



HAL
open science

Detection and characterization of porcine sapoviruses from asymptomatic animals in Irish farms

P.J. Collins, V. Martella, C. Buonavoglia, H. O'Shea

► **To cite this version:**

P.J. Collins, V. Martella, C. Buonavoglia, H. O'Shea. Detection and characterization of porcine sapoviruses from asymptomatic animals in Irish farms. *Veterinary Microbiology*, 2009, 139 (1-2), pp.176. 10.1016/j.vetmic.2009.05.013 . hal-00520665

HAL Id: hal-00520665

<https://hal.science/hal-00520665>

Submitted on 24 Sep 2010

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Accepted Manuscript

Title: Detection and characterization of porcine sapoviruses from asymptomatic animals in Irish farms

Authors: P.J. Collins, V. Martella, C. Buonavoglia, H. O'Shea

PII: S0378-1135(09)00264-8
DOI: doi:10.1016/j.vetmic.2009.05.013
Reference: VETMIC 4448

To appear in: *VETMIC*

Received date: 15-12-2008
Revised date: 1-5-2009
Accepted date: 28-5-2009



Please cite this article as: Collins, P.J., Martella, V., Buonavoglia, C., O'Shea, H., Detection and characterization of porcine sapoviruses from asymptomatic animals in Irish farms, *Veterinary Microbiology* (2008), doi:10.1016/j.vetmic.2009.05.013

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1 **Detection and characterization of porcine sapoviruses from asymptomatic**
2 **animals in Irish farms.**

3

4 P.J. Collins¹, V.Martella², C. Buonavoglia², H. O'Shea^{1*}.

5

6 Department of Biological Sciences, Cork Institute of Technology, Rossa Avenue,
7 Bishopstown, Cork, Ireland¹.

8

9 Department of Veterinary Public Health, Faculty of Veterinary Medicine of Bari, S.p.
10 per Casamassima Km 3, 70010 Valenzano, Bari, Italy².

11

12 *Corresponding author. Mailing address: Department of Biological Sciences, Cork
13 Institute of Technology, Rossa Avenue, Bishopstown, Cork, Ireland.

14 Telephone: +353-21-4326370, Fax: 00353-21-4326951.

15 E-mail: helen.oshea@cit.ie

16

17

18

19

20

21

22

23

24

25

26

1 **Abstract:**

2 Caliciviruses are an important cause of gastroenteritis in humans and animals.
3 Molecular analysis of the polymerase and capsid genes of porcine caliciviruses,
4 sapoviruses (SaVs) and noroviruses (NoVs), has demonstrated a broad range of
5 genetic diversity but information on their epidemiology and pathogenic role in pigs is
6 limited. In this study, 292 faecal samples were obtained from 4-5 to 8-9 week old
7 asymptomatic pigs from 4 porcine herds in Ireland during 2005-2007 and were
8 screened by RT-PCR using calicivirus-specific primers. Only 7 samples from 2
9 porcine herds tested positive for porcine calicivirus. By sequence analysis of the
10 partial RNA-dependent RNA polymerase (RdRp) fragment, 6 samples from one such
11 herd were closely related to each other (>98% nucleotide identity) and were
12 characterised as genogroup (GG) III (Cowden-like) porcine SaVs. These viruses
13 demonstrated an amino acid (aa) identity of 81.3-98.6% to GGIII SaVs. Conversely,
14 one calicivirus strain, 9/07/Ire (identified from a different herd in 2007), was distantly
15 related to GIII SaVs and displayed 94.6-98.6% aa identity to rare K7-like porcine
16 caliciviruses, representatives of a potential novel SaV genogroup (GGVII), described
17 previously in Japan and the USA. Circulation of SaVs in asymptomatic animals might
18 be a mechanism of virus persistence in porcine populations and should be considered
19 with respect to understanding the epidemiology of these viruses in porcine herds.

20

21

22

23

24

25

26

1 **Introduction:**

2 Caliciviruses are small, non-enveloped viruses, 27-38nm in diameter containing a
3 single-stranded 7.3-8.4 kb plus-sense RNA genome (Green et al., 2000). Caliciviruses
4 are associated with a wide range of economically important diseases in animals and
5 humans (Ohlinger et al., 1990; Geissler et al., 1997; Green et al., 2000).

6

7 Viruses in the family *Caliciviridae* are divided into at least 4 genera, *Norovirus*,
8 *Sapovirus*, *Vesivirus* and *Lagovirus* (Green et al., 2001). Novel caliciviruses, likely
9 representing additional candidate genera, have been identified in bovines and in
10 primates (Smiley et al., 2002; Oliver et al., 2006; Farkas et al., 2008). NoVs and SaVs
11 have emerged as the leading cause of food- and waterborne, acute, non-bacterial
12 gastroenteritis in humans worldwide (Green et al., 2006).

13

14 Sapoviruses were first found in stools of diarrheic children in 1976, by direct EM
15 (Flewett et al., 1976; Madeley et al., 1976). In 1980, a porcine SaV (Cowden strain)
16 was identified, together with rotavirus and astrovirus particles, in the faecal samples
17 of diarrhoeic piglets by EM in the United States (Saif et al., 1980). Porcine SaVs have
18 been subsequently identified in South Korea, Venezuela, Hungary, Italy and Belgium,
19 suggesting that they have a worldwide distribution in porcine populations (Kim et al.,
20 2006; Martinez et al., 2006; Reuter et al., 2006; Martella et al., 2008a; Mauroy et al.,
21 2008).

22

23 Sapoviruses are divided into genogroups (GG) based on sequence analysis of the
24 capsid protein (Farkas et al., 2004). Human SaVs have been classified into four such
25 genogroups (GI, II, IV and V) (Schuffenecker et al., 2001; Farkas et al., 2004;
26 Hansman et al., 2007). The majority of porcine SaVs resemble the prototype strain

1 Cowden, within genogroup GIII (Guo et al., 1999). Other SaVs of recent
2 identification, the porcine strains JJ681/00/US, K7/JP and 43/06-18p (Wang et al.,
3 2005a; Yin et al., 2006; Martella et al., 2008a), and the mink strain MEC151A3
4 (GenBank accession number AY144337) are likely prototypes of novel SaVs
5 although classification criteria have not been firmly established.

6

7 Some porcine caliciviruses are closely related to several human SaV and NoV strains
8 genetically, and this has raised public health concerns of potential cross-species
9 transmission and on the other hand is suggestive that the evolution of animal and
10 human SaVs is closely intermingled (Guo et al., 1999; Wang et al., 2005a, 2005b;
11 Martella et al., 2008b).

12

13 SaVs are suspected to play a role in the pathogenesis of enteritis in piglets.
14 Experimental infection of 4-day-old gnotobiotic pigs inoculated orally with wild-type
15 strain Cowden, GGIII, resulted in profuse diarrhoea, anorexia, and intestinal lesions
16 (Flynn et al., 1988). Also, large scale screening of stools from diarrhoeic piglets has
17 identified SaVs in as many as 32.5% of animals (Martella et al., 2008a), either alone
18 or in conjunction with group A and group C rotaviruses. However, SaVs are not
19 included routinely in the diagnostic algorithms for porcine pathogens and their impact
20 in the porcine livestock is unclear. Thus, epidemiological information on SaV
21 distribution in porcine populations and on SaV genetic heterogeneity is limited.

22

23 In this study, faecal samples obtained from asymptomatic piglets in Ireland were
24 screened by RT-PCR using calicivirus-specific primers. The sequence of the
25 calicivirus strains was determined and analysed in detail, in order to amass
26 information on their genetic features.

1 **Materials and Methods.**

2 **Sample collection and Preparation.** A total of 292 faecal samples were obtained
3 from 4-5 to 8-9 week old asymptomatic pigs from porcine herds in Ireland over 3
4 years, from 2005-2007. The samples were collected from 4 different herds in County
5 Cork. Eighty samples were collected in May 2005 (farm 1), 80 samples were collected
6 in September 2006 (farm 2), 102 samples in May 2007 (farm 3). Thirty samples (farm
7 4) were collected in March 2007, in County Dublin. All of the samples were
8 processed in order to extract the RNA, using standard phenol-chloroform method with
9 ethanol precipitation. The extracted nucleic acids were re-suspended in 100µl of
10 sterile DEPC H₂O and stored at -80°C prior to use. All of the samples had been
11 screened for the presence of group A (GARVs) and group C rotaviruses (GCRVs) by
12 RT-PCR (Collins et al., 2008), using oligonucleotide primers previously described
13 (Gentsch et al., 1992; Sanchez-Fauquier et al., 2003).

14

15 **Detection by RT-PCR of caliciviruses.** For the detection of caliciviruses, the
16 broadly-reactive primer pair p289/p290 (Jiang et al., 1999), which targets the
17 conserved motifs of RdRp region was used. For RT-PCR, the reaction was carried out
18 in 50ul volume using an Enhanced Avian Reverse transcriptase kit (Sigma-Aldrich) in
19 a one-step reaction. All reactions were carried out on a MJ researcher PTC-200
20 thermocycler (GMI Inc, Minnesota, USA).

21

22 The following conditions were used for RT-PCR using the primer set p289/p290
23 (Jiang et al., 1999): 45°C for 30 mins, 94°C for 5 mins followed by 40 cycles of 94°C
24 for 1 min, 48°C for 1mins 20sec and 72°C for 1 min plus a final extension of 72°C for
25 10 min.

26

1 All samples which were positive by primer pair p289/p290, were also screened with
2 primer pair PEC45/PEC46, designed to recognize selectively porcine GIII SaVs (Guo
3 et al., 2001). The conditions for RT-PCR with primers PEC45 and PEC46 (Guo et al.,
4 2001) were as follows: 45°C for 30 mins, 94°C for 5 mins followed by 40 cycles of
5 94°C for 1 min, 55° C for 1 min and 72°C for 1 min plus a final extension of 72°C for
6 10 min.

7

8 **Sequence analysis of the porcine caliciviruses.** All samples positive by the
9 calicivirus-specific RT-PCR were subjected to sequencing. The PCR products were
10 purified using a QIAquick PCR purification kit (Qiagen Ltd, West Sussex, England)
11 and sequenced commercially at MWG Biotech (MWG-biotech, Ebersberg, Germany).

12

13 The sequences were assembled, edited and analyzed using the Bioedit software
14 package version 2.1 (Hall, 1999). Preliminary analysis was accomplished by
15 comparison with the sequences available in the database using the web-based
16 programs BLAST (<http://www.ncbi.nlm.nih.gov/BLAST>) and FASTA
17 (<http://www.ebi.ac.uk/fasta33>). Phylogenetic and molecular evolutionary analyses
18 were conducted using MEGA version 2.1 (Kumar et al., 2001). A phylogenetic tree,
19 based on the RdRp partial sequences, was elaborated with both parsimony and
20 distance methods, supplying a statistical support with bootstrapping of 500 replicates.

21 The partial RdRp sequences of the Irish porcine caliciviruses, 9/07/Ire and 8D/05/Ire
22 were registered in Gen Bank under the accession numbers, FJ975601 and FJ975602
23 respectively.

24

25 **Results and discussion.**

1 Out of 292 samples, 7 were found to be positive for calicivirus. Six samples
2 (7A/05/Ire, 16/05/Ire, 2A/05/Ire, 1M/05/Ire, 1D/05/Ire and 8D/05/Ire) were identified
3 in 2005, from a large commercial piggery (Farm 1) in County Cork and one sample
4 (9/07/Ire) was identified from an asymptomatic animal in a different commercial
5 piggery (Farm 3), in County Cork, Southern Ireland, 2007. In addition, a total of 13
6 out of 292 samples (4.4%) were found to contain GCRV, while 19 samples (6.5%)
7 contained GARV. There were no cases of mixed infections.

8

9 The samples positive for calicivirus by RT-PCR with primer pair p289/p290 (Jiang et
10 al., 1999) were screened with the GIII SaV-specific primers PEC45 and PEC46 (Kim
11 et al., 2006). Amplicons of 572 bp were obtained and successfully sequenced for 6
12 strains, all which had been identified in 2005 from the same herd (7A/05/Ire,
13 16/05/Ire, 2A/05/Ire, 1M/05/Ire, 1D/05/Ire and 8D/05/Ire). Despite numerous
14 attempts, it was not possible to amplify strain 9/07/Ire with the primer pair
15 PEC45/PEC46. Therefore, sequence data was obtained using the primer set
16 p289/p290 (Jiang et al., 1999).

17

18 Phylogenetic analysis was carried out with a selection of strains representative of the
19 *Sapovirus* genus, including human SaVs of genogroup GI, GII, GIV and GV, mink
20 SaV, porcine GIII SaV strains (Cowden-like) and porcine SaVs JJ681, K7/JP, QW19
21 and 43/06-18p (Wang et al., 2005a; Yin et al., 2006; Martella et al., 2008a).
22 Representatives of other genera were also used: Lagovirus/RHDV/GH/1988/GE,
23 Vesivirus/FCV/F9/1958/US, Norovirus/Norwalk/68/US, Becovirus/Nebraska/80/US
24 (Smiley et al., 2002) and Vesivirus/Ca48/1990/JP (Matsuura et al., 2002). In addition,
25 the sequences of unclassified porcine caliciviruses (200/05-32, K-11, 42/05-6c and
26 200/05-10-like) were included in the analysis (Yin et al., 2006; Martella et al., 2008a).

1 Based on inspection of the phylogenetic tree, 3 lineages were observed within GGIII.
2 Lineage I consisted of the majority of the strains, which included SaV strains from the
3 US, Korea, Venezuela, Italy and the Netherlands. Lineage II consisted of Korean and
4 Hungarian strains, while lineage III consisted of the American strain MM280 and the
5 Dutch strain VA10. GGI, II, IV and V included human SaVs. As supported by
6 sequence analysis of the capsid-coding gene, the calicivirus strains JJ681, K7/JP and
7 43/06-18p are likely prototypes of SaV distinct genogroups, GGVI, GGVII and
8 GGVIII, respectively (Wang et al., 2005a; Yin et al., 2006; Martella et al., 2008a). A
9 number of porcine caliciviruses of uncertain classification (200/05-32, K-11, 42/05-6c
10 and 200/05-10-like) did not segregate in the phylogenetic tree with any of the well-
11 defined SaV genogroups.

12
13 The 6 Irish SaV strains identified in 2005 (7A/05/Ire, 16/05/Ire, 2A/05/Ire, 1M/05/Ire,
14 1D/05/Ire and 8D/05/Ire) segregated within lineage I of GGIII, which also includes
15 the prototype porcine SaV strain Cowden. Amino acid (aa) identity (Table 1) of strain
16 8D/05/Ire was 98.6% to representatives of lineage I of GGIII SaVs, while it was 81.3-
17 94.6% to representatives of lineages II and III of GGIII SaVs. Identity was 52-65.3%
18 to human GI, II, IV and V SaVs, 58.6-60% to QW19-like strains (GGVIII), 50.6% to
19 the GGVI strain JJ681, and 44-45.3% to the GVII strains LL26, K7/JP and K10/JP
20 and 44-48% to the unclassified porcine caliciviruses 200/05-32, K-11, 42/05-6c and
21 200/05-10.

22
23 The SaV strain 9/07/Ire was grouped with strains K7/JP, K10/JP and K16/JP in
24 genogroup GGVII. Strain 9/07/Ire displayed an aa identity (Table 1) of 94.6-98.6% to
25 K7-like caliciviruses. Amino acid identity to the GGVI strain JJ681 was 61.3%,
26 whereas it was 42.6-44% to GGIII (Cowden-like) strains and 41.3-42.6% to human

1 GGI, II, IV and V SaVs. Amino acid identity to the unclassified caliciviruses 200/05-
2 32, K-11, 42/05-6c and 200/05-10 ranged from 56 to 66.6%, while it was 41.3% to
3 GGVIII SaVs, QW19-like.

4

5 Overall the prevalence of SaVs in asymptomatic animals from the 4 Irish herds was
6 low (2.4%). SaVs were detected using broadly reactive primers for calicivirus (Jiang
7 et al., 1999) in 7/292 samples and 2 out of 4 herds. By using other primer sets or the
8 same primer pair, previous studies have also indicated a low prevalence rate of
9 calicivirus infections in piglets (Sugieda et al., 1998; Van der Poel et al., 2000; Wang
10 et al., 2005a). A study in Korea on piglets with enteritis indicated a low detection rate
11 (8.8%), with all the samples being characterised as GGIII (Kim et al., 2006).
12 Conversely, RT-PCR screening with primers p289/p290 of fecal samples from
13 diarrheic piglets in Italy detected caliciviruses in as high as 32.5% (68/209) of the
14 samples from 46/118 farms (38.9%), mostly in conjunction with GARVs and/or
15 GCRVs (Martella et al., 2008a). Analysis of fecal samples by RT-PCR with primers
16 p289/p290 revealed an almost identical frequency of detection from symptomatic and
17 asymptomatic animals (14% versus 19%, respectively) in Venezuela over a 10-year
18 time period and the majority of the strains were characterised as GGIII SaVs
19 (Martinez et al., 2006). These studies provide firm evidence that porcine SaVs are
20 common enteric viruses of piglets but do not clarify whether there is a significant
21 difference in calicivirus prevalence between symptomatic and asymptomatic animals.
22 Circulation of SaVs in asymptomatic pigs could mirror the epidemiology of enteric
23 caliciviruses in humans. Asymptomatic infections by noroviruses in humans have
24 been detected frequently and may be related to either genetic resistance (Harrington et
25 al., 2002; Huston et al., 2002, Marionneau et al., 2002; Huang et al., 2003; Huston et
26 al., 2003; Lindesmith et al., 2003), or to protective immunity preventing overt clinical

1 signs (Kapikian et al., 1978; Pang et al., 2000). Accordingly, asymptomatic virus
2 carriers would help maintain virus circulation in the population.

3

4 Experimental administration with the wild-type SaV strain Cowden by the oral route
5 caused diarrhoea, anorexia, and intestinal lesions in 4-day-old gnotobiotic pigs (Flynn
6 et al., 1988). This experimental evidence suggests a potential role for SaVs in the
7 aetiology of weaning and post-weaning enteritis of piglets, along with other common
8 enteric pathogens, such as GCRVs and chiefly GARVs (Saif and Jiang, 1990; Gouvea
9 et al., 1991; Saif et al., 1994; Will et al., 1994; Yuan et al., 2006; Martella et al.,
10 2008a). It is likely that piglets enteritis has a multi-factorial nature, with viruses,
11 bacteria and/or parasites acting in synergy, yet under the influence of other factors,
12 such as environmental and social stress due to overcrowding and/or incorrect
13 management. A clear understanding of this scenario is still hampered by the fact that
14 enteric viruses are not included routinely in the diagnostic algorithms of piglets
15 enteritis and the information is limited.

16

17 Molecular analyses of partial RNA polymerase gene sequences confirmed that the 7
18 Irish strains detected in this study were members of the *Sapovirus* genus. Due to the
19 existence of a preferential recombinational site downstream of the primer binding
20 region (Green et al., 2001; Katayama et al., 2002), analysis of the RdRp fragment can
21 not be used to definitively characterize SaVs or other caliciviruses and a definitive
22 characterization should rely on sequence analysis of the capsid-coding region (Green
23 et al., 2000a; Schuffenecker et al., 2001; Katayama et al., 2002). However, since the
24 RdRp region has proven to be a good target to design broadly reactive diagnostic
25 primers for caliciviruses (Jiang et al., 1999), and sequence information spanning this
26 region has been determined for a number of previously unrecognised caliciviruses,

1 attempts have been made to use the RdRp partial sequence to establish the genetic
2 relationships among the various SaV strains. Based on the analysis of this smaller
3 fragment, novel potential SaV genogroups have been defined using an identity cut off
4 of 80% (Wang et al., 2006; Martella et al., 2008a). Six strains (7A/05/Ire, 16/05/Ire,
5 2A/05/Ire, 1M/05/Ire, 1D/05/Ire and 8D/05/Ire) were characterised as GGIII
6 (Cowden-like), lineage I. Conversely, strain 9/07/Ire resembled (94.6-98.6%) K7-like
7 SaVs, that are tentatively proposed as a novel SaV genogroup (GGVII). While GIII
8 SaVs appear as the most common SaV strains worldwide, the K7-like SaVs seem to
9 be less common (Kim et al., 2006; Martinez et al., 2006; Martella et al., 2008a; Wang
10 et al., 2006a). Such rare SaV strains have been already detected in Japan and USA
11 from either symptomatic or asymptomatic animals (Wang et al., 2006a; Yin et al.,
12 2006). Their detection on the European continent indicates that they are not restricted
13 geographically and provides further evidence for the genetic heterogeneity of porcine
14 enteric caliciviruses.

15

16 Not unexpectedly, porcine NoVs were not detected in this study. This finding is in
17 agreement with other published data in which NoVs have been detected in healthy
18 finisher pigs, but not from nursing or post-weaning pigs (Sugieda et al., 1998; van der
19 Poel et al., 2000; Wang et al., 2005b, 2006; Martella et al., 2008a).

20

21 The 2006 Irish Regional Veterinary Surveillance Report demonstrated that
22 pneumonia, post-weaning multi-systemic wasting syndrome (PMWS), and enteritis
23 were the most frequently detected causes of mortality in pigs (Department of
24 Agriculture, Fisheries and Food). Given the evidence that caliciviruses, GARVs and
25 GCRVs (Collins et al., 2008) are circulating in piglets in Ireland, it will be important
26 to establish surveillance for enteric viral pathogens, including porcine caliciviruses, in

1 order to gather information on the genetic diversity of these viruses and, above all, in
2 order to plan more adequate measures of control for piglets enteritis. Accordingly, it
3 would be opportune to include SaVs in the diagnostic algorithms for porcine enteric
4 infections.

5

6 **References:**

7 Collins, P.J., Martella, V., O'Shea, H., 2008. Detection and characterization of group
8 C rotaviruses in asymptomatic piglets in Ireland. *J. Clin. Microbiol.* 46, 2973-9.

9

10 Farkas, T., Zhong, W.M., Jing, Y., Huang, P.W., Espinosa, S.M., Martinez, N.,
11 Morrow, A.L., Ruiz-Palacios, G.M., Pickering, L.K., Jiang, X., 2004. Genetic
12 diversity among sapoviruses. *Arch. Virol.* 149, 1309-1323.

13

14 Farkas, T., Sestak, K., Wei, C., Jiang, X., 2008. Characterization of a rhesus monkey
15 calicivirus representing a new genus of Caliciviridae. *J. Virol.* 82, 5408-16.

16

17 Flewett, T.H., Davies, H., 1976. Letter: Caliciviruses in man. *Lancet* 1 (7954) pp. 311.

18

19 Flynn, W.T., Saif, L.J., Moorhead, P.D., 1988. Pathogenesis of porcine enteric
20 calicivirus-like virus in four-day-old gnotobiotic pigs. *Am. J. Vet. Res.* 49, 819-825.

21

22 Geissler, K., Schneider, K., Platzer, G., Truyen, B., Kaaden, O.R., Truyen, U., 1997.
23 Genetic and antigenic heterogeneity among feline calicivirus isolates from distinct
24 disease manifestations. *Virus Res.* 48, 193-206.

25

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

4

5 Green, K.Y., Ando, T., Balayan, M.S., Berke, T., Clarke, I.N., Estes, M.K., Matson,
6 D.O., Nakata, S., Neill, J.D., Studdert, M.J., Thiel, H.J., 2000. Taxonomy of the
7 caliciviruses. *J. Infect. Dis.* 18, S322–S330.

8

9 Green, J., Vinje, J., Gallimore, C.I., Koopmans, M., Hale, A., Brown, D.W., 2000a.
10 Capsid protein diversity among Norwalk-like viruses. *Virus Genes.* 20, 227-36.

11

12 Green, K. Y., Chanock, R.M., Kapikian, A.Z., 2001. Human calicivirus. *In* H. P.
13 Knipe (ed.), *Fields virology*, 4th ed., vol. 1. Lippincott Williams & Wilkins,
14 Philadelphia, Pa. pp. 841-874.

15

16 Guo, M., Chang, K.O., Hardy, M.E., Zhang, Q., Parwani, A.V., Saif, L.J., 1999.
17 Molecular characterization of a porcine enteric calicivirus genetically related to
18 Sapporo-like human caliciviruses. *J. Virol.* 73, 9625-9631.

19

20 Guo, M., J. Hayes, K. O. Cho, A. V. Parwani, L. M. Lucas, and L. J. Saif. 2001.
21 Comparative pathogenesis of tissue culture-adapted and wild-type Cowden porcine

1 enteric calicivirus (PEC) in gnotobiotic pigs and induction of diarrhea by intravenous
2 inoculation of wild type PEC. *J. Virol* 75, 9239-51.

3

4 Gouvea, V., Allen, J.R., Glass, R.I., Fang, Z.Y., Bremont, M., Cohen, J., McCrae,
5 M.A., Saif, L.J., Sinarachatanant, P., Caul, E.O., 1991. Detection of group B and C
6 rotaviruses by polymerase chain reaction. *J. Clin. Microbiol.* 29, 519-523.

7

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

10

11 Hansman, G.S., Oka, T., Katayama, K., Takeda, N., 2007. Human sapoviruses:
12 genetic diversity, recombination, and classification. *Rev. Med. Virol.* 17, 133-41.

13

14 Harrington, P.R., Lindesmith, L., Yount, B., Moe, C.L., Baric, R.S., 2002. Binding of
15 Norwalk virus-like particles to ABH histo-blood group antigens is blocked by antisera
16 from infected human volunteers or experimentally vaccinated mice. *J. Virol.* 76,
17 12335-43.

18

19 Huang, P., Farkas, T., Marionneau, S., Zhong, W., Ruvoen-Clouet, N., Morrow, A.L.,
20 2003. Noroviruses bind to human ABO, Lewis, and secretor histo-blood group
21 antigens: identification of 4 distinct strain-specific patterns. *J. Infect. Dis.* 188, 19-31.

22

- 1 Huston, A.M., Atmar, R.L., Graham, D.Y., Estes, M.K., 2002. Norwalk virus
2 infection and disease is associated with ABO histo-blood group type. *J. Infect. Dis.*
3 185, 1335-7.
- 4
- 5 Huston, A.M., Atmar, R.L., Marcus, D.M., Estes, M.K., 2003. Norwalk virus-like
6 particle hemagglutination by binding to h histo-blood group antigens. *J. Virol.* 77,
7 405-15.
- 8
- 9 Jiang, X., Huang, P.W., Zhong, W.M., Farkas, T., Cubitt, D.W., Matson, D.O., 1999.
10 Design and evaluation of a primer pair that detects both Norwalk- and Sapporo- like
11 caliciviruses by RT-PCR. *J. Virol. Methods.* 83, 145-154.
- 12
- 13 Kapikian, A.Z., Greenberg, H.B., Cline, W.L., Kalica, A.R., Wyatt, R.G., James Jr,
14 H.D., 1978. Prevalence of antibody to the Norwalk agent by a newly developed
15 immune adherence hemagglutination assay. *J. Med. Virol.* 2, 281-94.
- 16
- 17 Katayama, K., Shirato-Horikoshi, H., Kojima, S., Kageyama, T., Oka, T., Hoshino, F.,
18 2002. Phylogenetic analysis of the complete genome of 18 Norwalk-like viruses.
19 *Virology.* 299, 225-39.
- 20
- 21 Kim, H.J., Cho, H.S., Cho, K.O., Park, N.Y., 2006. Detection and molecular
22 characterisation of porcine enteric calicivirus in Korea, genetically related to
23 sapoviruses. *J. Vet. Med. B: Infect. Dis Vet. Public Health.* 53, 155-159.
- 24
- 25 Kumar, S., Tamura, K., Jakobsen, I.B., Nei, M., 2001. MEGA2: Molecular
26 Evolutionary Genetics Analysis software. *Bioinformatics.* 17, 1244-1245.

1

2 Lindesmith, L., Moe, C., Marionneau, S., Ruvoen, N., Jiang, X., Lindblad, L., 2003.

3 Human susceptibility and resistance to Norwalk virus infection. *Nat Med.* 9, 548-53.

4

5 Madeley, C.R., Cosgrove, B.P., 1976. Letter: Caliciviruses in man. *Lancet* 1 (7952)

6 pp. 199–200.

7

8 Marionneau, S., Ruvoen, N., Le Moullac-Vaidye, B., Clement, M., Cailleau-Thomas,

9 A., Ruiz-Palacois, G., 2002. Norwalk virus binds to histo-blood group antigens

10 present on gastroduodenal epithelial cells of secretor individuals. *Gastroenterology.*

11 122, 1967-77.

12

13 Martella, V., Banyai, K., Lorusso, E., Decaro, N., Bellacicco, A.L., Mari, V., Saif, L.,

14 Costantini, V., Grazia De, S., Pezzotti, G., Lavazza, A., Buonavoglia, C., 2008a.

15 Genetic heterogeneity within porcine enteric caliciviruses identified from diarrhoeic

16 piglets. *Virus Genes.* 36, 365-373.

17

18 Martella, V., Lorusso, E., Banyai, K., Decaro, N., Corrente, M., Elia, G., Cavalli, A.,

19 Radogna, A., Costantini, V., Saif, L.J., Lavazza, A., Trani Di, L., Buonavoglia, C.,

20 2008b. Identification of a Porcine Calicivirus Related Genetically to Human

21 Sapoviruses. *J. Clin. Microbiol.* 46, 1907-1913.

22

23 Martinez, M., Alcalá, A., Carruyo, G., Botero, L., Liprandi, F., Ludert, J., 2006.

24 Molecular detection of porcine enteric caliciviruses in Venezuelan farms. *Veterinary*

25 *Microbiology.* 116, 77-84.

26

- 1 Matsuura, Y., Tohya, Y., Nakamura, K., Shimojima, M., Roerink, F., Mochizuki, M.,
2 Takase, K., Akashi, H., Sugimura, T., 2002. Complete nucleotide sequence, genome
3 organization and phylogenetic analysis of the canine calicivirus. *Virus Genes*. 25, 67-
4 73.
- 5
- 6 Mauroy, A., Scipioni, A., Mathijs, E., Miry, C., Ziant, D., Thys, C., Thiry, E., 2008.
7 Noroviruses and sapoviruses in pigs in Belgium. *Arch. Virol.* 153, 1927-1931.
- 8
- 9 Ohlinger, V.F., Haas, B., Meyers, G., Weiland, F., Thiel, H.J., 1990. Identification
10 and characterization of the virus causing rabbit hemorrhagic disease. *J. Virol.* 64,
11 3331–3336.
- 12
- 13 Oliver, S.L., Asobayire, E., Dastjerdi, A.M., Bridger, J.C., 2006. Genomic
14 characterization of the unclassified bovine enteric virus Newbury agent-1 (Newbury1)
15 endorses a new genus in the family of Caliciviridae. *Virology*. 350, 240-50.
- 16
- 17 Pang, X.L., Honma, S., Nakata, S., Vesikari, T., 2000. Human caliciviruses in acute
18 gastroenteritis of young children in the community. *J. Infect. Dis.* 181, S288-294.
- 19
- 20 Reuter, G., Biro, H., Szucz, G., 2006. Enteric caliciviruses in domestic pigs in
21 Hungary. *Arch. Virol.* 152, 611-614.
- 22
- 23 Saif, L.J., Bohl, E.H., Theil, K.W., Cross, R.F., House, J.A., 1980. Rotavirus-like,
24 calicivirus-like, and 23-nm virus-like particles associated with diarrhea in young pigs,
25 *J. Clin. Microbiol.* 12, 105–111.
- 26

- 1 Saif, L. J., Jiang, B., 1990. Non-group A rotaviruses of humans and animals. *In* R. F.
2 Ramig (ed.), Rotaviruses. Springer-Verlag, Berlin, Germany. pp.339-371.
3
- 4 Saif, L.J, Rosen, B., Parwani, A., 1994. Animal Rotaviruses. *In*: A. Kapikian, Editor,
5 Viral Infections of the Gastrointestinal Tract, Marcel Dekker, Inc., New York. pp.
6 279–367.
7
- 8 Sanchez-Fauquier, A., Roman, E., Colomina, J., Wilhelmi, I., Glass, R.I., Jiang, B.,
9 2003. First detection of group C rotavirus in children with acute diarrhea in Spain.
10 Arch. Virol. 148, 399-404.
11
- 12 Schuffenecker, I., Ando, T., Thouvenot, D., Lina, B., Aymard, M., 2001. Genetic
13 classification of “Sapporo-like viruses.” Arch. Virol. 146, 2115-2132.
14
- 15 Smiley, J. R., K. O. Chang, J. Hayes, J. Vinje, and L. J. Saif. 2002. Characterization
16 of an enteropathogenic bovine calicivirus representing a potentially new calicivirus
17 genus. J. Virol. 76, 10089-98.
18
- 19 Sugieda, M., Nagaoka, H., Kakaishima, Y., Ohshita, T., Nakamura, S., Nakajima, S.,
20 1998. Detection of Norwalk-like virus genes in the caecum contents of pigs. Arch.
21 Virol. 143, 1215-1221.
22
- 23 Van Der Poel, W.H., Vinje, J., van Der Heide, R., Herrera, M.I., Vivo, A., Koopmans,
24 M.P., 2000. Norwalk-like calicivirus genes in farm animals. *Emerg. Infect. Dis.* 6, 36-
25 41.
26

- 1 Wang, Q.H., Han, M.G., Funk, J.A., Bowman, G., Janies, D.A., Saif, L.J., 2005a.
2 Genetic diversity and recombination of porcine sapoviruses. *J. Clin. Microbiol.* 43,
3 5672–5963.
4
- 5 Wang, Q.H., Han, M.G., Cheetham, S., Souza, M., Funk, J.A., Saif, L.J., 2005b.
6 Porcine noroviruses related to human noroviruses. *Emerg. Infect. Dis.* 11, 1874–1881.
7
- 8 Wang, O.H., Costantini, V., Saif, L.J., 2006. Porcine enteric caliciviruses: Genetic and
9 antigenic relatedness to human caliciviruses, diagnosis and epidemiology. *Vaccine.*
10 25, 5453-5466.
11
- 12 Wang, Q. H., M. Souza, J. A. Funk, W. Zhang, and L. J. Saif. 2006a. Prevalence of
13 Noroviruses and Sapoviruses in Swine of Various Ages Determined by Reverse
14 Transcription-PCR and Microwell Hybridization Assays. *J. Clin. Microbiol.* 44, 2057-
15 62.
16
- 17 Will, L. A., Paul, P.S., Proescholdt, T.A., Aktar, S.N., Flaming, K.P., Janke, B.H.,
18 Sacks, J., Lyoo, Y.S., Hill, H.T., Hoffman, L.J., 1994. Evaluation of rotavirus
19 infection and diarrhoea in Iowa commercial pigs based on an epidemiologic study of a
20 population represented by diagnostic laboratory cases. *J. Vet. Diagn. Investig.* 6, 416-
21 422.
22
- 23 Yin, Y., Tohya, Y., Owaga, Y., Numazawa, D., Kato, K., Akashi, H., 2006. Genetic
24 analysis of calicivirus genomes detected in intestinal contents of piglets in Japan.
25 *Arch. Virol.* 151, 1749-1759.
26

1 Yuan, L., Stevenson, G.W., Saif, L.J., 2006. Rotavirus and reovirus. *In* B. E. Straw,
2 J.J. Zimmerman, S. D’Allaire, and D.J. Taylor (ed.), Diseases of swine, 9th ed.
3 Blackwell Publishing Professional, Ames, IA. pp. 435-454.

4

5 **Figure Legends.**

6 **Fig. 1:** Neighbor-joining phylogenetic tree based on partial (266 nt) sequence of the
7 RdRp of SaV, RHDV, NB, Norwalk, FCV and CaCV strains. The Irish SaV strains
8 described in this study are in boldface. Phylogenetic analysis was carried out with a
9 selection of representative strains as described by Martella et al., 2008a. Within Fig.
10 1, the proposed new genogroups within the *Sapovirus* genus based on porcine SaV
11 strains JJ681, K7/JP, QW19 and 43/06-18p (Wang et al., 2005a; Yin et al., 2006;
12 Martella et al., 2008a) are indicated as GGVI, GGVII and GGVIII.

13

14 **Table 1:** RdRp comparison (% amino acid identity) between the Irish SaV strains
15 8D/05 and 9/07 detected in this study and representatives of the sapovirus genus
16 within the Caliciviridae family. The highest identity values for each strain to reference
17 viruses are in bold.

18

19

20

21

22

Strain	8D/05/Ire	9/07/Ire	Strain Identity
8D/05/Ire	-	44	
9/07/Ire	44	-	
AY615806/Po/PECIVA116	98.6	44	GGIII PoSaVs Lineage I
AY633966/Po/Yaracuy/1999/VE	98.6	44	GGIII PoSaVs Lineage I
AF182760/Po/Cowden	98.6	44	GGIII PoSaVs Lineage I
AY425671/Po/LL14	98.6	44	GGIII PoSaVs Lineage I
DQ389628/Po/JB-SC55/04/Korea	94.6	44	GGIII PoSaVs Lineage II
DQ383274/Po/swine-Id3/2005/HUN	94.6	44	GGIII PoSaVs Lineage II
AY615807/Po/SWECI/VA10	82.6	42.6	GGIII PoSaVs Lineage III
AY826425/Po/OH-QW152/03/US	81.3	42.6	GGIII PoSaVs Lineage III
AY144332/MinkMEC/Canada 151B	61.3	42.6	Mink SaV
AF405715/Hu/Arg39/GV	52	41.3	GV SaV
AY826424/Po/MI-QW19/02/US	60	41.3	QW19-like SaVs (GVIII SaV?)
AY615811/Po/SWECII/VA103	58.6	41.3	QW19-like SaVs (GVIII SaV?)
Po/43/06-18p3/It	60	41.3	QW19-like SaVs (GVIII SaV?)
AY157866/Hu/Mex11718/Mex/GIV	61.3	42.6	GGII+GGIV Hu SaVs
AY157863/Hu/cruise ship/GII-3	61.3	42.6	GGII+GGIV Hu SaVs
AF435809/Hu/Mex340/GII-2	60	42.6	GGII+GGIV Hu SaVs
AY237420/Hu/Mc10/GII-4	61.3	42.6	GGII+GGIV Hu SaVs
AY603425/Hu/C12/GII-5	61.3	42.6	GGII+GGIV Hu SaVs
U95645/Hu/London/29845/GII-1	58.6	41.3	GGII+GGIV Hu SaVs
U65427/Hu/Sapporo/GI-1	58.6	42.6	GGI Hu SaVs
U95643/Hu/Houston/86/GI-1	58.6	42.6	GGI Hu SaVs
U73124/Hu/Parkville/GI-2	65.3	42.6	GGI Hu SaVs
U95644/Hu/Houston/90/GI-2	64	41.3	GGI Hu SaVs
AY615812/Po/SWECIII/VA24a	44	57.3	200/05-32-like SaVs
AB223002/Po/K15/JP	45.3	58.6	200/05-32-like SaVs
AB223005/Po/K24/JP	44	60	200/05-32-like SaVs
AB223000/Po/K11/JP	45.3	56	K11-like SaVs
AY615814/Po/SWECIII/VA112	44	57.3	42/05-6c-like SaVs
AY974192/Po/OH-JJ681/2000/US	50.6	61.3	JJ681-like SaVs (GGVI SaV?)
AB223004/Po/K19/JP	48	66.6	200/05-10-like SaVs
AY615813/Po/SWECIII/VA59	46.6	65.3	200/05-10-like SaVs
AY974195/Po/OH-LL26/US	45.3	94.6	K7-like SaVs (GGVII SaV?)
AB221130/Po/K7/JP	45.3	98.6	K7-like SaVs (GGVII SaV?)
AB221131/Po/K10/JP	44	96	K7-like SaVs (GGVII SaV?)

1

2

3

4

Fig 1:

