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Molecular characterization of a new porcine rotavirus P genotype found in an asymptomatic pig in Slovenia

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Abstract

Rotaviral RNA was detected in the stool sample of an asymptomatic fattening pig at a Slovenian pig farm. To characterize the rotavirus, RT-PCR was used, employing primers specific for the VP7, VP4 and NSP4 genes. Specific products were purified and the sequencing reaction was performed for the molecular analysis of amplified genes. Nucleotide and amino acid sequences of the VP7 gene were found highly identical (85.3–88.1% and 90.7–91.6%) to G1 genotype strains. Phylogenetic and molecular analyses of the VP7 antigen regions revealed the sample to be from a new lineage of G1 genotype. In the molecular analysis of the VP4 gene, only 70.9% nucleotide (76.2% amino acid) identity was found with the most related rotavirus VP4 gene from GenBank. Following this, the NSP4 gene was also analyzed. After the phylogenetic analysis, it clustered with the NSP4 B genotype, but also seemed to represent a new lineage of this genotype. This new rotavirus strain, named P21-5, differed greatly from all rotaviruses characterized so far in all three genes analyzed. The virulence of this strain is not clear yet and has to be investigated.

Keywords: Porcine rotavirus; P genotype; G genotype; Slovenia

Introduction

Rotaviruses are the major cause of acute diarrhea in animals, infants and children under 5 years old. In humans, approximately 440,000 deaths per year are caused worldwide by rotaviruses, and in animals there is also an economic impact on pig breeding (Kapikian et al., 2001; Parashar et al., 2003). Rotaviruses are unenveloped; the viral particles are three layered and their genome consists of 11 dsRNA segments. Six structural proteins (VP1–VP4, VP6 and VP7) and six non-structural viral proteins (NSP1–NSP6) are encoded in the viral genome. Rotaviruses are classified into 7 groups (A–G) based on the antigen specificity of the VP6 gene (Estes and Cohen, 1989). The most common group which infects humans and animals is group A. According to the VP4 (and VP4-gene) and VP7 (and VP7-gene) antigenic and molecular characterization, group A rotaviruses are further classified into different P and G types. Rotavirus strains are characterized with a dual classification system with G and P serotype or G and P genotype. Whilst G serotype and genotype match, there are differences between P serotypes and genotypes. Thus, it is important to be aware of this difference in the classification of rotaviruses (Parashar et al., 1998). At molecular level, strains with more than 89% identities in amino acid sequence are considered to be of the same genotype (Gorziglia et al., 1990). So far, 15 G and 26 P genotypes have been described (Estes, 2001; Hoshino et al., 2002; Liprandi et al., 2003; Martella et al., 2003, 2006; McNeal et al., 2005; Rahman et al., 2005; Rao et al., 2000). The most frequent genotype combinations in humans are G1 P[8], G2 P[4], G3 P[8], G4 P[8]. These are also the most predominant global genotypes (Gentsch et al., 1996). In the mid-nineties, a new emerging rotavirus G9 in combination with P[6] or P[8] genotype was spreading across all the continents. The origin of this genotype was probably in animals (Hoshino et al., 2005). It

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was speculated that animals are a reservoir for new rotaviruses following the interspecies transmission between humans and animals (Cook et al., 2004; Iturriza Gómara et al., 2003b). In addition, the infection of one cell with two different rotaviruses could produce a new rotavirus with a reassorted genome. Some evidence of genome reassortments has been previously observed (Gentsch et al., 2005). Recently, a full genome analysis of lapine and human rotavirus strains (30/06 and B4106) demonstrated that a child with severe gastroenteritis had been infected with a rotavirus with an entirely lapine genome complement (Matthijnssens et al., 2006).

Several different G and P genotypes have been found in pigs. The most prevalent porcine G genotypes are G3, G4, G5 and G11. In P typing, mostly P[6] and P[7] types have been found, but some other G and P types have also been detected in lower percentages in pigs (Barreiros et al., 2003; Martella et al., 2001; Ghosh et al., 2006; Teodoroff et al., 2005; Winiarczyk et al., 2002).

In this report, a new P genotype of porcine rotavirus is described, with a phylogenetic analysis of three of the genes in this strain which we name "P21-5". A comparison of VP4, VP7 and NSP4 genes of the P21-5 strain with the published sequences of all known G and P genotypes reported to date is presented. These analyses clearly demonstrate that the porcine P21-5 strain is carrying a new P genotype, bearing a very low nucleotide and amino acid identity with all known P types described so far. Furthermore, a comparison of the NSP4 gene with other published NSP4 genotypes shows this strain to differ in more than 10% of the amino acids coded.

Results

The 943-nucleotide and resultant 313-amino acid sequence of the VP7 gene were analyzed and compared with all 15 known G genotype sequences. As shown in Table 1, the strain P21-5 was most similar to other human or animal G1 types. The highest identity (88.1% nucleotide and 91.6% amino acid) was found with a human isolate AU007. The phylogenetic analysis confirmed a close relationship of Slovenian P21-5 porcine isolate to other G1 genotypes. It clustered with G1 genotypes in a monophyletic branch (Fig. 1). The VP7 amino acid sequence was compared with other animal G1 strains and different G1 strains belonging to four proposed human G1 lineages (Berois et al., 2003; Diwakarla and Palombo, 1999; Jin et al., 1996). The P21-5 strain was more closely related to the animal G1 strains as shown in Fig. 2. Animal G1 strains, including P21-5, were grouped separately from human representative strains of lineages I–IV. Furthermore, the P21-5 strain represents a separate lineage within animal G1 strains (Fig. 2). When comparing antigenic regions in the VP7 protein, significant differences ranging from 9% to 30% of amino acids were found in A, B, C, E and F regions, compared to the strain Wa (Fig. 3). Potential glycosylation sites at positions 69 (data not shown) and 238 in region F were also present in the P21-5 strain (Fig. 3). The most amino acid differences among analyzed epitopes were observed in regions A and C. It can be seen in antigen region A that the P21-5 strain is more similar to animal G1 strains than to human G1 isolates. All animal G1 strains have the amino acid asparagine conserved at positions 91 and 94, sites which tend to be associated with the binding of neutralizing antibodies (Green and Kapikian, 1992).

Table 1
Nucleotide and amino acid VP7 sequence identities of the Slovenian P21-5 strain with different G genotypes obtained from GenBank

Strain	Origin	G genotype	Sequence identity with P21-5 strain	
			Nucleotide	Amino acid
T449	Bovine	1	85.9	91.3
AU007	Human	1	88.1	91.6
C60	Porcine	1	85.3	91.6
KU	Human	1	88.0	90.7
AU19	Human	1	86.3	91.3
KUN	Human	2	72.6	73.8
AU-1	Human	3	74.2	79.8
Gottfried	Porcine	4	74.8	75.0
CC117	Porcine	5	74.2	78.2
NCDV	Bovine	6	74.0	79.8
Ch-2	Avian	7	61.6	57.8
B37	Human	8	69.8	74.4
WI61	Human	9	73.6	78.9
B223	Bovine	10	72.1	76.0
YM	Porcine	11	72.2	78.2
L26	Human	12	72.8	75.0
L338	Equine	13	72.4	74.4
FI23	Equine	14	74.5	78.5
Hg18	Bovine	15	72.1	75.0

More differences were found when the VP4 gene was compared with the prototypes of all P genotypes known to date (Table 2). After BLAST-ing the whole VP4 nucleotide sequence into GenBank, only short VP4 fragments with low identity were found. The nucleotide sequence was translated and the identity search was repeated, but this time with the deduced amino acid sequence. The highest identity (76.2%) found with an amino acid sequence in GenBank was with the rotavirus strain TUCH, which was of P[24] genotype specificity. The phylogenetic analysis also clearly demonstrated a new phylogenetic branch in the phylogenetic tree of all known P genotypes (Fig. 4).

The molecular analysis of the NSP4 gene, which encodes rotavirus enterotoxin, was also performed. The highest nucleotide sequence identity (91%) was found with the human strain A_G4_120 (data not shown). This human strain also revealed the highest amino acid identity (95%) with the P21-5 strain. All other strains in GenBank showed lower than 90% amino acid identity of their NSP4 gene with that of P21-5 (data not shown). In a phylogenetic tree of NSP4, the amino acid sequences of P21-5 and A_G4_120 were clustered separately from other NSP4 genotypes, but were closely related to the NSP4 genotype B (Fig. 5). It has been shown in previous studies that the amino acid region 135–141 of the NSP4 protein bears a certain degree of intraspecies conservation whilst having some interspecies variation (Mohan and Atreya, 2000). In Fig. 6, the NSP4 amino acid region 114–141 of different porcine, human and bovine strains is aligned and compared. The enterotoxin domain (aa 114–135) appears to be conserved among all rotavirus strains, including P21-5. Only one amino acid at position 135 (histidine) was replaced with tyrosine in the P21-5 strain. A

larger number of differences were found in the amino acid region of 135–141. It would seem that the P21-5 strain has some porcine specific sequence, although there are some altered amino acid residues that were also found in bovine (methionine at position 135) and human strains (lysine at position 137). Interestingly, in this specific region, the same amino acid sequence was found in the human A_G4_120 strain.

Discussion

The prevalence of rotavirus infection was investigated to determine the importance of asymptomatic shedding of rotaviruses. A pig farm from which the described rotavirus strain was obtained experienced no problems with diarrhea among pigs.

In this report, a novel strain of rotavirus was identified and genetically characterized. It was shown that this new rotavirus strain, the Slovenian rotavirus P21-5 strain, differed greatly in all three analyzed genes (VP4, VP7 and NSP4) from other strains in GenBank. The highest difference rate was found in the VP4 gene, where only 76.2% of amino acid identity was observed with the most related P genotype (Table 2). As the nucleotide and amino acid sequences shared very low identity with all other published P genotypes, rotavirus P21-5 was considered to represent a new P genotype. This was also

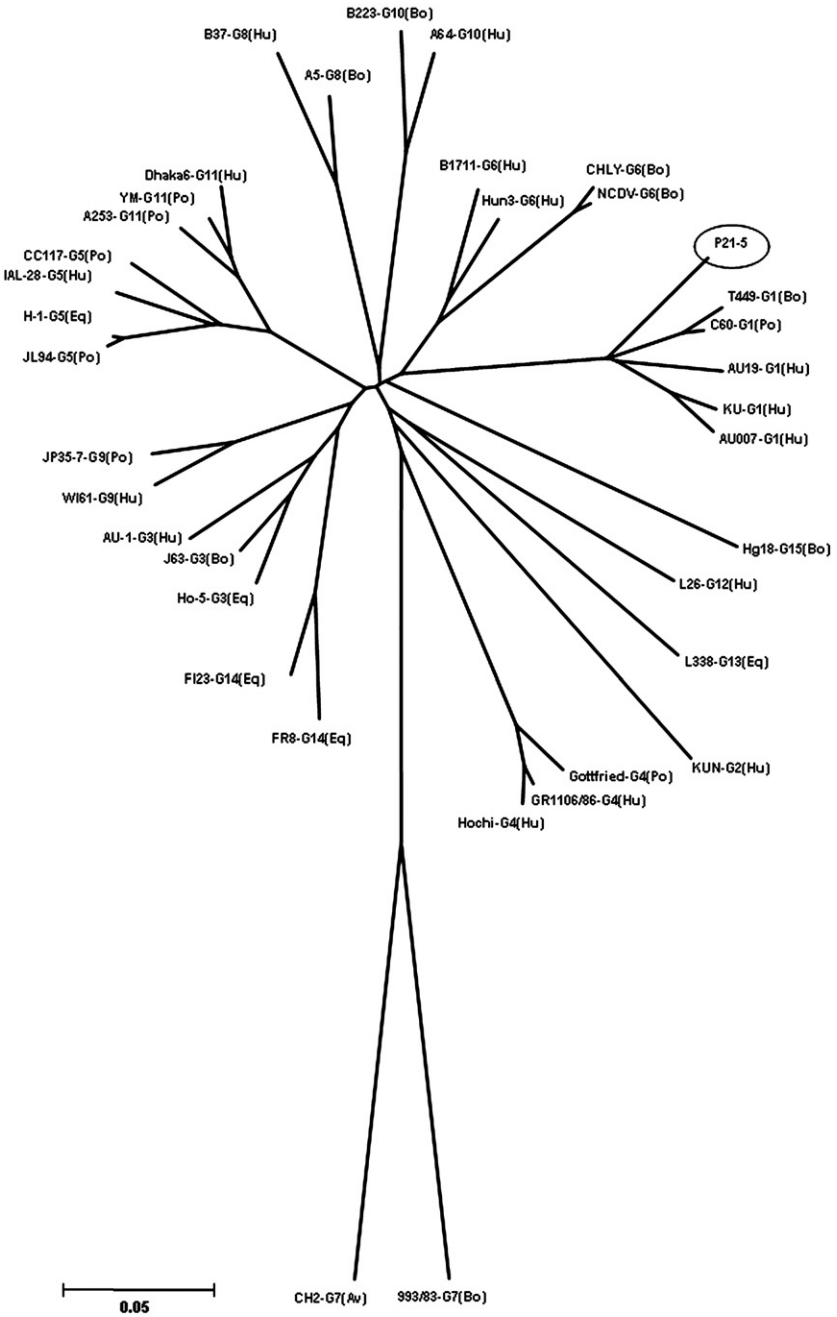

Fig. 1. Neighbor-joining phylogenetic tree based on the VP7 amino acid sequences of all known G genotypes (Av—avian, Bo—bovine, Eq—equine, Hu—human, Po—porcine).

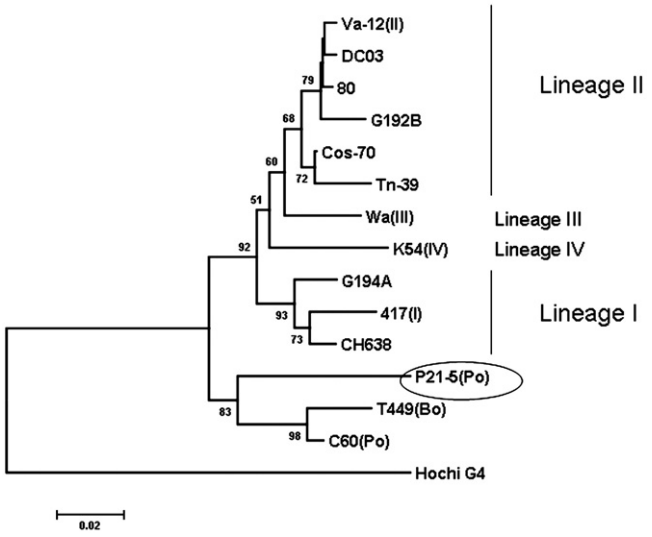

Fig. 2. Neighbor-joining phylogenetic tree based on the VP7 amino acid sequences of various human and animal G1 rotavirus strains with designation of G1 I–IV lineages. Animal G1 rotavirus strains are clustered separately to human G1 I–IV lineages (Po—porcine, Bo—cattle).

confirmed by the phylogenetic analysis of the VP8* fragment. It is clearly demonstrated that the porcine P21-5 strain from Slovenia represents a new branch in the phylogenetic tree of all known P genotypes (Fig. 4). The fragment VP8* of the VP4 gene contains the greatest amino acid divergence which correlates to the differentiation between P genotypes (Gentsch et al., 1992; Larralde and Gorziglia, 1992; Larralde et al., 1991). Therefore, the VP8* fragment of the VP4 gene was used for phylogenetic analysis. As shown by the calculations in Table 2 and as confirmed by the phylogenetic analysis (Fig. 4), there was enough information for the classification of the P21-5 strain into a different P genotype.

In the VP7 gene, P21-5 was identified as being of G1 genotype. Only a few G1 genotype strains have been detected in animals. In 1992, the bovine rotavirus strain T449 was identified as G1 genotype using an immunoperoxidase focus neutralization assay and this was confirmed with whole gene nucleotide and amino acid sequence analysis (Blackhall et al., 1992). Also, porcine C60 and C95 strains were assigned as G1 by a cross-neutralization test and characterized with nucleotide and amino acid sequence analysis (Ciarlet and Liprandi, 1994).

Table 2
The VP4 nucleotide (2265 nt length) and amino acid (754 aa length) sequence identities of the Slovenian P21-5 strain with different P genotypes obtained from GenBank

Strain	Origin	P genotype	Sequence identity with P21-5 strain	
			Nucleotide	Amino acid
NCDV	Bovine	1	70.8	74.9
SA-11	Simian	2	70.7	75.4
CU-1	Dog	3	69.2	73.8
DS-1	Human	4	68.1	68.9
B641	Bovine	5	66.6	69.7
Gottfried	Porcine	6	67.8	69.3
CRW-8	Porcine	7	69.8	73.6
Hochi	Human	8	66.6	66.1
K8	Human	9	64.6	64.5
69 M	Human	10	69.9	75.3
B223	Bovine	11	56.6	56.0
H-2	Equine	12	69.6	74.2
MDR-13	Porcine	13	68.9	71.0
JP13-3 [a]	Porcine	Unusual 13 or 22	60.6	54.2
PA169	Human	14	63.6	64.9
Lp14	Sheep	15	70.4	74.5
EB	Mouse	16	66.1	71.1
PO-13	Pigeon	17	60.8	57.9
L338	Equine	18	70.3	71.7
4F	Porcine	19	69.6	71.7
EHP	Mouse	20	66.1	72.9
Hg18	Bovine	21	70.0	72.1
3489/3[a]	Lapine	22	61.4	54.6
A34[a]	Porcine	23	66.6	63.9
Tuch	Simian	24	70.9	76.2
Dhaka6	Human	25	63.2	62.7
134/04-15	Porcine	26	69.2	72.1

[a] Amino acid identity was calculated based on VP8* region of VP4 gene.

Unfortunately there are no reports on P genotypes of T449, C60 and C95 strains. The only described animal G1 genotype with a characterized VP4 gene is the porcine SW20/21 strain with P[7] specificities (El-Attar et al., 2001). It would therefore appear that the G1 rotavirus genotype is not a common diarrhea-inducing pathogen in animals. Although nucleotide and amino acid identities with other G1 strains were low, the phylogenetic analysis ranged it in a monophyletic branch with other G1 strains, which was statistically supported by a bootstrap of 100% (Fig. 1). Phylogenetic analysis of animal and human G1 strains shows that the G1 lineages determined by Jin et al.

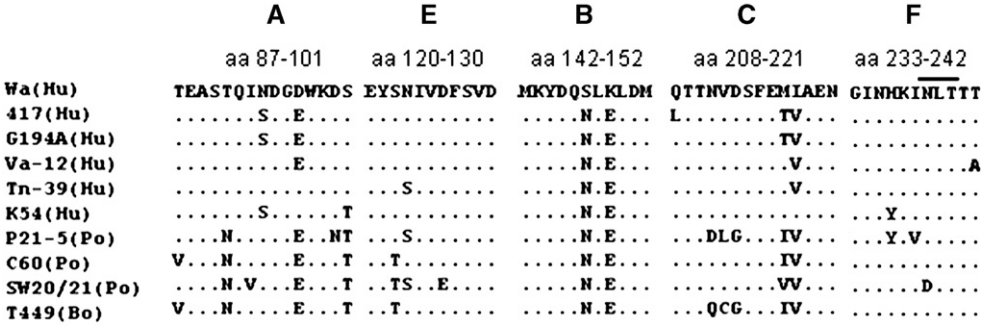

Fig. 3. Antigenic sites of human and animal G1 genotypes compared with the P21-5 strain (aa 87–101: region A; aa 120–130: region E; aa 142–152: region B; aa 208–221: region C; aa 233–242: region F). Potential glycosylation site is marked with a line above the amino acid sequence in antigen region F.

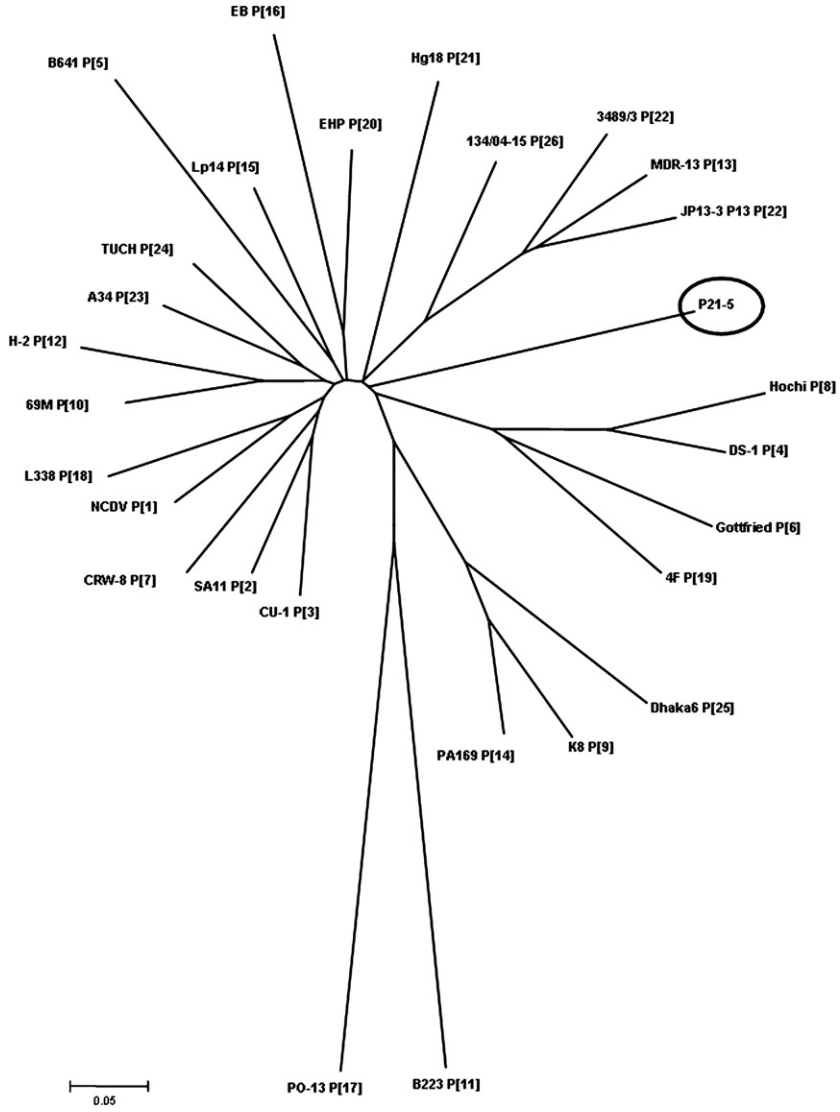

Fig. 4. Neighbor-joining phylogenetic tree of the VP8* amino acid sequences of all known P genotypes.

(1996) do not include animal G1 strains (Fig. 2). Animal strains also seem to be substantially different from human G1 isolates in antigen regions. In the antigen region A, the difference between animal and human G1 strains was found at amino acid 91; which is asparagine and not threonine as it is in human strains. Asparagine is also present at amino acid position 94 in the P21-5 strain and in all animal strains (Fig. 3). This specific amino acid was associated with the designation of human G1 lineages II and monotype 1a (Ciarlet and Liprandi, 1994; Diwakarla and Palombo, 1999). In the study of Green and Kapikian (1992), the antigenic site A was characterized as an immunodominant protective epitope. In the same study it was also shown that the presence of serum antibodies that blocked binding of monoclonal antibodies mapping to asparagine at position 94 was correlated with resistance to illness or shading in adults. The importance of these findings has to be investigated for porcine G1 strains. Nevertheless, Ciarlet and Liprandi (1994) were able to demonstrate that antisera to porcine G1 strains neutralized strain Wa effectively.

Rotaviruses are divided into six genotypes (A–F) based on the genetic diversity of the NSP4 gene (Cunliffe et al., 1997; Horie et al., 1997; Iturriza-Gómara et al., 2003a; Mori et al., 2002). The NSP4 gene sequence classifies the P21-5 strain into the genotype B branch of the phylogenetic tree (Fig. 5). Nevertheless, it was placed separately from other strains of the B genotype, together with human strain A_G4_120 (Fig. 4). In a study of NSP4 genes including various rotavirus strains, Horie et al. (1997) detected three different alleles among the NSP4 group A rotaviruses, which correspond to the NSP4 genotypes A, B and C. Based on amino acid sequences, an average genetic distance of 5.7% within each allele was determined. The differences ranged from 0.8% to 9.9%. As the NSP4 gene of the P21-5 strain differed more than 10% from all other published NSP4 genes (with the exception of the A_G4_120 isolate), it could represent a new NSP4 genotype, together with human A_G4_120 NSP4. It is interesting that both strains, A_G4_120 and P21-5, were detected in the stools of individuals with asymptomatic rotavirus infection. As the NSP4 glycoprotein is

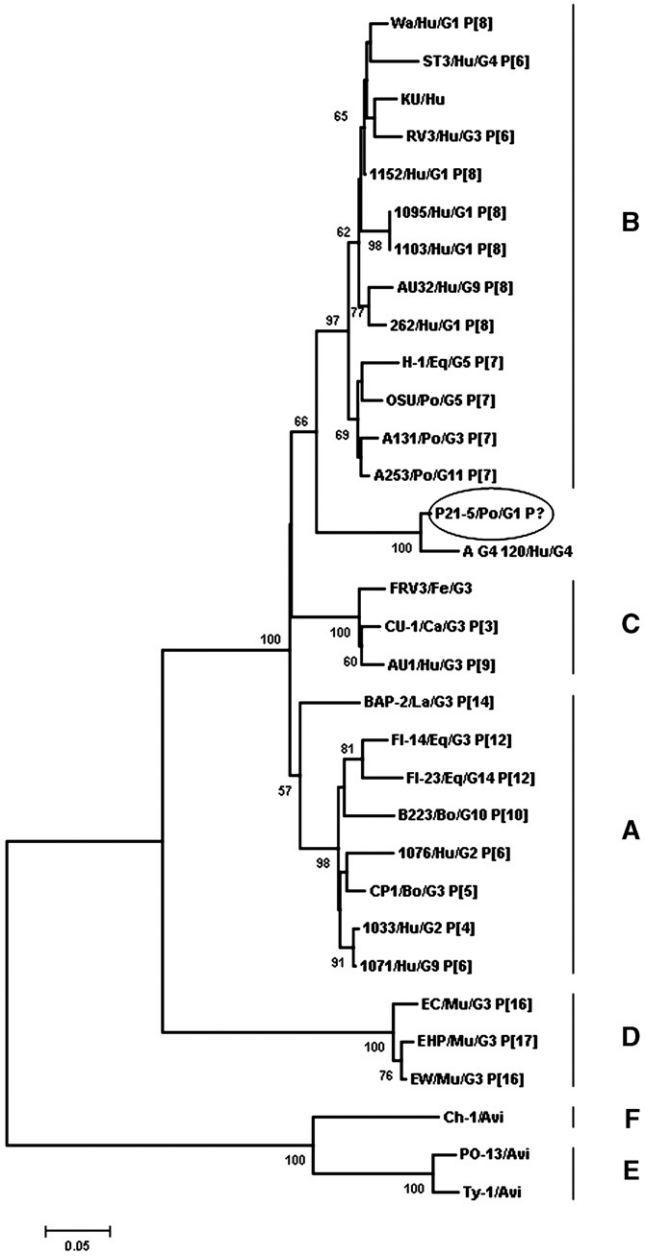

Fig. 5. Neighbor-joining phylogenetic tree based on the NSP4 amino acid sequences of all known NSP4 genotypes (Avi—avian, Bo—bovine, Ca—canine, Eq—equine, Fe—feline, Hu—human, La—lapine, Mu—murine, Po—porcine).

one of the major virulent factors of rotaviruses (Hoshino et al., 1995), further investigations must be performed to elucidate the antigenic and functional characteristics of the NSP4 gene in those two isolates. In the NSP4 amino acid region of 131–140, some changes of amino acid residues were associated with differences in pathogenicity of two porcine strains (OSU and Gottfried) in neonatal mice (Zhang et al., 1998). It was reported that amino acid substitution at position 135 (valine to alanine) and 138 (proline to serine) lead to attenuation of OSU and Gottfried strains. These amino acids at positions 135 and 138 were also commonly found in other porcine strains. In contrast to this finding, some other observations failed to evidence that these or other amino acid substitutions in NSP4 were associated with attenuation of virulent rotavirus strains (Chang et al., 1999; Lee et al., 2000; Oka et al., 2001; Ward et al., 1997). However, the study by Zhang et al. (1998) demonstrated that specific mutations at positions 135 and 138 in NSP4 caused attenuation of infective rotavirus particles as they also altered the enterotoxin function of purified enterotoxin, given intraperitoneally into mice or tested on cell culture. As this study was done with porcine strains, the NSP4 amino acid sequence of the P21-5 strain was compared with findings of this study. Surprisingly, in the NSP4 sequence of the P21-5 strain, methionine and asparagine were found at amino acid positions 135 and 138, respectively (Fig. 6). So far, these amino acids have not been found in any other porcine strains, yet have been found in the human A_G4_120 strain (Fig. 6). The significance of this methionine at position 135 and asparagine at position 138 is not yet known. To obtain more information concerning the implications or importance of these two amino acids for virulence and enterotoxin function, additional testing has to be performed. Such testing should employ both infective virus particles and also purified enterotoxin with amino acid substitutions at positions 135 and 138. Nevertheless, in any rotaviral infection of animals or humans, other factors also have to be considered in the development of disease symptoms. As the NSP4 amino acid region of 135–142 has been characterized as an intraspecies conserved region of various rotavirus strains, it is interesting to find the exact amino acid sequence match in this region between the human A_G4_120 and the porcine P21-5 strains. The NSP4 sequence of the strain A_G4_120 is published only in the GenBank database, with no journal publications on this strain. It would be interesting to have an insight into the molecular features of the other genome segments of the A_G4_120 strain to see if this is a human–animal reassortant, a zoonotic transmitted animal strain, or human rotavirus strain with unique NSP4 gene.

Because the sample was tested retrospectively, it was not possible to test additional samples of this pig or others, being in closer contact with the pig infected with the rotavirus P21-5 strain. It would be interesting to screen the pigs with symptomatic and asymptomatic rotavirus infection for the prevalence of this strain.

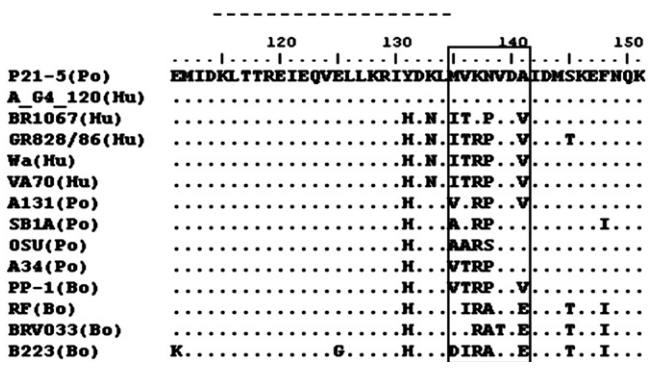

Fig. 6. Alignment of the NSP4 amino acid region 110–151. Comparison of rotavirus sequences detected in pigs (Po), cattle (Bo) or humans (Hu). Intraspecies conserved region is boxed and enterotoxin domain (aa114–135) is marked with a dotted line.

With intensified monitoring of rotaviruses in pigs and also in other animals, it is probable that further novel P and G genotypes will be detected. Gathering the information on the full spectrum of rotaviruses circulating in our environment will remain an important task for rotavirus surveillance, outbreak control, and both animal and public health.

Materials and methods

The porcine rotavirus strain P21-5 was detected during an epidemiological survey of rotaviruses in various pig farms in Slovenia. The asymptomatic rotavirus infection in pigs was being investigated to determine the importance of asymptomatic shedding of rotaviruses. On 14th of January 2004, stool samples of asymptomatic pigs at a pig farm near Ljubljana were collected. A total of 61 stool samples were examined: 20 from suckling (up to 3 weeks old), 21 from weanling (3 to 10 weeks old) and 20 from fattening pigs (more than 10 weeks old). Of 61 collected stool samples, 22 were positive using the RT-PCR method for group A rotavirus detection described by Gouvea and colleagues (Gouvea et al., 1990). The positive results were: 5 from suckling pigs, 9 from weanling pigs, and 8 from fattening pigs.

In order to isolate and characterize the rotaviral RNA, a 10% stool suspension in PBS buffer (0.2 M, pH=7.4) was prepared and clearance was performed with a 10-min centrifugation at 1.6×*g*. RNA was then isolated from the supernatant using TRIzol reagent (Invitrogen™), following the manufacturer's instructions. Rotaviral RNA was amplified in a one tube two enzyme commercial system RT-PCR (Access RT-PCR, Promega), following the manufacturer's instructions. For the detection of rotaviruses in the stool sample, the group A rotavirus VP7 gene specific primers Beg9/End9 (Gouvea et al., 1990) were used. In addition, the VP8* region of the VP4 and NSP4 genes were amplified, using the con2/con3 (Gentsch et al., 1992) and NSP4 primers (Cunliffe et al., 1997), respectively. The protocols for RT-PCR were used as instructed by the articles describing these primers.

Amplified VP7 and VP8* products were further used as a template for genotyping G and P, respectively. For VP7, specific primers for determining the human and animal genotypes G1–G6 and G8–G10 were used as described previously (Gouvea et al., 1990, 1994). In addition, P typing of the VP8* fragment with primers of genotype P[4], P[6], P[8], P[9], P[10], P[14] specificity was performed (Arista et al., 1999; Gentsch et al., 1992). The P genotype of the P21-5 strain could not be typed, thus VP8* fragment was selected for nucleotide sequence analysis.

Specific fragments amplified in the RT-PCR were purified using WIZARD PCR Preps chemistry and spin columns as described by the manufacturer (Promega). The purified products were then directly sequenced by termination with fluorescently labeled dideoxynucleotides (BigDye Terminator Cycle Sequencing Kit, PE Applied Biosystems). The products of the sequencing reactions were purified with Centri-sep purification columns (Princeton Separation) and analyzed on nucleic acid analyzer (ABI PRISM 310, PE Applied Biosystems).

Forward and reverse sequences were aligned and corrected using the Vector NTI Advance™ program for DNA and protein sequence analysis (Invitrogen™). For the phylogenetic analysis, both nucleotide and deduced amino acid sequences were used. The amino acid sequence alignments of the VP4, VP7 and NSP4 genes of P21-5 strain with those obtained from GenBank were performed using the Mega 3.1 program package. The neighbor-joining phylogenetic tree was constructed based on the Kimura-2 parameter distance calculation method. All three phylogenetic trees, processed separately for the VP8*, VP7 and NSP4 genes, were statistically supported using bootstrapping with over 1000 replicates.

The nucleotide sequence data reported in this paper for the rotaviral VP4, VP7 and NSP4 genes have been deposited in the GenBank database and assigned the accession numbers DQ629926 (VP4 gene), DQ629928 (VP7 gene) and DQ629927 (NSP4 gene).

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References

Arista, S., Vizzi, E., Alaimo, C., Palermo, D., Cascio, A., 1999. Identification of human rotavirus strains with the P[14] genotype by PCR. J. Clin. Microbiol. 37, 2706–2708.

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

Berois, M., Libersou, S., Russi, J., Arbiza, J., Cohen, J., 2003. Genetic variation in the VP7 gene of human rotavirus isolated in Montevideo-Uruguay from 1996–1999. J. Med. Virol. 71, 456–462.

Blackhall, J., Bellinzoni, R., Mattion, N., Estes, M.K., La Torre, J.L., Magnusson, G., 1992. A bovine rotavirus serotype 1: serologic characterization of the virus and nucleotide sequence determination of the structural glycoprotein VP7 gene. Virology 189, 833–837.

Chang, K.O., Kim, Y.J., Saif, L.J., 1999. Comparison of nucleotide and deduced amino acid sequences of NSP4 genes of virulent and attenuated pairs of group A and C rotaviruses. Virus Genes 18, 229–233.

Ciarlet, M., Liprandi, F., 1994. Serological and genomic characterization of two porcine rotaviruses with serotype G1 specificity. J. Clin. Microbiol. 32, 269–272.

Cook, N., Bridger, J., Kendall, K., Iturriza Gomara, M., El-Atar, L., Gray, J., 2004. The zoonotic potential of rotavirus. J. Infect. 48, 289–302.

Cunliffe, N.A., Woods, P.A., Leite, J.P.G., Das, B.K., Ramachandran, M., Bhan, M.K., Hart, C.A., Glass, R.I., Gentsch, J.R., 1997. Sequence analysis of NSP4 gene of human rotavirus allows classification into two main genetic groups. J. Med. Virol. 53, 41–50.

Diwakarla, C.S., Palombo, E.A., 1999. Genetic and antigenic variation of capsid protein VP7 of serotype G1 human rotavirus isolates. J. Gen. Virol. 80, 341–344.

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. Lippincott William and Wilkins, Philadelphia, pp. 1747–1785.
Estes, M.K., Cohen, J., 1989. Rotavirus gene structure and function. Microbiol. Rev. 53, 410–449.
El-Attar, L., Dhaliwal, W., Howard, C.R., Bridger, J.C., 2001. Rotavirus cross-species pathogenicity: molecular characterization of a bovine rotavirus pathogenic for pigs. Virology 291, 172–182.
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.
Gentsch, J.R., Woods, P.A., Ramachandran, M., Das, B.K., Leite, J.P., Alfieri, A., Kumar, R., Bhan, M.K., Glass, R.I., 1996. Review of G and P typing results from a global collection of rotavirus strains: implications for vaccine development. J. Infect. Dis. 174, 30–36.
Gentsch, J.R., Laird, A.R., Bielfeld, B., Griffin, D.D., Bányai, K., Ramachandran, M., Jain, V., Cunliffe, N.A., Nakagomi, O., Kirkwood, C.D., Fischer, T.K., Parashar, U.D., Bresee, J.S., Jiang, B., Glass, R.I., 2005. Serotype diversity and reassortment between human and animal rotavirus strains: implications for rotavirus vaccine programs. J. Infect. Dis. 192, 146–159.
Ghosh, S., Varghese, V., Samajdar, S., Bhattacharya, S.K., Kobayashi, N., Naik, T.N., 2006. Molecular characterization of a porcine group A rotavirus strain with G12 genotype specificity. Arch. Virol. 151, 1329–1344.
Gorziglia, M., Larralde, G., Kapikian, A.Z., Chanock, R.M., 1990. Antigenic relationships among human rotaviruses as determined by outer capsid protein VP4. Proc. Natl. Acad. Sci. 87, 7155–7159.
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., 1994. Identification of bovine and porcine rotavirus G types by PCR. J. Clin. Microbiol. 32, 1338–1340.
Green, K.Y., Kapikian, A.Z., 1992. Identification of VP7 epitopes associated with protection against human rotavirus illness or shedding in volunteers. J. Viorol. 66, 548–553.
Horie, Y., Masamune, O., Nakagomi, O., 1997. Three major alleles of rotavirus NSP4 proteins identified by sequence analysis. J. Gen. Virol. 78, 2341–2346.
Hoshino, Y., Saif, L.J., Kang, S.-Y., Sereno, M.M., Chen, W.-K., Kapikian, A.Z., 1995. Identification of group A rotavirus genes associated with virulence of a porcine rotavirus and host range restriction of a human rotavirus in the gnotobiotic piglet model. Virology 209, 274–280.
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., Honma, S., Jones, R.W., Ross, J., Santos, N., Gentsch, J.R., Kapikian, A.Z., Hesse, R.A., 2005. A porcine G9 rotavirus strain shares neutralization and VP7 phylogenetic sequence lineage 3 characteristics with contemporary human G9 rotavirus strains. Virology 332, 177–188.
Iturriza-Gómara, M., Anderton, E., Kang, G., Gallimore, C., Phillips, W., Desselberger, U., Gray, J., 2003a. Evidence for genetic linkage between the gene segments encoding NSP4 and VP6 proteins in common and reassortant human rotavirus strains. J. Clin. Microbiol. 41, 3566–3573.
Iturriza Gómara, M., Desselberger, U., Gray, J., 2003b. Molecular epidemiology of rotaviruses: genetic mechanisms associated with diversity. In: Desselberger, U., Gray, J. (Eds.), Viral Gastroenteritis. Elsevier, Amsterdam, pp. 317–344.
Jin, Q., Ward, R.L., Knowlton, D.R., Gabbay, Y.B., Linhares, A.C., Rappaport, R., Woods, P.A., Glass, R.I., Gentsch, J.R., 1996. Divergence of VP7 genes of G1 rotaviruses isolated from infants vaccinated with reassortant rhesus rotaviruses. Arch. Virol. 141, 2057–2076.
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. Lippincott William and Wilkins, Philadelphia, pp. 1787–1834.
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., Li, B., Kapikian, A.Z., Gorziglia, M., 1991. Serotype-specific epitope(s) present on the VP8 subunit of rotavirus VP4 protein. 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.
Liprandi, F., Gerder, M., Bastidas, Z., Lopez, 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, 373–378.
Martella, V., Pratelli, A., Greco, G., Tempesta, M., Ferrari, M., Losio, M.N., Buonavoglia, C., 2001. Genomic characterization of porcine rotaviruses in Italy. Clin. Diagn. Lab. Immunol. 8, 129–132.
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.
Martella, V., Ciarlet, M., Bányai, K., Lorusso, E., Cavalli, A., Corrente, M., Elia, G., Arista, S., Camero, M., Desario, C., Decaro, N., Lavazza, A., Buonovoglia, C., 2006. Identification of a novel VP4 genotype carried by a serotype G5 porcine rotavirus strain. Virology 346, 301–311.
Matthijnssens, J., Rahman, M., Martella, V., Xuelei, Y., De Vos, S., De Leener, K., Ciarlet, M., Buonavoglia, C., Van Rast, M., 2006. Full genomic analysis of human rotavirus strain B4106 and lapine rotavirus strain 30/96 provides evidence for interspecies transmission. J. Virol. 80, 3801–3810.
McNeal, M.M., Sestak, K., Choi, A.H.-C., 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.
Mohan, K.V., Atreya, C.D., 2000. Comparative sequence analysis identified mutations outside the NSP4 cytotoxic domain of tissue culture-adapted ATCC-Wa strain of human rotavirus and a novel inter-species variable domain in its C-terminus. Arch. Virol. 145, 1789–1799.
Mori, Y., Borgan, M.A., Ito, N., Sugiyama, M., Minamoto, N., 2002. Diarrhea-inducing activity of avian rotavirus NSP4 glycoproteins, which differ greatly from mammalian rotavirus NSP4 glycoproteins in deduced amino acid sequence, in suckling mice. J. Virol. 76, 5829–5834.
Oka, T., Nakagomi, T., Nakagomi, O., 2001. A lack of consistent amino acid substitutions in NSP4 between rotaviruses derived from diarrheal and asymptomatically-infected kittens. Microbiol. Immunol. 45, 173–177.
Parashar, U.D., Bresee, J.S., Gentsch, J.R., Glass, R.I., 1998. Rotavirus. Emerg. Infect. Dis. 4, 561–570.
Parashar, U.D., Hummelman, E.G., Bresee, J.S., Miller, M.A., Glass, R.I., 2003. Global illness and deaths caused by rotavirus disease in children. Emerg. Infect. Dis. 9, 565–571.
Rahman, M., Matthijnssens, J., Nahar, S., Podder, G., Sack, D.A., Azim, T., Van Rast, M., 2005. Characterization of a novel P[25], G11 human group A rotavirus. J. Clin. Microbiol. 43, 3208–3212.
Rao, C.D., Gowda, K., Yugandhar Reddy, B.S., 2000. Sequence analysis of VP4 and VP7 genes of nontypable strains identifies a new pair of outer capsid proteins representing novel P and G genotypes in bovine rotaviruses. Virology 276, 104–113.
Teodoroff, T.A., Tsunemitsu, H., Okamoto, K., Ketsuda, K., Kohmoto, M., Kawashima, K., Nakagomi, K., Nakagomi, O., 2005. Predominance of porcine rotavirus G9 in Japanese piglets with diarrhea: close relationship of their VP7 genes with those of recent human G9 strains. J. Clin. Microbiol. 43, 1377–1384.
Ward, R.L., Mason, B.B., Bernstein, D.I., Sander, D.S., Smith, V.E., Zandle, G.A., Rappaport, R.S., 1997. Attenuation of a human rotavirus vaccine candidate did not correlate with mutations in the NSP4 protein gene. J. Virol. 71, 6267–6270.
Winiarczyk, S., Paul, P.S., Mummidi, S., Panek, R., Gradzki, Z., 2002. Survey of porcine rotavirus G and P genotype in Poland and the United States using RT-PCR. J. Vet. Med. B 49, 373–378.
Zhang, M., Zeng, C.Q.-Y., Dong, Y., Ball, J.M., Saif, L.J., Morris, A.P., Estes, M.K., 1998. Mutations in rotavirus nonstructural glycoprotein NSP4 are associated with altered virus virulence. J. Virol. 72, 3666–3672.