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Smith, N., Power, U. F., & McKillen, J. (2018). Phylogenetic analysis of porcine reproductive and respiratory syndrome virus isolates from Northern Ireland. *Archives of Virology*. Advance online publication. https://doi.org/10.1007/s00705-018-3886-7

Published in: Archives of Virology

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Document Version: Peer reviewed version

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Phylogenetic analysis of porcine reproductive and respiratory syndrome virus isolates from Northern Ireland

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11 Abstract

To investigate the genetic diversity of porcine reproductive and respiratory syndrome virus (PRRSV) in Northern Ireland, the ORF5 gene from 9 field isolates was sequenced and phylogenetically analysed. The results revealed relatively high diversity amongst isolates with 87.6-92.2% identity between farms at the nucleotide level and 84.1-93.5% identity at the protein level. Phylogenetic analysis confirmed that all 9 isolates belonged to the European (type 1) genotype, and formed a cluster within the subtype 1 subgroup. This study provides the first report on PRRSV isolate diversity in Northern Ireland.

Porcine reproductive and respiratory syndrome virus (PRRSV), the causative agent of porcine reproductive and respiratory syndrome (PRRS), is a small, enveloped, positive sense, single stranded RNA virus (genus Arterivirus). It is responsible for inducing reproductive failure in sows and respiratory disease in growing pigs [1]. Two genotypes of PRRSV are recognised internationally, European (type 1) and North American (type 2). The type 2 virus was originally isolated in pig herds in America in 1987 [2], while type 1 was first identified in European pigs in 1991 [3]. Although the first outbreaks occurred close together, the two genotypes have high sequence divergence and share only 50-70% genetic identity [4–7]. There are at least 4 subtypes of type 1 PRRSV, with different worldwide and European distributions, as well as variations in pathogenicity [8]. Similarly, type 2 PRRSV is further divided into several lineages [9].

The high strain diversity of PRRSV is consistent with other RNA viruses, and results in rapidly emerging virus variants that lead to recurrent disease outbreaks and increased difficulty with virus control [10-12]. As the island of Ireland is geographically isolated we hypothesised that Northern Ireland (NI) may have circulating strains of virus that are different from those found in Great Britain and the rest of Europe. The genotype of the virus has obvious implications for diagnostics and vaccination, with only limited protection afforded against heterologous strains. In this study, the ORF5 gene from PRRSV field isolates in NI was sequenced and compared with those from modified live virus (MLV) vaccine sequences and other European isolates. The genetic diversity of PRRSV strains in NI relative to vaccine and European wild type strains was examined and the implications for PRRSV-associated disease management discussed.

Seven lung samples and 2 mesenteric lymph node (MLN) samples were obtained
from 9 pigs from 5 farms in NI. Ten percent w/v tissue homogenates were prepared
and RNA was extracted using standard methods. A previously described primer set
[13] was used to amplify a 780 bp PCR product containing the complete ORF5 gene

of PRRSV and resultant amplicons were sequenced commercially. Raw sequence reads were analysed and trimmed using Geneious version 6 [14]. Clustal W was used to align the NI nucleotide sequences with a selection of chronologically and geographically varied wildtype PRRSV type 1 isolates from subtypes 1-4 as well as MLV sequences from GenBank. Phylogenetic trees were generated using the neighbour-joining method [15] in MEGA 6 [16] with 1000 iterations for bootstrapping. Predicted protein sequences were also aligned using Clustal W. The sequences of the predicted GP5 proteins were searched for motifs associated with N-linked glycosylation [17] and a previously described neutralising epitope [18, 19].

All field isolates from NI were analysed and confirmed as type 1, subtype 1 PRRSV
isolates by sequencing and BLASTn analysis. Comparison of aligned sequences
showed the expected high degree of variability among sequences (Table 1). Analysis
of nucleotide sequences from NI isolates showed 87.62-92.2% similarity between
farms. Within farms there was a high level of sequence conservation (99.5-100%).

Nucleotide sequence homology between NI strains and selected European sequences ranged from 76.2% (subtype 2) to 92.7% (subtype 1). The NI strains were all subtype 1 and had between 82.5% and 92.7% similarity with the other sequences in this subtype. Nucleotide similarity was lower between the NI sequences and other European subtypes (76.2-80% with subtype 2, 79.2-83.5% with subtype 3 and 77.6 -82.1% with subtype 4). NI sequences were also compared to the ORF5 sequences of 4 commercially available PRRSV MLV vaccines. Sequence comparison showed nucleotide homology ranging from 84.7-92.9%. The majority of NI PRRSV isolates were collected in 2015 (8 sequences from 4 farms) and these clustered together as a distinct subtype 1 subgroup on the phylogenetic tree as part of a larger subgroup (Figure 1). One sequence from farm 13320-12 collected in 2012 clustered with UK and North American strains, most closely clustering with EuroPRSSV (Accession no. AY366525) [20].

The predicted proteins were 201 amino acids in length for all NI sequences, with homology ranging from 74.1-100% with the other international sequences (Table 1), reflecting the differences evident at the nucleotide level. NI PRRSV protein sequences were 81.6-91.5% identical to vaccine strains.

The presence of a neutralisation epitope located in the N-terminus of the GP5 ectodomain was observed between residues 29-35 (WSFADGN) (Fig. 2), as previously described [19]. The MLV vaccine-derived sequences and 3 other subtype 1 sequences had a slightly different motif of WSFVDGN. Interestingly, the 2 NI sequences from farm 1776-15 had differences in the neutralisation epitope at residues 30 and 35, resulting in a motif of WPFADGA. All ORF5 sequences displayed 3 potential N-linked glycosylation sites, at residues 37-39 (Asn-Ser-Ser), 46-48 (Asn-Leu-Ser) and 53-55 (Asn-Gly-Thr). One NI sequence from farm 5612-15 displayed an additional N-linked glycosylation site at residues 38-40 (Asn-Ser-Thr), which overlapped with the sequen at residues 37-39 to contain the sequence Asn-Asn-Ser-Thr.

ORF5 was targeted for phylogenetic analysis as it encodes the most variable structural protein, GP5 [13, 21]. GP5 is also the major target for virus neutralising antibodies
[22] and, as such, is important in relation to protection derived from previous infection or vaccination. Alignment of the complete ORF5 sequences revealed

nucleotide homology ranging from 87.6-100% between NI field strains. The difference in homology between these virus strains is consistent with the genetic diversity previously reported for PRRSV isolates in the United Kingdom [23]. Italy [13], Denmark [24], the United States [6], China [25] and Poland [26]. Phylogenetic analyses confirmed that all NI PRRSV isolates are of the European genotype, and placed the 2015 NI sequences into a distinct cluster within this group (Fig. 1). As expected, virus sequences from the same farms were closely related.

Interestingly, sequence diversity was observed between the ORF5 nucleotide and predicted protein sequences of circulating NI isolates and those of MLV vaccine sequences. MLV vaccines are capable of reducing the clinical signs associated with PRRSV infection, as well as viremia and viral shedding [27]. However, the efficacy of commercially available PRRSV type 1 MLV vaccines is variable and is characterised by a delayed neutralising antibody response [28]. Importantly, the genetic diversity of circulating strains may result in diminished protection afforded by the vaccines. For example, in vaccine efficacy studies, vaccination of pigs with MLV vaccine resulted in only partial protection against challenge with a heterologous East European PRRSV type 1 subtype 3 strain (Lena strain) [29]. The ORF5 of the Lena sequence was found to be 88% identical to the MLV vaccine at the protein level. The levels of amino acid homology between NI isolates was as low as 81.6% (farm 13320-12). It is not known what level of protection would be provided by MLV vaccines against this Northern Irish field isolate. However, such large differences may have a significant impact on vaccine efficacy. Consequently, continued monitoring of local PRRSV sequence variation is necessary. Nonetheless, vaccination against PRRSV with MLV vaccines remains one of the most important tools for control of the virus.

Changes in neutralising epitopes were shown to alter the effectiveness of neutralising antibodies [30]. A number of studies have described neutralisation epitopes in type 2 PRRSV [18, 31]. While the putative neutralising epitopes have not been as well documented for type 1 PRRSV we explored one epitope situated between residues 29-35 [19, 22]. The NI isolates from farm 1776-15 had mutations in amino acids in this neutralisation epitope compared to the majority of subtype 1 strains and the vaccine sequences. This suggests that vaccine efficacy may be compromised in NI. However, further studies are evidently required to determine the significance of these amino acid changes on vaccine efficacy.

As well as resulting in variation in neutralising epitopes, genetic variation can lead to changes in N-linked glycosylation sites and this can have an effect on the recognition of the neutralisation epitope [32]. Three potential N-linked glycosylation sites were identified in the GP5 ectodomain of NI PRRSV strains at residues 37-39, 46-48 and 53-55. This is consistent with Pesente et al. [13] and Frossard et al. [23], who identified the same predicted glycosylation sites on the GP5 protein of Italian and British PRRSV isolates, respectively. Interestingly, in one NI PRRSV strain an additional N-linked glycosylation site that overlapped with the sequon present at residue 37 was identified. Glycosylation of the viral envelope protein is a mechanism for immune evasion and several studies demonstrated a role for PRRSV GP5 glycosylation modification in evading host immune responses [33, 34]. Indeed, removal of N-glycosylation sites surrounding the neutralisation epitope of PRRSV GP5 resulted in increased sensitivity to neutralising antibodies [33] and convalescent-

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phase serum [17]. Consistent with the NI strains, the majority of subtype 1 sequences studied had predicted N-glycosylation sites at residues 37-39, 46-48 and 53-55. Importantly, all but one of the MLV vaccine strains (Porcilis) had no predicted N-glycosylation at residues 37-39, suggesting that the neutralising epitope may be more immunogenic for these vaccine strains. The presence of N-glycosylation at this site in the NI field isolates could compromise the immunity provided by the vaccines.

In conclusion, despite the genetic diversity observed between NI PRRSV isolates, these strains mostly clustered together in a distinct group within the European genotype. These data demonstrated relatively high genetic variability among PRRSV strains in NI and this variability poses significant challenges to the control of PRRS through vaccination. The geographical isolation of the island of Ireland may be a positive factor in terms of prevention of the introduction of diverse strains of PRRSV. However, the diversity between Northern Irish PRRSV strains evident in this study suggests that a more in depth surveillance on an all-island basis will be important in understanding, and locally controlling, PRRSV disease in Ireland.

Acknowledgements

The authors would like to thank the Department of Agriculture, Environment and Rural Affairs (DAERA) for funding this study.

Funding

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This study was part of a PhD studentship funded by the Department of Agriculture, Environment and Rural Affairs (DAERA) for Northern Ireland.

Conflict of Interest

All the authors declare that they have no conflict of interests.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

References

- Rossow KD (1998) Veterinary Pathology Online. Vet Pathol 35:1-20. 1.
- Keffaber K. (1989) Reproductive failure of unknown etiology. Am Assoc 2. Swine Pr Newsl 1–10.
- Wensvoort G, Terpstra C, Pol JM, et al (1991) Mystery swine disease in The 3. Netherlands: the isolation of Lelystad virus. Vet Q 13:121-130.
- Allende R, Lewis TL, Lu Z, et al (1999) North American and European porcine 4. reproductive and respiratory syndrome viruses differ in non-structural protein coding regions. Gen Virol 80:307-15
- Meng X. (2000) Heterogeneity of porcine reproductive and respiratory 5. syndrome virus: implications for current vaccine efficacy and future vaccine development. Vet Microbiol 74:309-329.
- Fang Y, Schneider P, Zhang WP, et al (2007) Diversity and evolution of a 6. newly emerged North American Type 1 porcine arterivirus: Analysis of isolates collected between 1999 and 2004. Arch Virol 152:1009-1017.

	183	7.	Dokland T (2010) The structural biology of PRRSV. Virus Res 154:86–97.
1	184	8.	Stadejek T, Stankevicius A, Murtaugh MP, Oleksiewicz MB (2013) Molecular
2	185		evolution of PRRSV in Europe: Current state of play. Vet Microbiol 165:21-
3	186		28.
4	187	9	Brar MS Shi M Murtaugh MP Leung FCC (2015) Evolutionary
5	188	2.	diversification of type 2 porcine reproductive and respiratory syndrome virus I
7	189		Gen Virol 96:1570–1580
8	109	10	Marozin S. Gragory, V. Comoron K. et al. (2002) Antigonia and genetic
9	101	10.	diversity emong swine influenze viruses in Europe, I Can Virel 82:725, 745
10	191	11	Middler SE Deneri K. Dener L et al. (2012) Dimension and example a statistical
11	192	11.	Midgley SE, Banyai K, Buesa J, et al (2012) Diversity and zoonotic potential
⊥∠ 12	193	10	of rotaviruses in swine and cattle across Europe. Vet Microbiol 156:238–245.
14	194	12.	Gunn L, Collins PJ, O'Connell MJ, O'Shea H (2015) Phylogenetic
15	195		investigation of enteric bovine coronavirus in Ireland reveals partitioning
16	196		between European and global strains. Ir Vet J 68:31.
17	197	13.	Pesente P, Rebonato V, Sandri G, et al (2006) Phylogenetic analysis of ORF5
18	198		and ORF7 sequences of porcine reproductive and respiratory syndrome virus
19	199		(PRRSV) from PRRS-positive Italian farms: a showcase for PRRSV
∠0 21	200		epidemiology and its consequences on farm management. Vet Microbiol
22	201		114:214–24.
23	202	14.	Kearse M. Moir R. Wilson A. et al (2012) Geneious Basic: An integrated and
24	203		extendable desktop software platform for the organization and analysis of
25	204		sequence data Bioinformatics 28.1647–1649
26	205	15	Saitou N Nei M (1987) The neighbour-joining method: a new method for
27	205	15.	reconstructing phylogenetic trees. Mol Biol Evol 4:406–425
∠o 29	200	16	Tamura K Stachar G Datarson D at al (2013) MEGA6: Molacular
30	207	10.	avalutionery constitution on the second distribution of the second distribu
31	200	17	Wei 77 Lin T. San L.C. et al. (2012). N.Linhad Characterian of CD5 of
32	209	17.	wei ZZ, Lin I, Sun LC, et al (2012) N-Linked Glycosylation of GP5 of
33	210		Porcine Reproductive and Respiratory Syndrome Virus Is Critically Important
34	211		for Virus Replication In Vivo. J Virol 86:9941–9951.
35	212	18.	Ostrowski M, Galeota J a, Jar a M, et al (2002) Identification of Neutralizing
37	213		and Nonneutralizing Epitopes in the Porcine Reproductive and Respiratory
38	214		Syndrome Virus GP5 Ectodomain Identification of Neutralizing and
39	215		Nonneutralizing Epitopes in the Porcine Reproductive and Respiratory
40	216		Syndrome Virus GP. J Virol 76:4241–4250.
41	217	19.	Wissink EHJ, van Wijk H a R, Kroese M V., et al (2003) The major envelope
42 43	218		protein, GP5, of a European porcine reproductive and respiratory syndrome
44	219		virus contains a neutralization epitope in its N-terminal ectodomain. J Gen
45	220		Virol 84:1535–1543.
46	221	20.	Ropp SL, Wees CEM, Fang Y, et al (2004) Characterization of emerging
47	222		European-like porcine reproductive and respiratory syndrome virus isolates in
48	223		the United States. J Virol 78:3684–3703.
49 50	224	21	Meng XI Paul PS Halbur PG Morozov I (1995) Sequence comparison of
50	225	21.	open reading frames 2 to 5 of low and high virulence United States isolates of
52	225		porcine reproductive and respiratory syndrome virus. I Can Virol 76:3181
53	220		2100
54	227	22	J100. Weilend E. Wieszerek Krokmen M. Kohl D. et al. (1000) Menaderal
55	228	22.	weiland E, wieczorek-Kronmer M, Koni D, et al (1999) Monocional
56	229		antibodies to the GP5 of porcine reproductive and respiratory syndrome virus
ン/ 58	230		are more effective in virus neutralization than monoclonal antibodies to the
59	231	~ -	GP4. Vet Microbiol 66:1/1–186.
60	232	23.	Frossard J-P, Hughes GJ, Westcott DG, et al (2013) Porcine reproductive and
61			
62			
63			

- respiratory syndrome virus: genetic diversity of recent British isolates. Vet
 234 Microbiol 162:507–18.
- 235 24. Kvisgaard LK, Hjulsager CK, Kristensen CS, et al (2013) Genetic and antigenic characterization of complete genomes of Type 1 Porcine Reproductive and Respiratory Syndrome viruses (PRRSV) isolated in Denmark over a period of 10 years. Virus Res 178:197–205.
- ⁷ 239 25. Li Y, Wang X, Jiang P, et al (2009) Genetic variation analysis of porcine reproductive and respiratory syndrome virus isolated in China from 2002 to 2007 based on ORF5. Vet Microbiol 138:150–155.
- 1124226.Stadejek T, Oleksiewicz MB, Scherbakov A V., et al (2008) Definition of12243subtypes in the European genotype of porcine reproductive and respiratory13244syndrome virus: Nucleocapsid characteristics and geographical distribution in14245Europe. Arch Virol 153:1479–1488.
- 1524627.Renukaradhya GJ, Meng X-J, Calvert JG, et al (2015) Live porcine17247reproductive and respiratory syndrome virus vaccines: Current status and future18248direction. Vaccine 33:4069–4080.
- 19
20
21
2228.Charerntantanakul W (2012) Porcine reproductive and respiratory syndrome
virus vaccines: Immunogenicity, efficacy and safety aspects. World J Virol
1:23.
- 23 252 29. Trus I, Bonckaert C, van der Meulen K, Nauwynck HJ (2014) Efficacy of an attenuated European subtype 1 porcine reproductive and respiratory syndrome virus (PRRSV) vaccine in pigs upon challenge with the East European subtype 3 PRRSV strain Lena. Vaccine 32:2995–3003.
- 28 256 30. Kim W II, Yoon KJ (2008) Molecular assessment of the role of envelope 29 257 associated structural proteins in cross neutralization among different PRRS
 30 258 viruses. Virus Genes 37:380–391.
 31 250 21 Diagonal data and the DDD and the DDD and the DDD and the DDD and the DDD.
- 259 31. Plagemann PGW, Rowland RRR, Faaberg KS (2002) The primary neutralization epitope of porcine respiratory and reproductive syndrome virus strain VR-2332 is located in the middle of the GP5 ectodomain. Arch Virol 147:2327–2347.
- ³⁶ 263 32. Faaberg KS, Hocker JD, Erdman MM, et al (2006) Neutralizing antibody responses of pigs infected with natural GP5 N-glycan mutants of porcine reproductive and respiratory syndrome virus. Viral Immunol 19:294–304.
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- 45 270 34. Vu HLX, Kwon B, Yoon K-J, et al (2011) Immune evasion of porcine
 46 271 reproductive and respiratory syndrome virus through glycan shielding involves both glycoprotein 5 as well as glycoprotein 3. J Virol 85:5555–64.

274 Legends

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- 51 275 Fig. 1 Neighbour-joining phylogenetic tree based on complete PRRSV ORF5 52 276 nucleotide sequences. Scale bar indicates an evolutionary distance of 10.0 nucleotides 53 per position in the sequences. Sequences from the Northern Irish strains from this 277 54 study are in blue, while MLV vaccine sequences are in red. Tree is rooted with VR-278 55 279 2332 type 2 PRRSV sequence. 56
- 57 280 58 281

Fig. 2 Alignment of the predicted amino acid sequence of the PRRSV GP5 protein of the Northern Irish strains and selected European and vaccine strains. Three potential

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N-linked glycosylation sites are denoted with an arrow (\downarrow) and the neutralisation epitope (WSFADGN) is highlighted by a box.



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	1 10	20	30 40	V 50 V 60
13320/12 Northern Ireland	MTCEHKLGRS I IPHSC	EWWEELLCTGS	EWSEADGNGNSSTYOY	YNI TIČE I NGTNWVAŠHEDWA
7130/15 A Northern Ireland	MICSHKSGRFLTPHSC	YWWLFFLCTGS	FWSFADGNGNSSTYOY	YNLTICELNGTETLSSNFYWA
7130/15 B Northern Ireland	MICSHKSGRFLTPHSC	YWWLFFLCTGS	FWSFADGNGNSSTYOY	YNLTICELNGTEALSSNFYWA
1776/15 C Northern Ireland	MTCFHKLGRFLTPHYC	FWWLFLLCTGL	SWP FADGAONS STYOY I	YNLTICELNGTDWLSGHFTWA
1776/15 D Northern Ireland	MTCFHKLGRFLTPHYC	FWWLFLLCTGL	SWP FADGAONS STYOY I	YNLTICELNGTDWLSGHFTWA
5216/15 Northern Ireland	MICSHKSGRFLTPHSC	YWWLFFLCTGS	FWSFADGNGNNSTYQYI	YNLTICE LNGTDWLSGH FDWA
5133/15 A Northern Ireland	MRCSHKLGRFLTPHSC	FWWLFLLCTGL	SWSFADGNGNSSTYQSI	YNLTICELNGTEWLSGHFDWA
5133/15 C Northern Ireland	MRCSHKLGRFLTPHSC	FWWLFLLCTGL	SWSFADGNGNSSTYQSI	YNLTICELNGTEWLSGHFDWA
5133/15 B Northern Ireland	MRCSHKLGRFLTPHSC	FWWLFLLCTGL	SWSFADGNGNSSTYQSI	YNLTICELNGTEWLSDHFDWA
180-09-ST1-UK-2009	MKCSHRLGRSLIPHSC	FWWLFLLCIGL	HWSFADGNGNSSTYLYI	YNLTICELNGTRWLSSHFDWA
304-06-ST1-UK-2006	MKCSHKLGRSLIPHSC	FWWLFLLCIGL	HWSFADGNGNSSTYQYI	YNLTICELNGTHWLSSHFDWA
598-04-ST1-UK-2004	MRCSHKLGRSLIPHSC	FWWLFLLCIGL	HWSFADGNGNSSTYQYI	YNLTICELNGTHWLSSHFDWA
EUROPERSV-ST1-USA	MRCSYKLGRSLILHSC	SWWFFLLCTGL	SWSFADGNGNNSTYQYI	YNLTICELNGTNWLSGHFDWA
5D03-15_P3-511-05A-2003	MRCSHKLGRSLIPHSC	FWWLFLLCIGL	SWSFADGNGNNSTYQT	YNL TICELNGTAWLSGHFDWA
AS10EU-ST1-Theiland-2010	MRCSHKLGRFLIPHSC	FWWLFLLCTGL	SWSFADGNGDSSTTQT	YNLTICELNGTDWLSSHFGWA
abustad.ST1-Mathaclands.1001	MRCSHKLGRFLTPHSC	EWWLELLCTGL	SWSFADGNGDSSTTOT	VNI TICELNGTDWLSSHEGWA
1008-07-ST1-UK-2007	MRCSHKLGRFLTPHSC	EWWLELLCIGL	SWSFADGNGSSSTYOV	YNI TICELNGTDWLSSHEGWA
1135-05-ST1-UK-2005	MRCSHKLGRFLTPHSC	FWWLFLLCIGL	SWSFADGNGSSSTYOY	YDLTICELNGTDWLSSHEGWA
(F991509 Porcilis PRRS	MRCSHKIGRFITPHSC	FWWFFLLCTGL	SWSFADGNGNSSTYOY	YNI TICELNGTDWISSHEGWA
H3-ST1-UK-1991	MRCSHKLGRFLTPHSC	FWWLFLLCTGL	SWSFADGNGNSSTYQYI	YNLTICELNGTDWLSSHFGWA
59755-13-ST1-UK-2013	MRCSHKLGCFSTTQSC	FWWFILLCTGL	SWS FADGNGNS S TYQY I	YNLTICELNGTGWLSNHFDWA
0x1-ST1-UK-1994	MRCSHKLGRFLTPQSC	FWWLFLLCTGL	SWSFADGNGDSSTYQYI	YNLTICE LNGTDWLSDHFVWA
59812-13-ST1-UK-2013	MRCFHKLERFSTPHSC	FWRLFLLCTGL	FWSFADGNGNNSTYQYI	YNLTICE LNGTDWLSGR FDWA
59816-13-ST1-UK-2013	MRCSHKLGRFLTPHSC	FWWLFLLCTGL	SWSFADGNGNSSTYQYI	YNLTICELNGTDWLSGHFDWA
NY4-ST1-UK-1994	MICSHKLGRFLTPHSC	FWWLFLLCTGL	SWSFADGNGNSSTYQYI	YNLTICELNGTDWLSGHFDWA
52-05-ST1-UK-2005	MRCSHKSGRFLTPRSC	FWWFFFLCTGL	SWSFADGNGNSSTYQYI	YNLTICQLNGTDWLSGRFDWA
216-06-ST1-UK-2006	MRCSHKSGRFLTPRSC	FWWFFFLCTGL	SWSFADGNGNSSTYQYI	YNLTICELNGTDWLSGRFDWA
51-08-511-UK-2008	MRCSHKLGRSLILRSC	FWWLFLLCTGL	SWSFADGNGNSSTYQY I	YNLTICELNGTNWLSGHFDWA
20.07.ST1.UK.2007	MRCSKKSGKFLIPKSC	EWWLELLCTGL	SWSFADGNGNSSTTOTI	YNI TICELNGTDWI SNHEDWA
2099-07-511-08-2007	MPCSHKSGPLLTPHSC	EWWLELLCTGL	SWSEADGNGNSSTYOT	VNI TICELNGTEWISSHEDWA
Cresa2982-ST1-Spain-2005	MRCSHKIERELTPHSC	EWWLELLCTGL	SWSFADGNGNSSAVOVI	YNI TICELNGTEWLSSHERWA
DO345725 UNISTRAIN PRRS	MRCSHKLERFLTPHSC	EWWLELLCTGL	SWSEVDGNDSSSTYOV	YNI TICELNGTEWI PSHEDWA
DO345726 Pyrsyac-183	MRCSHKLECFLTPHSC	FWWLFLLCTGL	SWSFVDGNDSSSTYOY	YNLTICELNGTESLSSHFDWA
Cresa3267-ST1-Portugal-2006	MRCSHKLERFLTPHSC	FWWLFLLCTGL	SWSFVDGNDDSSTYQYI	YNLTICELNGTEWLSSHFDWA
CT988004 Ingelvac PRRSFLEX	MKCSCKLGHFLTPHSC	FWWLFLLCTGL	SWSFVDGNDDSSTSQYI	YNLTICELNGTEWLSGHFDWA
1264-08-ST1-UK-2008	MRCSHKLERFLIQRSC	LWWLFLLYTGL	PWSFVDGNDNSSTYQYI	YNLTICELNGTEWLSGHFDWA
13V117-ST1-Belgium-2013	MRCSHKLGRFLTPHSC	FWWLFLLCTGL	SWSFADGNGNSSTYQYI	YNLTICELNGTTWLSDHFYWA
V3140-ST1-South Korea	MRCSHKLECFLTPHSC	FCWLFFLCIGL	SWSFADGNANSSTYQYI	YNLTICELNGTTWLSTNFHWV
NEU12-ST1-China-2012	MRCSHKLEHFLTLHSC	FWWLFLLCTGL	SWSFVDGNGNSSTYQYI	YNLTICELNGTDWLSNNFYWA
NVDC-NM1-2011-ST1-China-2011	MRCSHKLEHFLTPHSC	FWWLFLLCTGL	SWSFADGNGNSSTYQYI	YNLTICELNGTDWLSEHFYWA
ACTO-511-AUSTRIA-2015	MRCSHKSGLFLIPHSC	FWLPFLLCTGL	SWSFADGNGNSSTTQTI	YNLTICEL SCTAWLSNIE YWA
MELIO0.5.ST1.Chips.2009	MRCSTRLOCFLIPHSC	EWWLELLCTGL	SWSFANGND I SSTTUTI	VNMTICELSGTAWLSNHFTWA
ena.ST3.Belarus.2007	MRCSRTIGOPSTHHSV	IWWIELLCTGL	SWSFADGNGNSSTVIVI	VNI TICELNGTEWI VNHEDWA
Zad-1-ST3-Belarus2004	MRCSRTLGOPSTHHEY	LWWLFLLCIGL	SWSFADGNGNSSTYOY	YDLTICELNGTNWLASRESWY
(uz-34-ST3-Belarus-2004	PSTHHSY	IWWIFIICTGI	SWSFADGNGNSSTYOY	YNI TICELNGTKWI TSHEDWA
/os-ST3-Belarus-2004	MKCSHKLGOPLTLHSC	SWWLFLLCTGL	SWS FADGNGNS S TYOY I	YNLTICELNGTAWLEDHEDWA
OBU-1-ST3-Belarus-2004	MRCSHMLGQPSTLHSC	FWWLFLLCTGL	SWSFADGNNDSSTYOY	YNLTICE LNGTAWL FDH FDWA
Bel-ST2-Belarus-2004	MKCSHKLGQPLILHSC	FWWLFLLCTGL	SWSFADGNGNSSTYQY	YNLTICE LNGTAWLSTHFDWA
Soz(f2)-ST3-Belarus-2006	MRCSHRSGQPLTLYSY	SWWLFLLCTGL	SWSFVDGNGNSSTYQYI	YNLTICELNGTAWLSSHFDWA
PK-ST4-Belarus-2002	RSTSSSY	FWWLFLLCTGL	SWSFADGNGNSSTYQYI	YNLTICE LNGTDWLSNK FYWA
JIb-7M-ST4-Latvia-2009	PLIPCSY	FWWLFLLCTGL	SWSFADGSGNSSTYQYI	YNLTICE LNGTAWLSYKFHWA
Okt-ST4-Belarus-2010	MRCSHRLGLPLTLHSC	SWWLFFLCTGL	SWSFANGNGNSSTYQFI	YNMTICE LNGTEWLSGNFDWA
/as-2-512-Belarus-2005	FSTPHNC	SWWPFLLSIGL	FWSFAAANGNSSTHQYI	YNLTICELNGTDWLQGKFEWA
/K-S12-Kussia-2005	MRCSHRLGSFSTPHFC	SWWLFLLSTGL	SWSSAVSNGNSS FYQY I	YNL TICELNGTDWLRNHFDWA
Sidenan-ST2-Kussia-2013	MACSHALUSISTPYFC	EWWIEELSTGL	EWSSAAAS CNSSTYRY	VNLTICELNGTEWISGDEDWA
hus-512-Litriuania-2000	MSTPRSC	- WWLFFLSIGL	T T P T C C P C C A A A C C A A A C C A C A C C C A C	THE FICE LING TEWESOR FDWA

Table 1. The minimum and maximum percentage homologies of PRRSV ORF5 nucleotide and protein sequences. Comparisons were within and between Northern Ireland farms, between Northern Ireland sequences and type 1 PRRSV subtypes (1 – 4) and between Northern Ireland sequences and selected MLV vaccine sequences.

ORF5 sequences compared	Minimum nucleotide homology	Maximum nucleotide homology	Minimum protein homology	Maximum protein homology
NI – NI (between farms)	87.6	92.2	84.1	93.5
NI – NI (within farms)	99.5	100	99	100
NI – Subtype 1	82.5	92.7	82.1	92.5
NI – Subtype 2	76.2	80	74.1	82.6
NI – Subtype 3	79.2	83.5	75.6	86.1
NI – Subtype 4	77.6	82.1	77.6	85.3
NI – Porcilis PRRS MLV	90.3	92.9	87.6	91.5
NI – Pyrsvac-183	87.3	89.8	83.6	90.1
NI – UNISTRAIN PRRS	87.6	89.9	85.1	91
NI – Ingelvac PRRSFLEX	84.7	88.1	81.6	87.6