1) were identified as P[6] by PCR genotyping and the P[6] genotype was subsequently confirmed by sequence analysis of the VP8* gene sequences. The VP6 genogroup, predictive of VP6 subgroup, was determined by amplification and direct sequencing of a 379-bp fragment, spanning from aa 241 to 367 of the VP6, by using primer pair VP6F–VP6R (Iturriza-Gómara et al., 2002).

Determination of the dsRNA electropherotype (e-type)

Viral dsRNA was subjected to electrophoresis on 10% polyacrylamide gel and visualized by silver staining to determine the e-type. All the sample with visible e-types revealed 4:2:3:2 long patterns that are typical of animal group A rotaviruses (data not shown).

*Sequence and phylogenetic analysis of the VP8**

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 (Gentsch et al., 1992). For direct sequencing of PCR amplicons, three distinct amplicons were analyzed and a consensus sequence was determined. Sequences were assembled and analyzed using Bioedited software package (Department of Microbiology, North Carolina State University, USA) (Hall, 1999). The GenBank accession numbers assigned to the PoRVs analyzed in the present study are as follows: AY955303 (221/04-7); AY955302 (221/04-13); AY955307 (221/04-19); AY955308 (221/04-20); AY955309 (221/04-21); AY955310 (134/04-7); AY955301 (134/04-8); AY955299 (134/04-10); AY955300 (134/04-11); AY955304 (ES51/02); AY955305 (ES51/03); AY955306 (ES51/04).

Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 2.1 (Arizona State University, USA) (Kumar et al., 2001). A phylogenetic tree based on the VP8* was generated with both parsimony and distance methods, supplying a statistical support with bootstrapping over 100 replicates.

Amplification and sequence analysis of the NSP4 and NSP5/6 genes

The nearly full-length NSP4 gene was amplified with primer pair 10Beg16–10End722 (Lee et al., 2000), while the NSP5/NSP6 full-length gene was amplified with the primer pair described by Mohan and Atreya (2001). Partial sequences of the genes were determined by direct sequencing. The sequences of the NSP4 gene (approximately nt 20 to 720) are available under accession numbers DQ186199 and DQ189230 to DQ189240. The sequences of the NSP5/6 gene (aaproximately nt 50 to

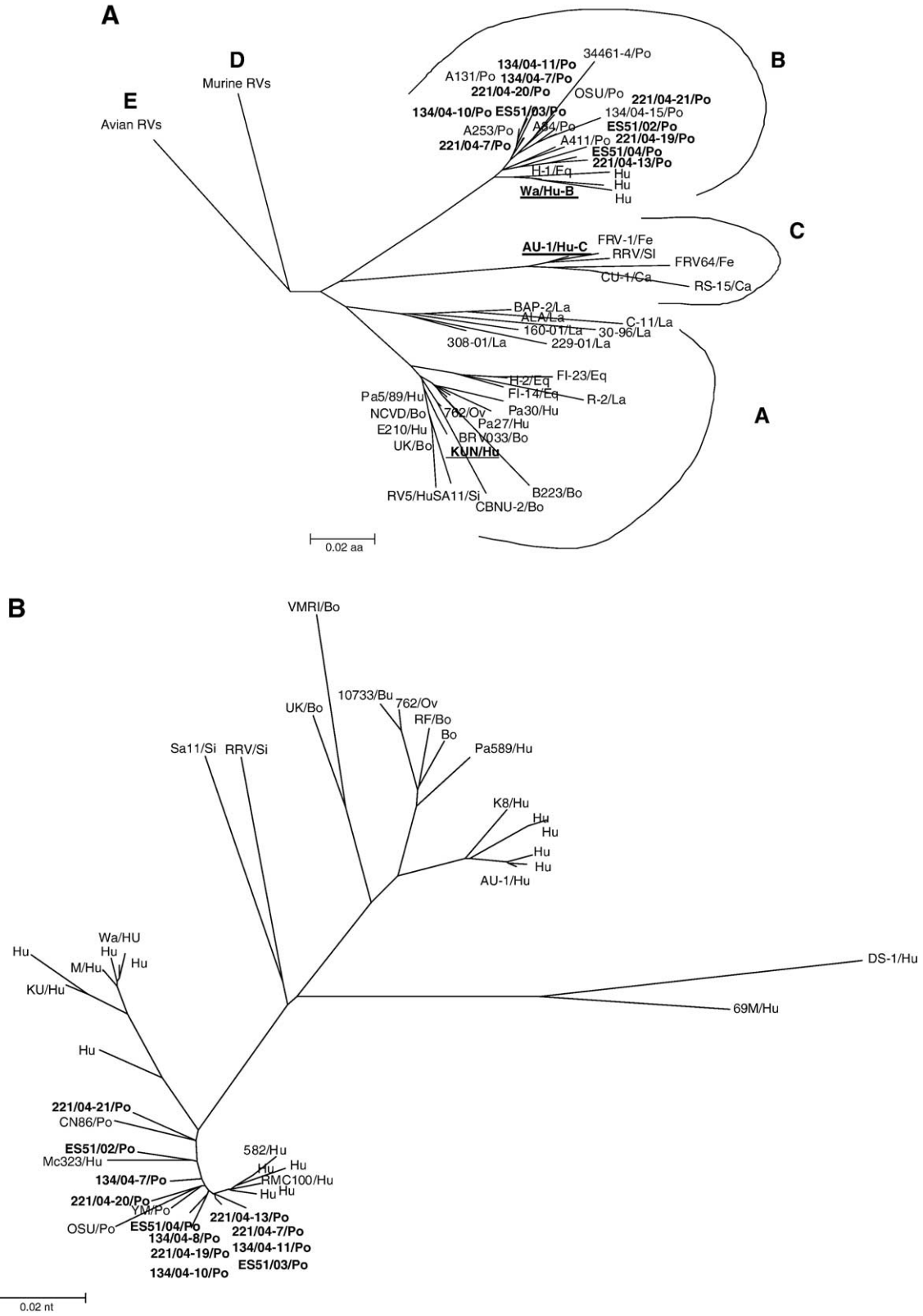

Fig. 4. Phylogenetic tree of the NSP4 and NSP5/6 genes of the P6 porcine strains (shown in boldface type). (A) NSP4 tree displaying the relationships among a selection of animal and human strains representative of the various NSP4 genetic groups. The branch of the murine (genogroup D) and avian (genogroup E) strains is not to scale. The prototypes of the NSP4 genogroups A, B and C (strains KUN, Wa and AU-1, respectively) are boldfaced and underlined. (B) NSP5 tree displaying the relationships among a selection of animal and human strains. The tree is inferred from the nucleotide alignment. Abbreviations: hu, human; po, porcine; bo, bovine; mu, murine; eq, equine; si, simian; ov, ovine; fe, feline; ca, canine; la, lapine.

650) are available under accession numbers DQ189243 to DQ189254.

Acknowledgments

The work was supported by grants from Ministero della Sanità (Progetto Finalizzato 2003, "Diversità genetica ed antigenica dei rotavirus, studio dei meccanismi evolutivi ed implicazioni ai fini diagnostici e vaccinali" and Progetto Finalizzato 2003, "Il ruolo del suino quale serbatoio e vettore di zoonosi: valutazione del rischio e proposte per nuove strategie") and from Ministero dell'Università (Fondi di Ateneo ex 60%). We thank Filomena Cariola, Caterina Nanna, Nicoletta Tutino and Donato Narcisi for their expert technical assistance. We are extremely grateful to Professor Leland Eugene Carmichael for continued encouragement throughout our studies.

References

Adah, M.I., Rohwedder, A., Olaleyly, O.D., Werchau, H., 1997. Nigerian rotavirus serotype G8 could not be typed by PCR due to nucleotide mutation at the 3′ end of the primer binding site. Arch. Virol. 142, 1881–1887.

Arias, C.F., Romero, P., Alvarez, V., López, S., 1996. Trypsin activation pathway of rotavirus infectivity. J. Virol. 70, 5832–5839.

Bányai, K., Gentsch, J.R., Griffin, D.D., Holmes, J.L., Glaa, R.I., Szücs, G., 2003. Genetic variability among serotype G6 human rotaviruses: identification of a novel lineage isolated in Hungary. J. Med. Virol. 71, 124–134.

Bányai, K., Martella, V., Jakab, F., Melegh, B., Szücs, G., 2004. Human genotype P[6] rotavirus strains detected in Hungary are phylogenetically distinct from strains within the three subtypes of serotype P2[6]. J. Clin. Microbiol. 42, 4338–4443.

Barnes, G.L., Lund, J.S., Adams, L., Mora, A., Mitchell, S.V., Caples, A., Bishop, R.F., 1997. Phase I trial of a candidate rotavirus vaccine (RV3) derived from a human neonate. J. Paediatr. Child Health 33, 300–304.

Barnes, G.L., Lund, J.S., Mitchell, S.V., De Bruyn, L., Piggford, L., Smith, A.L., Furmedge, J., Masendycz, P.J., Bugg, H.C., Bogdanovic-Sakran, N., Carlin, J.B., Bishop, R.F., 2002. Early phase II trial of human rotavirus vaccine candidate RV3. Vaccine 20, 2950–2956 (S0264-410X (02)00235-9).

Berreiros, M.A., Alfieri, A.A., Alfieri, A.F., Medici, K.C., Leite, J.P., 2003. An outbreak of diarrhoea in one-week-old piglets caused by group A rotavirus genotypes P[7],G3 and P[7],G5. Vet. Res. Commun. 27, 505–512.

Bohl, E.H., Theil, K.W., Saif, L.J., 1984. Isolation and serotyping of porcine rotaviruses and antigenic comparison with other rotaviruses. J. Clin. Microbiol. 19, 105–111.

Ciarlet, M., Ludert, J.E., Liprandi, F., 1995. Comparative amino acid sequence analysis of the major outer capsid protein (VP7) of porcine rotaviruses with G3 and G5 serotype specificities isolated in Venezuela and Argentina. Arch. Virol. 140, 437–451.

Cunliffe, N.A., Gondwe, J.S., Broadhead, R.L., Molyneux, M.E., Woods, P.A., Bresee, J.S., Glass, R.I., Gentsch, J.R., Hart, C.A., 1999. Rotavirus G and P types in children with acute diarrhea in Blantyre, Malawi, from 1997 to 1998: predominance of novel P[6]G8 strains. J. Med. Virol. 57, 308–312.

Das, M., Dunn, S.J., Woode, G.N., Greenberg, H.B., Rao, C.D., 1993. Both surface proteins (VP4 and VP7) of an asymptomatic neonatal rotavirus strain (I321) have high levels of sequence identity with the homologous proteins of a serotype 10 bovine rotavirus. Virology 194, 374–379. doi:10.1006/viro.1993.1271.

Das, B.K., Gentsch, J.R., Cicirello, H.G., Woods, P.A., Gupta, A., Ramachandran, M., Kumar, R., Bhan, M.K., Glass, R.I., 1994. Characterization of rotavirus strains from newborns in New Delhi, India. J. Clin. Microbiol. 32, 1820–1822.

Estes, M.K., 2001. Rotaviruses and their replication. In: Knipe, D.M., Howley, P.M., Griffin, D.E., Lamb, R.A., Martin, M.A., Roizman, B., Strais, S.E. (Eds.), Fields Virology, 4th ed. Lipincott William and Wilkins, Philadelphia, pp. 1747–1785.

Flores, J., Midthun, K., Hoshino, Y., Green, K., Gorziglia, M., Kapikian, A.Z., Chanock, R.M., 1986. Conservation of the fourth gene among rotaviruses recovered from asymptomatic newborn infants and its possible role in attenuation. J. Virol. 60, 972–979.

Flores, J., Pérez-Schael, I., Blanco, M., White, L., García, D., Vilar, M., Cunto, W., Gónzales, R., Urbina, C., Boher, J., Méndez, M., Kapikian, A.Z., 1990. Comparison of reactogenicity and antigenicity of M37 rotavirus vaccine ad rhesus-based quadrivalent vaccine. Lancet 336, 330–334.

Gentsch, J.R., Glass, R.I., Woods, P., Gouvea, V., Gorziglia, M., Flores, J., Das, B.K., Bhan, M.K., 1992. Identification of group A rotavirus gene 4 types by polymerase chain reaction. J. Clin. Microbiol. 30, 1365–1373.

Gorziglia, M., Hoshino, Y., Buckler-White, A., Blumentals, I., Glass, R., Flores, J., Kapikian, A.Z., Chanock, R.M., 1986. Conservation of amino acid sequence of VP8 and cleavage region of 84-kDa outer capsid protein among rotaviruses recovered from asymptomatic neonatal infection. Proc. Natl. Acad. Sci. U.S.A. 83, 7039–7043.

Gorziglia, M., Green, K., Nishikawa, K., Taniguchi, K., Jones, R., Kapikian, A.Z., Chanock, R.M., 1988. Sequence of the fourth gene of human rotaviruses recovered from asymptomatic or symptomatic infections. J. Virol. 62, 2978–2984.

Gorziglia, M., Nishikawa, K., Hoshino, Y., Taniguchi, K., 1990. Similarity of the outer capsid protein VP4 of the Gottfried strain of porcine rotavirus to that of asymptomatic human rotavirus strains. J. Virol. 64, 414–418.

Gouvea, V., Glass, R.I., Woods, P., Taniguchi, K., Clark, H.F., Forrester, B., Fang, Z.-Y., 1990. Polymerase chain reaction amplification and typing of rotavirus nucleic acid from stool specimens. J. Clin. Microbiol. 28, 276–282.

Gouvea, V., Santos, N., Timenetsky, M.C., 1994a. Identification of bovine and porcine rotavirus G types by PCR. J. Clin. Microbiol. 32, 1338–1340.

Gouvea, V., Santos, N., Timenetsky, M.C., 1994b. VP4 typing of bovine and porcine group A rotaviruses by PCR. J. Clin. Microbiol. 32, 1333–1337.

Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment and analysis program for Windows 95/98/NT. Nucleic Acids Symp. Ser. 41, 95–98.

Hoshino, Y., Kapikian, A.Z., 2000. Rotavirus serotypes: classification and importance in rotavirus epidemiology, immunity and vaccine development. J. Health Popul. Nutr. 18, 5–14.

Hoshino, Y., Wyatt, R.G., Flores, J., Midthun, K., Kapikian, A.Z., 1985. Serotypic characterization of rotaviruses derived from asymptomatic human neonatal infections. J. Clin. Microbiol. 21, 425–430.

Hoshino, Y., Jones, R.W., Kapikian, A.Z., 2002. Characterization of neutralization specificities of outer capsid spike protein VP4 of selected murine, lapine, and human rotavirus strains. Virology 299, 64–71.

Hoshino, Y., Jones, R.W., Ross, J., Santos, N., Kapikian, A.Z., 2003. Human rotavirus strains bearing VP4 gene P[6] allele recovered from asymptomatic or symptomatic infections share similar, if not identical, VP4 neutralization specificities. Virology 316, 1–8. doi:10.1016/S0042-6822(03)00543-9.

Iturriza-Gómara, M., Isherwood, B., Desselberger, U., Gray, J.J., 2001. Reassortment in vivo: driving force for diversity of human rotavirus strains isolated in the United Kingdom between 1995 and 1999. J. Virol. 75, 3696–3705.

Iturriza-Gómara, M., Wong, C., Blome, S., Desselberger, U., Gray, J., 2002. Rotavirus subgroup characterisation by restriction endonuclease digestion of a cDNA fragment of the VP6 gene. J. Virol. Methods 105, 99–103 (S0166-0934(02)00087-3).

Iturriza-Gómara, M., Desselberger, U., Gray, J.J., 2003. Molecular epidemiology of rotaviruses: genetic mechanisms associated with diversity. In: Desselberger, U., Gray, J.J. (Eds.), Viral Gastroenteritis. Perspectives in Medical Virology. Elsevier, Ámsterdam, pp. 317–344.

Iturriza-Gómara, M., Kang, G., Mammen, A., Jana, A.K., Abraham, M., Desselberger, U., Brown, D., Gray, J., 2004. Characterization of G10P[11] rotaviruses causing acute gastroenteritis in neonates and infants in Vellore, India. J. Clin. Microbiol. 42, 2541–2547.

Kapikian, A.Z., Hoshino, Y., Chanock, R.M., 2001. Rotaviruses. In: Knipe, D.M., Howley, P.M., Griffin, D.E., Lamb, R.A., Martin, M.A., Roizman, B., Strais, S.E. (Eds.), Fields Virology, 4th ed. Lipincott William and Wilkins, Philadelphia, pp. 1787–1833.

Kirkwood, C.D., Coulson, B.S., Bishop, R.F., 1996. G3P2 rotaviruses causing diarrhoeal disease in neonates differ in VP4, VP7 and NSP4 sequence from G3P2 strains causing asymptomatic neonatal infection. Arch. Virol. 141, 1661–1676.

Kumar, S., Tamura, K., Jakobsen, I.B., Nei, M., 2001. MEGA2: molecular evolutionary genetics analysis software. Bioinformatics 17, 1244–1245.

Larralde, G., Gorziglia, M., 1992. Distribution of conserved and specific epitopes on the VP8 subunit of rotavirus VP4. J. Virol. 66, 7438–7443.

Larralde, G., Kapikian, L.B., Gorziglia, M., 1991. Serotype-specific epitope (s) present on the VP8* subunit of rotavirus VP4. J. Virol. 65, 3213–3218.

Lee, C.-N., Wang, Y.-L., Kao, C.-L., Zao, C.-L., Lee, C.-Y., Chen, H.-N., 2000. NSP4 gene analysis of rotaviruses recovered from infected children with and without diarrhea. J. Clin. Microbiol. 38, 4471–4477.

Li, B., Gorziglia, M., 1993. VP4 serotype of the Gottfried strain of porcine rotavirus. J. Clin. Microbiol. 31, 3075–3077.

Liprandi, F., Gerder, M., Bastidas, Z., López, J.A., Pujol, F.H., Ludert, J.E., Joelsson, D.B., Ciarlet, M., 2003. A novel type of VP4 carried by a porcine rotavirus strain. Virology 315, 373–380. doi:10.1016/S0042-6822(03)00534-8.

Martella, V., Ciarlet, M., Camarda, A., Pratelli, A., Tempesta, M., Greco, G., Cavalli, A., Elia, G., Decaro, N., Terio, V., Bozzo, G., Camero, M., Buonavoglia, C., 2003. Molecular characterization of the VP4, VP6, VP7, and NSP4 genes of lapine rotaviruses identified in Italy: emergence of a novel VP4 genotype. Virology 314, 358–370. doi:10.1016/S0042-6822(03)00418-5.

Martella, V., Ciarlet, M., Baselga, R., Arista, S., Elia, G., Lorusso, E., Banyai, K., Terio, V., Madio, A., Ruggeri, F.M., Falcone, E., Camero, M., Decaro, N., Buonavoglia, C., 2005. Sequence analysis of the VP7 and VP4 genes identifies a novel VP7 gene allele of porcine rotaviruses, sharing a common evolutionary origin with human G2 rotaviruses. Virology 337, 111–123. doi: 10.1016/j.virol.2005.03.031.

McNeal, M.M., Sestak, K., Choi, A.H., Basu, M., Cole, M.J., Aye, P.P., Bohm, R.P., Ward, R.L., 2005. Development of a rotavirus-shedding model in rhesus macaques, using a homologous wild-type rotavirus of a new P genotype. J. Virol. 79, 944–954.

Midthun, K., Halsey, N.A., Jett-Goheen, M., Clements, M.L., Steinhoff, M., King, J.C., Karron, R., Wilson, M., Burns, B., Perkis, V., Samorodin, R., Kapikian, A.Z., 1991. Safety and immunogenicity of human rotavirus vaccine strain M37 in adults, children, and infants. J. Infect. Dis. 164, 792–796.

Mohan, K.V., Atreya, C.D., 2001. Nucleotide sequence analysis of rotavirus gene 11 from two tissue culture-adapted ATCC strains, RRV and Wa. Virus Genes 23, 321–329.

Nakagomi, O., Nakagomi, T., 1993. Interspecies transmission of rotaviruses studied from the perspective of genogroup. Microbiol. Immunol. 37, 337–348.

Nakagomi, O., Nakagomi, T., 2002. Genomic relationships among rotaviruses recovered from various animal species as revealed by RNA–RNA hybridization assays. Res. Vet. Sci. 73, 207–214.

Nakagomi, O., Isegawa, Y., Ward, R.L., Knowlton, D.R., Kaga, E., Nakagomi, T., Ueda, S., 1994. Naturally occurring dual infection with human and bovine rotaviruses as suggested by the recovery of G1P8 and G1P5 rotaviruses from a single patient. Arch. Virol. 137, 381–388.

Nakagomi, T., Horie, Y., Koshimura, Y., Greenberg, H.B., Nakagomi, O., 1999. Isolation of a human rotavirus strain with super-short RNA pattern and a new P2 subtype. J. Clin. Microbiol. 37, 1213–1216.

Pager, C.T., Alexander, J.J., Steele, A.D., 2000. South African G4P[6] asymptomatic and symptomatic neonatal rotavirus strains differ in their NSP4, VP8*, and VP7 genes. J. Med. Virol. 62, 208–216.

Pérez-Schael, I., Blanco, M., García, D., White, L., Alfonzo, E., Crespo, I., Cunto, W., Pittman, A.L., Kapikian, A.Z., Flores, J., 1994. Evaluation of the antigenicity and reactogenicity of varying formulations of the rhesus rotavirus-based quadrivalent and the M37 rotavirus vaccine candidates. J. Med. Virol. 42, 330–337.

Rácz, M.L., Kroeff, S.S., Munford, V., Caruzo, T.A.R., Durigon, E.L., Hayashi, Y., Gouvea, V., Palombo, E.A., 2000. Molecular characterization of porcine rotaviruses from southern region of Brazil: characterization of an atypical genotype G9 strain. J. Clin. Microbiol. 38, 2443–2446.

Rahman, M., Matthijnssens, J., Nahar, S., Podder, G., Sack, D.A., Azim, T., Van Ranst, M., 2005. Characterization of a novel P[25],G11 human group a rotavirus. J. Clin. Microbiol. 43, 208–212.

Ramachandran, M., Das, B.K., Vij, A., Kumar, R., Bhambal, S.S., Kesari, N., Rawt, H., Bahl, L., Thakur, S., Woods, P.A., Glass, R.I., Bhan, M.K., Gentsch, J.R., 1996. Unusual diversity of human rotavirus G and P genotypes in India. J. Clin. Microbiol. 34, 436–439.

Rao, C.D., Gowda, K., Reddy, B.S.Y., 2000. Sequence analysis of VP4 and VP7 genes of nontypeable strains identifies a new pair of outer capsid proteins representing novel P and G genotypes in bovine rotaviruses. Virology 276, 104–113. doi:10.1006/viro.2000.0472.

Santos, N., Hoshino, H., 2005. Global distribution of rotavirus serotypes/genotypes and its implication for the development and implementation of an effective vaccine. Rev. Med. Virol. 15, 29–56.

Santos, N., Riepenhoff-Talty, M., Clark, H.F., Offit, P., Gouvea, V., 1994a. VP4 genotyping of human rotavirus in the United States. J. Clin. Microbiol. 32, 205–208.

Santos, N., Gouvea, M., Timenetsky, M.C., Clark, H.F., Riepenhoff-Talty, M., Gabarg-Chenon, A., 1994b. Comparative analysis of VP8* sequences from rotaviruses possessing M37-like VP4 recovered from children with and without diarrhoea. J. Gen. Virol. 75, 1775–1780.

Steele, A.D., van Niekerk, M.C., Mphahlele, M.J., 1995. Geographic distribution of human rotavirus VP4 genotypes and VP7 serotypes in five South African regions. J. Clin. Microbiol. 33, 1516–1519.

Teodoroff, T.A., Tsunemitsu, H., Okamoto, K., Katsuda, K., Kohmoto, M., Kawashima, K., Nakagomi, T., Nakagomi, O., 2005. Predominance of porcine rotavirus G9 in Japanase piglets with diarrhea: close relationship of their VP7 genes with those of recent human G9 strains. J. Clin. Microbiol. 43, 1377–1384.

Timenetsky, M.C., Santos, N., Gouvea, V., 1994. Survey of rotavirus G and P types associated with human gastroenteritis in Sao Paulo, Brazil, from 1986 to 1992. J. Clin. Microbiol. 32, 2622–2624.

Vesikari, T., Ruuska, R., Koivu, H.P., Green, K.Y., Flores, J., Kapikian, A.Z., 1991. Evaluation of the M37 human rotavirus vaccine in 2- to 6-month-old infants. Pediatr. Infect. Dis. J. 10, 912–917.

Webster, R.G., 2002. The importance of animal influenza for human disease. Vaccine 20, 16–20. doi:10.1016/S0264-410X(02)00123-8.

Winiarczyk, S., Paul, P.S., Mummidi, S., Panek, R., Gradzki, Z., 2002. Survey of porcine rotavirus G and P genotype in Poland in the United States using RT-PCR. J. Vet. Med., B 49, 373–378.

Zaberezhny, A.D., Luoo, Y.S., Paul, P.S., 1994. Prevalence of P types among porcine rotaviruses using subgenomic VP gene probes. Vet. Microbiol. 39, 97–110. doi:10.1016/0378-1135(94)90090-6.