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ORIGINAL ARTICLE

Detection and characterisation of group A rotavirus in asymptomatic piglets in southern Ireland

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Abstract Porcine group A rotaviruses (GARV) are causative agents of enteritis in piglets and are a large reservoir of genetic material for the diversification of human GARVs. Accumulation of information on the genetic heterogeneity of porcine viruses is pivotal for readily characterising unusual human strains. Screening of 292 fecal samples, collected from 4–5- to 8–9-week-old asymptomatic pigs from four herds in Ireland between 2005 and 2007 resulted in 19 (6.5%) samples testing positive by reverse-transcription PCR (RT-PCR) for GARV. The strains were molecularly characterized to collate data on the VP7 and partial VP4 outer capsid genes. By sequence analysis of the VP7 gene, the Irish strains were identified as G2, G4, G5, G9 and G11 viruses. The G11 strains were closely related to other human and porcine G11 strains, while the G2 strains resembled porcine G2 viruses detected recently in Europe and southern Asia. The G4 strains were distantly related to other G4 human and animal strains, constituting a separate G4 VP7 lineage. Analysis of the G5 strains revealed that they were similar to a selection of G5 human and porcine strains, while the G9 strains resembled other porcine G9 viruses. By sequence analysis of the VP8* fragment of the VP4, the Irish viruses were characterised as P[6], P[7], P[13], P[13]/[22], P[26] and P[32].

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Introduction

Since its discovery in 1973, rotavirus has been documented as a major cause of acute gastroenteritis and deaths in infants and young children worldwide [26]. In humans, GARV is estimated to cause 138 million cases of gastroenteritis annually, resulting in approximately 870,000 deaths, mostly in developing countries [20].

Rotaviruses are classified into seven antigenically distinct groups (A to G). Groups A, B and C are associated with acute gastroenteritis in humans and animals, while groups D, E, F and G have been detected only in animals [3, 16, 27].

Rotavirus is a member of the family *Reoviridae*, genus *Rotavirus*, with a genome consisting of 11 double-stranded RNA segments, enclosed in a triple-layered capsid [44]. The outer capsid proteins VP7 and VP4 both elicit serotype-specific neutralising antibodies and are important for immune protection and vaccine development [3]. To date, 23 G types and 31 P types have been identified in humans and animals [1, 10, 11, 29, 40, 44, 54, 60–62, 64, 66]. Either polyvalent or monovalent vaccines, targeting the G and P types of the predominant human GARVs have been developed to prevent childhood gastroenteritis.

Group B and group C rotavirus (GCRV) have also been identified in pigs [55], but only GARVs have been firmly associated with enteritis. GARV accounts for up to 89% of all rotaviral diarrhoea in commercial pig operations

[15, 69]. GARV may also be detected in non-diarrheic piglets [34]. GARV infection of pigs has been recognised in both enzootic and epizootic forms of diarrhoea, resulting in economic losses in commercial piggeries [57].

Antigenic and molecular characterization of GARVs has revealed a broad heterogeneity in the VP7 and VP4 genes. The main G types previously identified in pigs are G3, G4, G5 and G11, associated with common P types, i.e. P[6] and P[7] [28]. Porcine GARV strains with additional, more unusual P types, P[13], P[19], P[23], P[26] and P[27], have also been described. Furthermore, porcine strains displaying human-like G types (G1, G2, G9 and G12) and P types (M37-like P[6] and P[8]) and bovine-like G types (G6, G8 and G10) and P types (P[1], P[5] and P[11]) have also been described [14, 18, 19, 37–40, 52].

Interspecies transmission, accumulation of point mutations, recombination and, chiefly, reassortment are responsible for the huge genetic heterogeneity of rotaviruses [25, 45, 46, 53]. The zoonotic potential of GARVs has been documented on several occasions [42]. Studying animal rotaviruses is fundamental for obtaining a better understanding of rotavirus ecology and the mechanisms by which rotaviruses evolve, but it is also important for improving the prophylactic tools for livestock. Along with cattle, pigs are regarded as important reservoirs for the diversification of human rotavirus [42]. Novel human strains of heterologous (porcine) origin or natural porcine-human reassortants may have arisen and spread successfully throughout human populations in Latin America, Southeast Asia, and Europe, demonstrating the impact of animal GARVs on human health [5, 13, 17, 31, 39, 51, 65, 67].

In this study, we investigated the occurrence of asymptomatic infections by GARVs in swine herds in Ireland between 2005 and 2007. Several collections of samples were screened by RT-PCR using GARV-specific primers, and the VP7 and VP4 gene sequences of the identified GARV strains were determined.

Materials and methods

Sample collection and preparation

A total of 292 faecal samples were obtained from 4–5- to 8–9-week-old asymptomatic pigs (i.e. with no diarrhoea) from porcine herds in Ireland over 3 years, from 2005 to 2007. The samples were collected from three different herds in County Cork. Eighty samples were collected in May 2005 (farm 1), 80 in September 2006 (farm 2) and 102 in May 2007 (farm 3). A collection of 30 samples (farm 4) was also obtained in March 2007 in County Dublin from 8–9 week old piglets.

RNA preparation

Total nucleic acids were extracted from the samples by a standard phenol–chloroform method, followed by ethanol precipitation. The extracted nucleic acids were resuspended in 100 µl of sterile DEPC H_2O, and stored at −80°C prior to use.

RT-PCR amplification of group A rotavirus

The extracted nucleic acids from the faecal samples were tested for the presence GARVs by RT-PCR, using oligonucleotide primers described previously [12].

RT-PCR amplification of the VP7 gene and prediction by PCR of the VP7 (G) type

The dsRNA extracted from the faecal samples was denatured in DMSO at 97°C for 5 min and immediately cooled on ice. For amplification of the VP7 gene, the primer pair Beg9/End 9 was used [15] in a one-step reverse-transcription (RT)-PCR protocol using an Enhanced Avian RT-PCR Kit (Sigma–Aldrich). The reaction was carried out in an MJ researcher PTC-200 thermocycler (GMI Inc, Minnesota, USA). For RT-PCR, the following conditions were applied: 45°C for 30 min, 70°C for 4 min, followed by 35 cycles of 94°C for 1 min, 55°C for 30 s and 68°C for 2 min plus a final extension of 68°C for 5 min. The amplicons were analyzed in 1.5% agarose gels following ethidium bromide staining and UV-light transillumination.

Prediction of the VP7 (G) type was carried out using a pool of primers including the specific oligonucleotides as described previously [6, 15, 19, 70]. The amplicons obtained in the first-round amplification were diluted 1:200 and used as templates for the second-round PCR. The thermal file was as follows: 94°C for 10 min, followed by 25 cycles of 94°C for 1 min, 55°C for 2 min, 72°C for 1 min and a final extension at 72°C for 10 min. The amplicons were analyzed in by electrophoresis 1.5% agarose gels, followed by ethidium bromide staining and UV-light transillumination.

RT-PCR amplification of the VP8* region and prediction by PCR of the VP4 (P) type

For amplification of the VP8* subunit of the VP4 gene, the primers Con2 and Con3 were used [12]. Thermal and reaction conditions for the amplification of the VP7 gene were identical to those described above.

Sequence analysis

The sequences were assembled, edited and analyzed using the Bioedit software package, version 2.1 [21]. Preliminary

analysis was accomplished by comparison with the sequences available in the database using the web-based programs BLAST (http://www.ncbi.nlm.nih.gov/BLAST) and FASTA (http://www.ebi.ac.uk/fasta33).

Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 2.1 (Arizona State University, USA) [32]. Phylogenetic trees, based on the VP7 and partial VP4 genes, were elaborated by using both parsimony and distance methods, supplying a statistical support with bootstrapping of 500 replicates.

Accession numbers

The partial VP7 sequences of strains 1/07/Ire, 2F/05/Ire, 10/07/Ire, 54/06/Ire, 2B/05/Ire, 60/07/Ire, 48/07/Ire and 61/07/Ire have been registered in GenBank under the accession numbers FJ617255, FJ617256, FJ617257, FJ617258, FJ617259, FJ617260, FJ492832 and FJ492831, respectively.

Results

RT-PCR screening for group A rotavirus

A total of 19 out of 292 samples (6.5%) were found to contain GARV. GARV-positive samples were detected in three of the four herds surveyed (2005–2007): in 6 of the 80 samples in the 2005 collection, in 4 of the 80 samples from 2006, and in 9 of the 102 samples from 2007, all of which had been collected in County Cork. GARV infection was not diagnosed in any of the 30 samples from the 2007 collection that were from County Dublin.

Sequence analysis of the gene encoding the outer capsid protein VP7

The VP7 gene sequences of 18 out of 19 GARV strains were determined. Preliminary analysis by BLAST and FASTA permitted identification of these strains as G2-like, G4, G5, G9 and G11 (Table 1).

Sequence analysis of the VP7 of G2 porcine rotaviruses

Four strains (25/07/Ire, 48/07/Ire, 61/07/Ire and 67/07/Ire) were characterised as G2-like. The Irish strains clustered tightly with porcine G2-like strains identified in Europe and Asia (Fig. 1) but separately from the human G2 strains, whose sequences appeared to be highly conserved. The Irish G2 porcine strains displayed 76.5 to 93% aa identity to G2 human and animal rotaviruses, with the highest aa identity being to the Spanish porcine strain 34461/4 (92.4–93%) (Table 2). In the VP7 antigenic regions A (aa 87 to 101), B (aa 141 to 152), C (aa 208 to 224) and F (235 to 242) [2, 8, 30, 47] (data not shown), the G2 strains differed in 7–8 residues from the G2 porcine strain 34461/4.

Table 1 Characterization of the G types using a pool of specific oligonucleotides as described previously [6, 15, 19, 70] in comparison to sequencing results obtained for the VP7 fragment of the Irish porcine GARV strains

Year	Sample no.	Gouvea [15]	Das [6]	Gouvea [19]	Winiarczyk [70]	G type	P type
2005	1C/05/Ire					G5	un
2005	2B/05/Ire					G9	P[13]/[22]
2005	2F/05/Ire	9				G5	P[26]
2005	2 J/05/Ire					un	un
2005	6B/05/Ire	9	9	5	4	G5	P[13]/[22]
2005	7F/05/Ire	9	9	5	4	G5	P[26]
2006	16/06/Ire	9		5		G9	P[6]
2006	48/06/Ire	9	9			G9	P[13]
2006	51/06/Ire	9	9			G9	P[13]
2006	54/06/Ire	9	9	5	4	G9	P[6]
2007	1/07/Ire		4			G4	P[13]
2007	2/07/Ire		4			G4	P[7]
2007	10/07/Ire	1				G5	un
2007	25/07/Ire		9			G2-like	un
2007	48/07/Ire		9			G2-like	P[32]
2007	60/07/Ire	9		11		G11	P[26]
2007	61/07/Ire					G2-like	P[32]
2007	67/07/Ire	1 and 3			10	G2-like	P[6]
2007	88/07/Ire		4			G4	P[13]

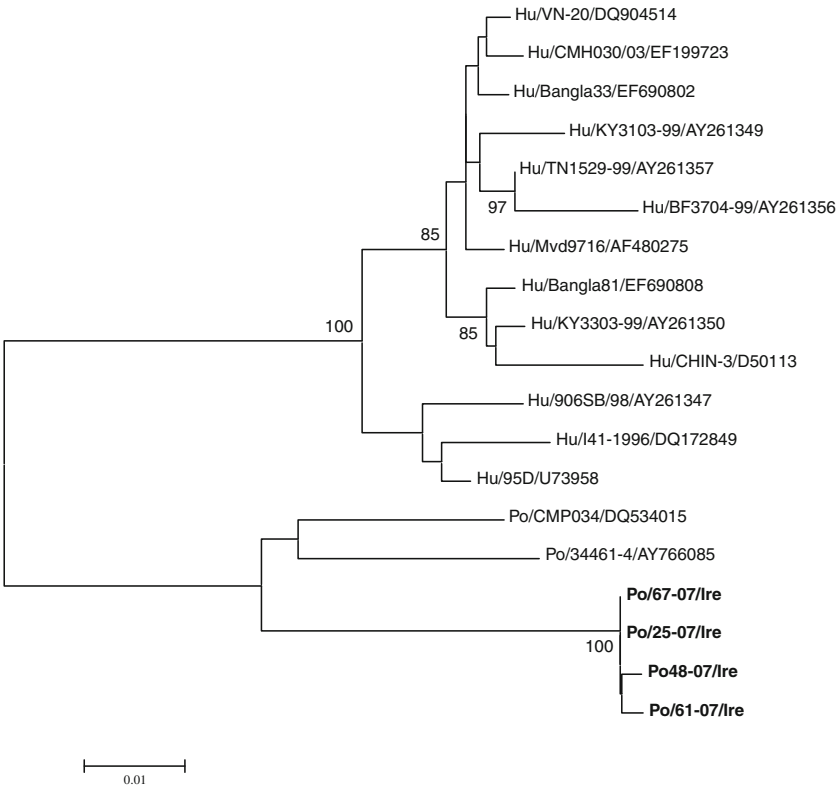


Fig. 1 Phylogenetic tree based on the VP7 nucleotide sequences of various porcine G2-like and human G2 porcine strains and porcine strains 25/07/Ire, 48/07/Ire, 61/07/Ire and 67/07/Ire. The Irish porcine strains are highlighted in *bold*

Table 2 Comparison of the VP7 amino acid (aa) sequences of two Irish G2-like porcine strains, 48/07/Ire and 61/07/Ire, to those of a selection of G2 human and porcine strains

Strain	Po/48/07/Ire	Po/61/07/Ire
Po/48/07/Ire	–	**94.9**
Po/61/07/Ire	**94.9**	–
Po/CMP034/Jp/DQ534015/G2	**91**	**90.4**
Po/34461-4/Italy/AY766085/G2	**93**	**92.4**
Hu/KY3303-99/AY261350/G2	84.4	83.8
Hu/TN1529-99/AY261357/G2	85.1	84.4
Hu/BF3704-99/AY261356/G2	83.1	82.5
Hu/Bangla81/EF690808/G2	84.4	83.8
Hu/Bangla33/EF690802/G2	84.8	84.1
Hu/CMH030/03/EF199723/G2	84.8	84.1
Hu/L-2/DQ478581/G2	77.2	76.5
Hu/VN-20/DQ904514/G2	84.1	83.4
Hu/CHIN-3/D50113/G2	82.1	81.5
Hu/Mvd9716/AF480275/G2	84.4	83.8
Hu/906SB/98/AY261347/G2	85.4	85.1

For each strain, the highest identity to the reference virus is shown in bold

Sequence analysis of the VP7 of G4 porcine rotaviruses

Using the VP7 sequences of a selection of human and animal G4 GARV strains, it was observed that the Irish G4 strains (1/07/Ire, 2/07/Ire and 88/07/Ire) were distantly related to other G4 human and animal strains, constituting a separate G4 VP7 lineage in the phylogenetic tree (Fig. 2). The strains 1/07/Ire and 2/07/Ire displayed 83.1–93.2% aa identity to G4 human and animal strains. The VP7 of Irish strains 1/07/Ire and 2/07/Ire showed 97.9% aa identity to each other and 90.8–93.2% aa identity to the human GARV strain Hu/G4-V/VN592/2003/Vietnam and the porcine GARV strains Po/G4-V/CMP121/Thailand and Po/G4-V/CMP77/Thailand (Table 3). In the VP7 antigenic regions A, B, C and F (data not shown), the Irish G4 strains differed in eight residues from the G4 reference strain Gottfried, in five residues from the human strain VN592/2003/Vietnam, and in five residues from the porcine strains CMP121/Thailand and CMP77/Thailand.

Sequence analysis of the VP7 of G5 porcine rotaviruses

Five G5 strains were identified in this study, with four strains being detected in 2005 (1C/05/Ire, 2F/05/Ire, 6B/05/Ire and 7F/05/Ire) and 1 strain in 2007 (10/07/Ire). The 2005 G5 strains (2F/05-like) were detected from the same herd and were highly similar to each other, while they exhibited only 94.6% aa identity to strain 10/07/Ire. The aa identity to G5 human and animal strains ranged from 91.1 to 95%. The highest identity (95%) for strain 2F/05 was found to the porcine strain 344-04-1. The highest identity for 10/07/Ire (93.9%) was to porcine strain CC117 (Table 4). In the VP7 antigenic regions A, B, C and F,

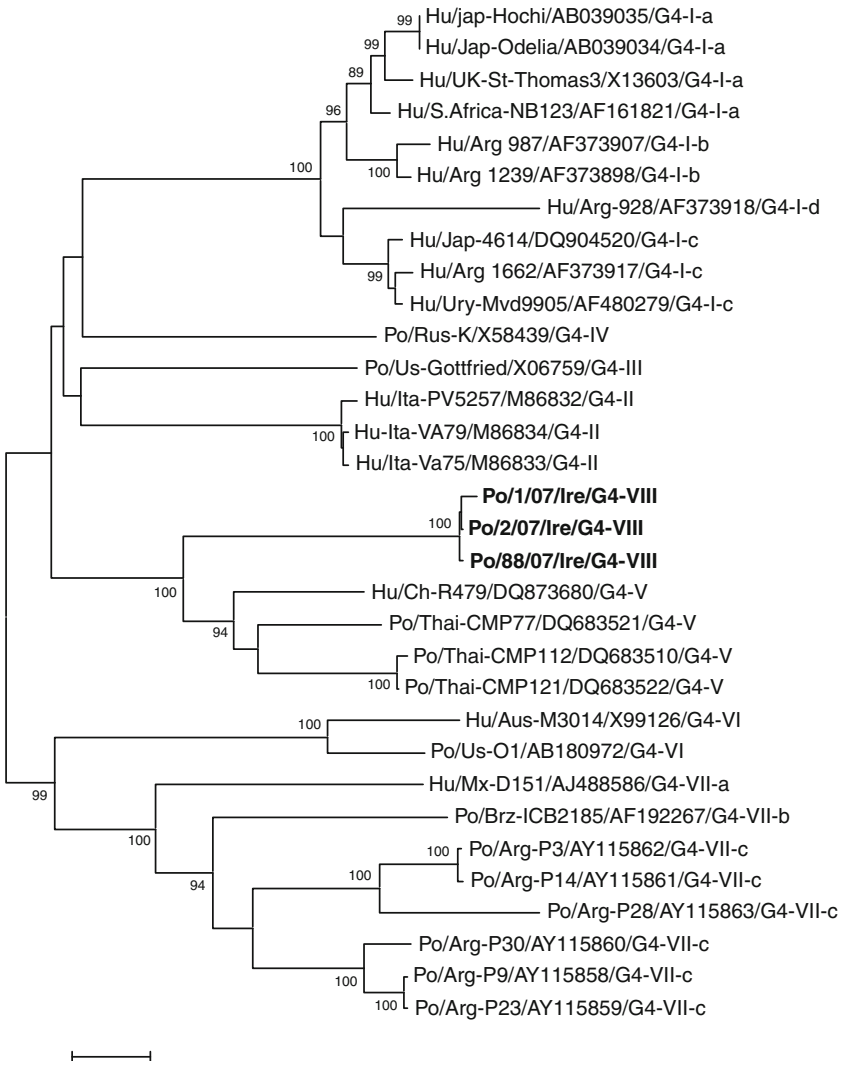


Fig. 2 Phylogenetic tree based on the VP7 nucleotide sequences of a selection of porcine and human G4 strains with porcine strains 1/07/Ire, 2/07/Ire and 88/07/Ire. The Irish porcine strains are highlighted in *bold*

strains 2F/05/Ire and 10/07 differed in four residues from the G5 reference strain OSU (data not shown).

Sequence analysis of the VP7 of G9 porcine rotaviruses

A phylogenetic tree based on a selection of human and animal GARV G9 strains was generated following as described by Teodoroff et al. [63] (Fig. 3). The G9 Irish strains (2B/05/Ire, 16/06/Ire, 48/06/Ire, 51/06/Ire, 54/06/Ire) clustered with G9 strains in lineage VI. The 2006 G9 strains (54/06/Ire-like) had been detected in the same herd and were highly similar to each other, and their amino acid sequences were 95.4% identical to that of strain 2B/05/Ire. The strains 2B/05/Ire and 54/06/Ire showed 89.1–97.1% aa identity to a selection of strains from the various G9 lineages, with the highest identity (94.9 and 97.1% aa, respectively) being to the Japanese strain JP29-6 within lineage G9-VIg (Table 5). In the VP7 antigenic regions A, B, C and F, the Irish G9 strains differed in either 3 or 4 residues from the closest G9 strain, JP29-6 (data not shown).

Sequence analysis of the VP7 of G11 porcine rotaviruses

One strain, 60/07/Ire, was characterised as G11, since it possessed 91.8–92.5% aa identity to porcine and human G11 GARV strains (Table 6). In the VP7 antigenic regions A, B, C and F, strain 60/07/Ire differed in seven residues from the G11 human strain Dhaka6 and in 6 residues from the G11 porcine strains A253 and YM (data not shown).

Sequence analysis of the gene encoding the outer capsid protein VP4

The VP4 sequence was determined for 15 of the 19 GARV samples, while the sequences from 4 samples were not usable for further analysis. Initial phylogenetic analysis

Table 3 Comparison of VP7 amino acid sequences of two Irish G4 porcine strains, 1/07/Ire and 2/07/Ire, to those of a selection of G4 human and porcine strains

Strain	1/07/Ire	2/07/Ire
Po/G4-III/Gottfried/US	87.5	89.1
Hu/G4-Ia/AF254139/Ireland	87.5	89.1
Hu/G4-Ia/Hochi/Japan	86.8	88.5
Hu/G4-Ia/ST3/US	86.8	88.5
Hu/G4-Ia/Odelia/Japan	87.1	88.1
Hu/G4-II/Va75/Italy	87.5	89.1
Hu/G4-II/VA79/Italy	87.1	88.8
Po/G4-IV/K/Russia	88.5	90.2
Hu/G4-V/VN592/2003/Vietnam.	**90.8**	**92.5**
Hu/G4-V/VN602/2003/Vietnam	90.8	92.5
Hu/G4-V/VN846/2003/Vietnam.	90.8	92.5
Hu/G4-V/VN-16/Vietnam	90.8	92.5
Hu/G4-V/R479/China	90.8	92.5
Po/G4-V/CMP121/Thailand	**91.5**	**93.2**
Po/G4-V/CMP77/Thailand	**91.5**	**93.2**
Po/G4-VI/O-1	83.1	84.7
Hu/G4-VI/M3014/Australia	83.7	85.4
Po/G4-VIIb/ICB2185/Brazil	86.1	87.8
Hu/G4-VII-a/D151/Mexico	84.4	86.1
Po/G4-VIII/Cork1/07/Ireland	–	**97.9**
Po/G4-VIII/Cork2/07/Ireland	**97.9**	–

For each strain, the highest identity to the reference virus is shown in bold

Table 4 Comparison of VP7 amino acid sequences of two Irish G5 porcine strains, 2F/05/Ire and 10/07/Ire, to those of a selection of G5 human and porcine strains

Strain	Po/10/07/Ire	Po/2F/05/Ire
Bo-EU541405-K8	91.4	92.3
Bo-EU541406-K33-2	92	93
Bo-EU541409-K291	91.4	92.3
Bo-EU873013-KV0407	92	93
Hu-EF077484-03'LL36755	92.3	93.3
Hu-EF159576-LL4260-china	93	94.3
Hu-LL3354-EF159575-China-G5	91.7	93
Po-DQ062572-134-04-15	91.1	92.6
Po-DQ515961-CMP178	92.7	93
Po-DQ813658-344-04-1	93	**95**
Po-G5-OSU-US	91.7	92.6
Po-L35054-A46	92.7	94
Po-L35056-CC117	**93.9**	**94.6**
Po-L35058-C134	**93.3**	94
Po-L35059-A34	91.1	91.6
Eq-AF242393-H-1	91.7	92.6
Po/10/07/Ire	–	**94.6**
Po/2F/05/Ire	**94.6**	–

For each strain, the highest identity to the reference virus is shown in bold

was constructed with the 15 strains identified in this study and those of the 31 P genotypes. This analysis identified the presence of P[6], P[7], P[13], P[13]/P[22], P[26] and P[32] strains (Fig. 4).

Sequence analysis of the VP8* region of P[13] and P[13]/[22] porcine GARVs

Four P[13] strains were identified in this study. Two strains were detected in 2006 (48/06/Ire and 51/06/Ire) and 2 in 2007 (1/07/Ire and 88/07/Ire). The highest nucleotide (nt) identity (Table 3) (81.7–81.8%) for strains 48/06/Ire and 1/07/Ire was found to the porcine strain MDR-13. Strain 1/07/Ire displayed 84.4–85.5% aa identity to porcine strains MDR-13 and JP13-3. Two P[13]/[22] strains (2B/05/Ire and 6B/05/Ire) were identified from the same herd in 2005. Strains 2B/05/Ire and 6B/05/Ire displayed the highest nt identity (87–87.2%) to the porcine strains JP13-3 and JP35-7. The highest aa identity for strains 2B/05/Ire and 6B/05/Ire was to the porcine strains JP13-3 and JP35-7 (87.8–88.5%, data not shown). In the phylogenetic tree, strains 2B/05/Ire, 6B/05/Ire and 60/07/Ire clustered with the porcine Japanese strains JP13-3 and JP35-7, which have been tentatively classified as P[13]/[22] [63]. Strains 48/06/Ire, 51/06/Ire, 1/07/Ire and 88/07/Ire clustered with the porcine P[13] reference strain MDR-13 (data not shown).

Discussion

GARVs are an important cause of acute gastroenteritis in humans and animals. In this study, evidence was collected for the occurrence of GARVs in asymptomatic piglets in Irish pig farms, and the genetic heterogeneity among the identified strains was examined. GARV were detected in 19/292 faecal specimens (6.5%) from three of the four swine herds over the 3 years of the study, suggesting the continual circulation of GARV in piggeries. GARV strains were detected from the three different herds in County Cork, farms 1, 2 and 3, respectively. No GARV strains were detected from farm 4, from a collection of 30 samples in County Dublin. This may be due to a higher age group of pigs included from this farm (8–9 weeks of age). GARVs were detected in 4–12-week-old piglets in 71.5% of the animals sampled in Italy [41]. The samples were also screened for the presence of GCRV [58], and in a previous study [4], evidence was obtained for the circulation of porcine GCRV in the same herds, since GCRV were also detected in 13 samples but not in conjunction with group A viruses.

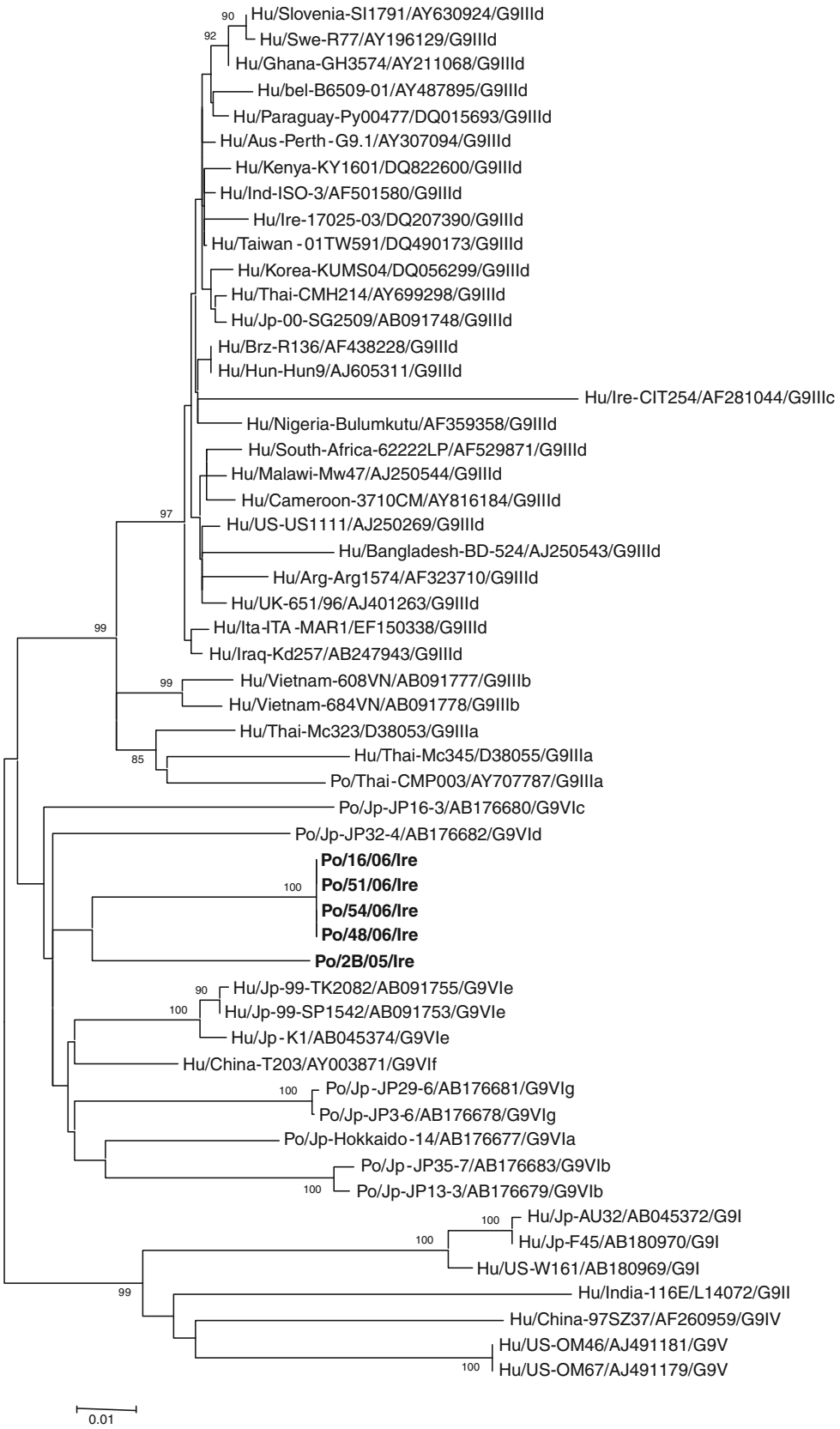


Fig. 3 Phylogenetic tree based on the VP7 nucleotide sequences of a selection of porcine and human G9 strains and porcine strains 2B/05/Ire, 16/06/Ire, 48/06/Ire, 51/06/Ire and 54/06/Ire. The Irish porcine strains are highlighted in *bold*

Table 5 Comparison of VP7 amino acid sequences of two Irish G9 porcine strains, 2B/05/Ire and 54/06/Ire, to those of a selection of G9 human and porcine strains

Strain	Po/2B/05/Ire	Po/54/06/Ire
Hu-USA-F45-AB180970-G9-I	92.2	93.9
Hu-India-116E-L14072-G9-II	89.4	90.9
Po-Thailand-CMP003-AY707787-G9-IIIa	92.8	95.1
Hu-608VN-AB091777-G9-IIIb	95	96.2
Hu-Ire-CIT254-AF281044-G9-IIIc	89.1	91.3
Hu-R77-AY196129-G9-IIId	94.5	96.1
Hu-97'SZ37-AF260959-G9-IV	93.5	94.8
Hu-OM46-AJ491181-G9-V	93.5	96.1
Po-Japan-Hokkaido-14-AB176677-G9-VIa	93.5	95.5
Po-Japan-JP35-7-AB176683-G9-VIb	92.8	95.1
Po-Japan-JP16-3-AB176680-G9-VIc	93.9	95.1
Po-Japan-jp32-4-AB176682-G9-VId	93.9	95.1
Hu-K-1-AB045374-G9-VIe	94.5	95.5
Hu-T203-AY003871-G9-VIf	94.5	96.8
Po-Japan-JP29-6-AB176681-G9-VIg	**94.9**	**97.1**
Po/2B/05/Ire	–	**95.4**
Po/54/06/Ire	**95.4**	–

For each strain, the highest identity to the reference virus is shown in bold

Table 6 Comparison of VP7 amino acid sequences of the Irish G11 porcine strain Cork60/07/Ire to those of a selection of G11 human and porcine strains

Strain	60/07/Ire
Po/G11P[7]/YM/Mexico	**92.1**
Matlab36-02/Hu/Bang/G11P[8]	91.8
CuK1/Ko/Hu/G11P[4]	**92.5**
GJ0703034/Hu/Ko/G11P[4]	**92.5**
Hu/Dhaka6/2001/BGD/G11P[25]	92.1
Hu/G11P[25]/04N368/Nepal	92.5
Po/G11P[7]/A253/Venezuela	92.1

For each strain, the highest identity to the reference virus is shown in bold

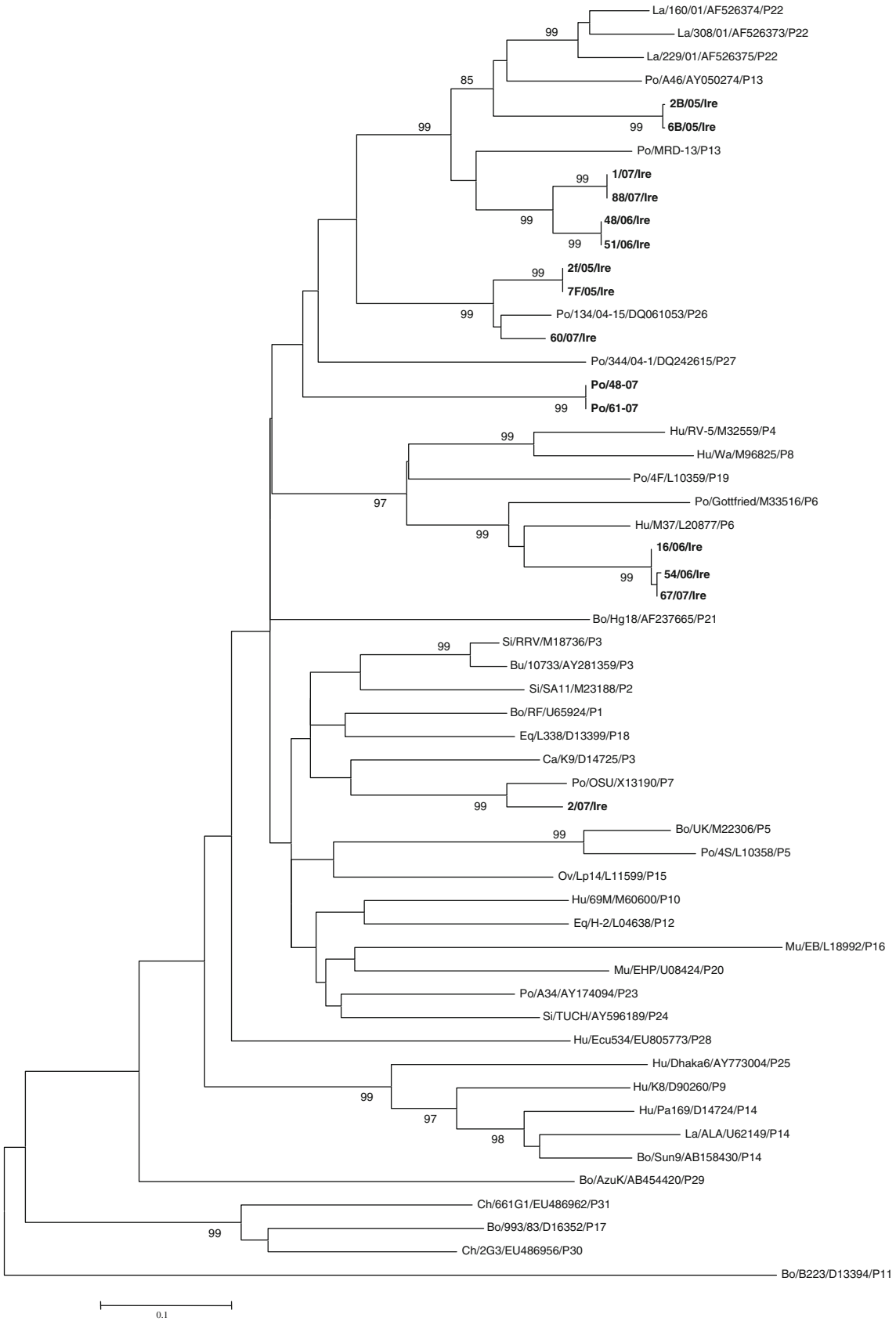
During a 3-year time span (2005–2007), 19 cases of asymptomatic infections in piglets were found to be associated with GARV infection. Asymptomatic infections by GARVs in swine have been identified in previous studies [34, 52]. Lingering maternally-derived immunity [22] or the circulation of naturally attenuated rotavirus strains [24, 48] could account for the occurrence of asymptomatic infections in those animals. Interestingly, the prevalence of GARVs in asymptomatic animals (6.5%) was low when compared to that reported in studies of symptomatic animals. In a study in 4- to 12-week-old piglets with enteritis in Italy, GARVs were detected in 71.5% of the sampled animals, and in 91.5% of the herds [41].

Fig. 4 Phylogenetic tree of the VP8* nucleotide sequences, displaying the relationship between the Irish porcine strains with those of all of the known 31 P genotypes. *Si* simian, *Eq* equine, *Po* porcine, *Ca* canine, *Bo* bovine, *Mu* murine, *Hu* human, *Ov* ovine, *La* lapine. The Irish porcine strains are highlighted in *bold* ▶

Characterization with several primer sets yielded inconsistent findings when compared with the results of sequence analysis (Table 1). In particular, the G2 strains were not correctly recognised or were mischaracterized. Visual inspection of the VP7 nucleotide (nt) sequence displayed 12 and 7 nt mismatches in the binding sites of the G2-specific primers aCT2 and 9T1-2, respectively [6, 15], which probably prevented recognition by the genotyping probes. There are two major reasons for these findings: there is limited information on the genetic heterogeneity of animal rotaviruses, and the various primers sets were established using the sequences of a few prototype viruses or of human viruses. These results provide strong evidence that genotyping of animal viruses with primer sets constructed for human viruses may result in mischaracterization and generate bias when collecting epidemiological data. In a study by Winiarczyk et al. [70], more than 3.2% of the strains were untypeable by G-typing PCR and 12.9% were untypable by P-typing PCR.

An interesting result of our investigation was that different strains were found to co-circulate at the same time in each herd, since the samples from each herd had all been collected on the same day. At least three strains were identified at farm 1 (G5P[26], G5P[13]/[22] and G9P[13]/[22]), two at farm 2 (G9P[6]-V and G9P[13]) and six at farm 3 (G2P[6], G2P[32], G4P[7], G4P[13], G5, G11P[26]). These findings are extremely intriguing, since they demonstrate the extreme genetic heterogeneity of these viruses, yet in a restricted environment. Also, the detection of such a variety of different strains in the same herd is not consistent with the hypothesis that asymptomatic infections are due to natural attenuation of the viruses, since the probability that all of the viruses were attenuated at the same time is low. Accordingly, other factors are more likely to be involved, such as age-related susceptibility to the disease or the establishment of a strong protective immunity in the animals.

Sequence analysis enabled characterization of four porcine GARVs as G2-like, all of which had been collected in 2007. The G2 Irish porcine strains resembled, although not closely, similar G2-like porcine GARVs detected recently in Europe and southern Asia, as evidenced by a number of mutations in the antigenic regions A through F. G2-like porcine strains were first identified from a Spanish piglet with enteritis [38] and subsequently in a 7-week-old


La/160/01/AF526374/P22
La/308/01/AF526373/P22
La/229/01/AF526375/P22
Po/A46/AY050274/P13
2B/05/Ire
6B/05/Ire
Po/MRD-13/P13
1/07/Ire
88/07/Ire
48/06/Ire
51/06/Ire
2f/05/Ire
7F/05/Ire
Po/134/04-15/DQ061053/P26
60/07/Ire
Po/344/04-1/DQ242615/P27
Po/48-07
Po/61-07
Hu/RV-5/M32559/P4
Hu/Wa/M96825/P8
Po/4F/L10359/P19
Po/Gottfried/M33516/P6
Hu/M37/L20877/P6
16/06/Ire
54/06/Ire
67/07/Ire
Bo/Hg18/AF237665/P21
Si/RRV/M18736/P3
Bu/10733/AY281359/P3
Si/SA11/M23188/P2
Bo/RF/U65924/P1
Eq/L338/D13399/P18
Ca/K9/D14725/P3
Po/OSU/X13190/P7
2/07/Ire
Bo/UK/M22306/P5
Po/4S/L10358/P5
Ov/Lp14/L11599/P15
Hu/69M/M60600/P10
Eq/H-2/L04638/P12
Mu/EB/L18992/P16
Mu/EHP/U08424/P20
Po/A34/AY174094/P23
Si/TUCH/AY596189/P24
Hu/Ecu534/EU805773/P28
Hu/Dhaka6/AY773004/P25
Hu/K8/D90260/P9
Hu/Pa169/D14724/P14
La/ALA/U62149/P14
Bo/Sun9/AB158430/P14
Bo/AzuK/AB454420/P29
Ch/661G1/EU486962/P31
Bo/993/83/D16352/P17
Ch/2G3/EU486956/P30
Bo/B223/D13394/P11
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diarrheic piglet in Thailand [29]. These viruses are genetically similar in the VP7 to G2 human viruses, and, according to the new classification system proposed recently by the RCWG (Rotavirus Classification Working Group) [44] (cutoff of 80% nt identity in the VP7), they fall unambiguously into the G2 genotype. However, analysis of the VP7 gene of porcine G2 strains from different geographical areas is revealing a remarkable genetic heterogeneity, much more pronounced than the diversity displayed by the human G2 strains, which, conversely, appear to be from a highly conserved group. This is consistent with a single bottleneck zoonotic event. This hypothesis is also supported by the genetic relatedness to bovine viruses displayed by the human G2 strains [44].

Three 2007 strains were characterized as G4 (1/07/Ire, 2/07/Ire and 88/07/Ire) but were found to be only distantly related to other animal and human G4 viruses. Based on a previous analysis of G4 GARVs [52], the Irish G4 strains appear to constitute a distinct lineage. The highest aa identity (91.5–93.2%) was found to a porcine strain, CMP77, and to porcine-like G4 human strains (CMP77-like), all which have been detected in Thailand, while identity to the prototype G4 porcine strain Gottfried was only 87.5–89.1%.

Interestingly, one 2005 strain (2B/05/Ire) and four 2006 strains (16/06/Ire, 48/06/Ire, 51/06/Ire and 54/06/Ire) were characterised as G9. The G9 Irish strains were found to be more closely related genetically to lineage VI G9 viruses, which encompasses both animal and human G9 strains [63]. Analysis of the VP7 genes of porcine G9 strains from various geographical settings has demonstrated that some porcine G9 strains are more closely related to recent human G9 strains, which have emerged since the mid-1990s worldwide, rather than to the "old" human G9 strains that were detected in the mid-1980s and are apparently now extinct [53, 63]. This piece of evidence, along with the fact that some G9 lineages are shared by both human and animal GARVs, strongly supports the hypothesis that recent human G9 strains may have originated from an unidentified animal (presumably porcine) host.

Five G5 strains (1C/05/Ire, 2F/05/Ire, 6B/05/Ire and 7F/05/Ire, 10/07/Ire) were also detected, and these displayed the highest aa identity to porcine strains CC117 and 344/04-1(93.9–95%), while identity to the prototype G5 strain OSU was only 91.7–92.6%. One strain, 60/07/Ire, identified in 2007, was characterised as G11, since it displayed 91.8–92.5% aa identity to a selection of porcine and human G11 GARV strains. Both G5 and G11 strains have also been identified in humans and are believed to have originated by inter-species transmission from pigs [7, 54, 59]. Accordingly, the G5 and G11 strains detected in this Irish survey were distantly related to the G5 and G11 prototypes, likely as a result of geographical/temporal or ecological patterns of diversification.

The 2006 Irish Regional Veterinary Surveillance Report demonstrated that enteritis, along with pneumonia and post-weaning multi-systemic wasting syndrome (PMWS), are the most frequently detected causes of mortality in pigs (Department of Agriculture, Fisheries and Food). Several studies have documented that GARVs may have a heavy impact on pig farms [28, 56]. As a consequence of either macroeconomic or local conjunctures in Ireland, a significant reduction in the number of Irish pigs and pig farmers has occurred in recent years. This area represents the fourth-largest sector in the Irish agricultural industry and is worth around 400 million euro per annum. Acquiring information on the epidemiology of GARVs and other viral enteric pathogens will be important in order to plan adequate measures of prophylaxis, to decrease the impact of enteric infections and to minimise the financial loss for pig farmers due to poor animal performance.

Currently, there is no vaccine licensed for the prevention of GARV-associated enteritis in piglets in Ireland. Given the broad genetic/antigenic heterogeneity of the GARV strains detected in this study, it remains unclear whether this diversity may pose a challenge for future prophylaxis programs for the prevention of enteritis in suckling and weaning piglets. Therefore, given the low frequency of GARV detected and the low number of farms surveyed, large-scale epidemiological investigations are required in order to better assess the genetic/antigenic diversity of porcine GARVs circulating in Irish pig farms. Analysis of these data would contribute to our knowledge and understanding of the epidemiology of GARV. Previous studies have also demonstrated a possible interaction of porcine and human rotavirus genes and suggest that reassortment could result in the introduction of novel or atypical strains, suggesting that pigs might play a role as a reservoir for the emergence of novel RV strains in humans [7, 9, 14, 23, 33, 35, 36, 43, 49, 50, 53, 63, 68].

In conclusion, this study provides evidence that porcine group A rotavirus may be detected in asymptomatic piglets, although much less frequently than in symptomatic animals. The evidence collected revealed a striking genetic heterogeneity of the GARVs circulating in Irish herds, with multiple strains being detected in animals from the same herd. Also, a marked genetic variation was observed between local porcine G2, G4, G5, G9 and G11 strains and GARVs of global origin.

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