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Porcine reproductive and respiratory syndrome virus: Genetic diversity of recent British isolates☆

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ABSTRACT

Porcine reproductive and respiratory syndrome (PRRS) continues to be a significant problem for European pig producers, contributing to porcine respiratory disease complex, neonatal piglet mortality, infertility and occasional abortion storms. PRRS virus (PRRSV), a member of the arterivirus family with two defined major genotypes, has been shown to be quite genetically diverse. In the present study, genetic analysis of multiple gene regions of over 100 viruses isolated in Britain between 2003 and 2007 revealed that the diversity of British strains is now far greater than during the early 1990s. All isolates belong to genotype 1 (European). While some recent isolates are still very similar to early isolates, a wide range of more diverse viruses is now also circulating. Interestingly, some isolates were found to be very similar to a modified-live vaccine strain, and it is suggested that use of the vaccine has affected the evolution pattern of PRRS virus strains in Britain. Evidence of deletions in one viral gene, ORF3, and of genome recombination was also seen. A molecular clock model using the ORF7 sequences estimates the rate of substitution as 3.8×10^{-3} per site per year, thereby dating the most recent common ancestor of all British viruses to 1991, coincident with the first outbreak of disease. Our findings therefore have implications for both the diagnostic and prophylactic methods currently being used, which are discussed.

1. Introduction

Porcine reproductive and respiratory syndrome (PRRS) was first described in the United States of America in 1987 (Keffaber, 1989), and subsequently in Europe in 1990 (Wensvoort et al., 1991). The causative agent, PRRS virus (PRRSV), is a single-strand positive-sense RNA virus of the order *Nidovirales*, family *Arteriviridae* (Rossow, 1998). The disease remains a significant problem worldwide, with a considerable economic impact for affected producers, due to increased mortality, treatment costs, and reduced weight gain and fertility (Neumann et al., 2005). More pathogenic variants have been described in the past, and since 2006, a highly pathogenic form of the virus has been circulating in China and other parts of Asia, causing a devastating economic impact (Zhou et al., 2008).

The mode of replication of the virus makes it prone to high rates of mutation and recombination (Gorbalenya et al., 2006). A high degree of diversity in the virus population has recently been reported in the United States (Fang et al., 2007), China (Li et al., 2009), Spain (Mateu

☆ The GenBank accession numbers for the new sequences described are listed in supplementary table.

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et al., 2006; Prieto et al., 2009), and eastern Europe (Stadejek et al., 2008). Despite the apparent increase in the number of cases of PRRS in Great Britain, little information has been available regarding the virus population since 1995 (Drew, 1996). Here, the geographical characteristics of Britain as an island, the licensed use of only one type of modified live vaccine, and the international trade conditions with limited import of live pigs into the country, argue for a relatively isolated evolutionary situation.

In this study, we aimed to obtain a comprehensive picture of the current diversity of PRRSV strains circulating in pig herds, using multiple gene regions, and compare this to the previous analysis with samples from the early 1990s. The strains analysed were obtained by virus isolation rather than molecular detection, thus avoiding any potential bias introduced by the choice of amplification primers.

2. Materials and methods

2.1. Virus isolation

PRRS viruses were isolated from clinical material (tissues or serum) submitted for PRRS diagnosis, using PRRS-free primary porcine alveolar macrophages (PAMs), labelled with monoclonal antibody SDOW17 (Nelson et al., 1993), and visualised by immunoperoxidase staining (Wensvoort et al., 1992). One hundred virus isolates from individual pigs from 62 farms were thus obtained for analysis between 2003 and 2008. These included 23 isolates from a targeted investigation of finisher pigs, described elsewhere (Jackson et al., 2006). Corresponding case histories and herd vaccination records were available.

2.2. Sequence analysis

Nucleic acid was extracted using the QIAamp® Viral RNA Mini Kit (QIAGEN), and used to generate cDNA using random primers and SuperScript™ II reverse transcriptase (Invitrogen). The cDNA was then used in PCR amplifications using primers specific for viral genes as described in Table 1. For nucleotide sequencing, the amplification products were purified using the QIAquick® gel extraction kit (QIAGEN). The Sanger sequencing reaction was performed using BigDye chemistry. The sequence data was analysed and assembled using SeqMan software version 7.1.0 (Lasergene, DNASTAR). Alignment of the sequences for comparison was performed with the ClustalW algorithm (Thompson et al., 1994), using MEGA software version 3.1 (Kumar et al., 2004). Phylogenetic and molecular evolutionary analyses (neighbour-joining method with bootstrap test) were conducted using MEGA software version 3.1. Predicted RNA folding patterns were investigated using mfold software (Mathews et al., 1999; Zuker, 2003).

Estimation of nucleotide substitution rates and time-scaled phylogenies were generated using BEAST v1.4.3 (Drummond and Rambaut, 2007). A model of the relaxed molecular clock was used as this has been shown to provide a better fit to viral sequence data (Drummond et al., 2006). Modelling was performed using the HKY85 model of nucleotide substitution with a gamma distribution of rate variation. Two separate runs of chain length 1×10^7 were combined to provide an effective sample size > 200 for all parameters. After a burn-in of 10% runs were combined to generate maximum clade credibility trees from a sample of 18,000 trees.

3. Results

In total, nucleotide sequence data was obtained from 147 recent virus isolates and 16 isolates from the 1990s. The ORF7 and ORF5 regions were examined initially, but later only ORF5, and for strains considered to be of particular interest, further regions were subsequently analysed, providing a total of 108 ORF7, 14 ORF6, 120 ORF5, 17 ORF4, and 54 ORF3 complete sequences. Where multiple isolates were available from the same farm, either from one time-point or several, the levels of similarity varied greatly, with some isolates having near-identical gene sequences, and others being radically different. Where duplicate or similar sequences were identified

Table 1
Oligonucleotide primers used for sequencing isolates of PRRS virus.

Primer	Position[a]	Gene target	Sequence[b]
ORF3_Fwd[c]	12,365–12,383	ORF3	CACGCCAGGTACCAGGCCA
ORF3_Rev[c]	13,236–13,255	ORF3	AAAGCATCTGCAGGTCCGCG
12500_Fwd	12,500–12,519	ORF3	TGTTTTTGGTTTCCATTGGC
12904_Rev	12,904–12,927	ORF3	CCCCCGTCTATTTGGTGGTGGTAA
ORF4_Fwd[c]	12,926–12,945	ORF4	GGCAATTGGATCCATTTGGA
ORF4_Rev[c]	13,526–13,545	ORF4	AGAAGCAAGCTTGCGGAGTC
13187_Fwd	13,187–13,210	ORF5	AGTACATCACGATAACGGCTAACG
ORF5_Fwd[c]	13,446–13,465	ORF5	GAGGTGGGATCCAACATTG
ORF5_Rev[c]	14,125–14,144	ORF5	CTAGCACAAGCTTTTGTGCG
ORF6_Fwd[c]	14,061–14,080	ORF6	CCCTTGACGAGCTCTTCGGC
ORF6_Rev[c]	14,630–14,649	ORF6	CCATCGGATCCGTACTTTTC
P71[d]	14,564–14,582	ORF7	GCTGTTAAACAGGGAGTGG
P72[d]	15,029–15,049	ORF7	CGCCCTAATTGAATAGGTGAC
ORF7_Fwd[c]	14,581–14,600	ORF7	GGTTAACCTCGTCGACTATG

[a] Position relative to the published sequence of the Lelystad virus (Genbank accession M96262).
[b] Sequence in 5′–3′ direction.
[c] Meulenberg et al. (1995).
[d] Guarino et al. (1999).