

# **Appearance of Novel PRRSV Isolates by Recombination in the Natural Environment**

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## **1. INTRODUCTION**

Porcine reproductive and respiratory syndrome virus (PRRSV) emerged in North America and Europe in the 1980's as a new viral disease of swine. PRRSV now is endemic in swine-rearing regions essentially worldwide. A better understanding of the mechanisms of genetic change may help to elucidate its evolutionary history and emergence as a swine pathogen.

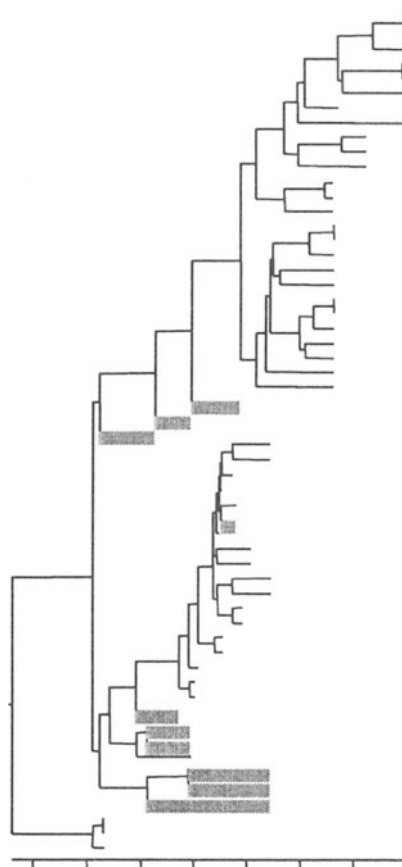
### **1.1 Initial Comments**

PRRSV appeared at approximately the same time in Europe and North America as the causative agent of reproductive disease in sows and interstitial pneumonia in young pigs (Collins et al. 1992, Wensvoort et al. 1991). Surprisingly, the genomic sequences of European and North American viruses were substantially divergent (Nelsen et al. 1999), suggesting that PRRSV was present for an unknown time in an undetected form, and also that it might be capable of rapid genetic change. Nucleotide sequence analysis of PRRSV isolates in Europe and North America revealed substantial genetic and antigenic variation within the viral populations in Europe and North America (Drew et al. 1997, Kapur et al. 1996). The genetic differences between European and North American PRRSV is nearly as great as the differences between either genotype and lactate dehydrogenase-elevating virus (Murtaugh et al. 1994). This striking

diversity suggests that PRRSV has evolved independently on two continents for an extended period or is evolving very quickly.

## **2. GENETIC DRIFT AND THE APPEARANCE OF NEW PHENOTYPES**

The population structure of PRRSV is changing rapidly. Genotypes of isolates present in the early 1990's are largely extinct or are present as minor groups. Figure 1 shows that the large majority of recent PRRSV isolates have evolved from a limited set of ancestral genotypes consistent with genetic drift. This pattern of genetic drift is observed regardless of the genetic region used for analysis, and in European forms of the virus.



*Figure 1.* Genetic drift in PRRSV. Clustal analysis of the ORF 5 of nonredundant North American isolates submitted to the Minnesota Veterinary Diagnostic Laboratory in 1997-98. Filled rectangles are 1989-92 isolates described in Kapur et al. 1996.

New phenotypes also are emerging. In the U.S., neurovirulent PRRSV was isolated in 1996 from farms with a history of PRRSV infection and previous or ongoing use of modified-live vaccine (Rossow *et al.* 1999). In 1999, a European-like PRRSV with approximately 95% similarity to Lelystad virus was discovered in the midwestern U.S. on a farm previously positive for PRRS (K.D. Rossow and K.S. Faaberg, unpublished observations). The emergence of phenotypes with new pathogenic characteristics and the geographic redistribution of existing strains illustrates the existence of broad genetic variation from which novel genotypes might arise.

## **2.1 Evolutionary Considerations**

Three mechanisms of genetic change contribute to the evolution of viruses: small local changes in nucleotide sequence due to natural mutation and polymerase infidelity, introduction of new genetic information by horizontal gene transfer from other organisms, and intra- or intergenomic reshuffling of subgenomic nucleic acids by recombination (Arber, 2000). In PRRSV, recombination has the potential to rapidly create new genotypes and phenotypes in the natural environment since the virus is distributed worldwide, because numerous genetically distinct variants coexist in local environments, and since greatly divergent genotypes characteristic of North American and European forms of the virus are now redistributed.

## **3. HOMOLOGOUS RECOMBINATION GENERATES NEW PRRSV ISOLATES**

To determine if recombination was contributing to genetic diversity in PRRSV, nucleotide sequencing was performed on a panel of 50 U.S. isolates and phylogenetic dendrograms from the ORF 5 and ORF 6 regions of the genomes were compared. A set of three natural isolates from the same geographic region appeared to be directly related according to the analysis of ORF 5, whereas a different relationship was inferred from analysis of ORF 6. Direct analysis of the nucleotide sequences of the three strains showed that the isolate NC-93-14 was a recombination between the NC-93-15 and NC-93-20 within ORF 5 (Figure 2). In this example, the predicted envelope glycoprotein of the recombinant virus contained ectodomains of isolate NC-93-14 and a cytoplasmic tail composed largely of isolate NC-93-20. In ORF 6 the sequences of isolates NC-93-14 and NC-93-20 are identical and different from isolate NC-93-15. All three isolates were

obtained within a six week time period in adjacent counties in North Carolina, USA, making recombination between these strains possible.

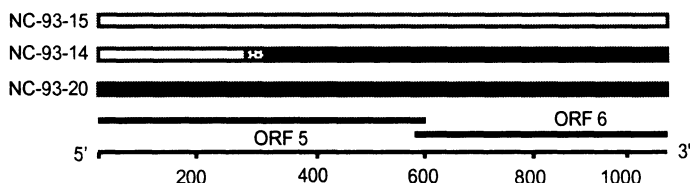


Figure 2. Diagrammatic representation of putative parental and recombinant PRRSV isolates obtained from the field and containing a recombination site in ORF 5. The stippled region shows the site at which recombination occurred.

Several European-type isolates obtained in recent years from Denmark demonstrate marked deletions or frank nonhomologous recombination as compared to earlier Danish isolates (Figure 3).

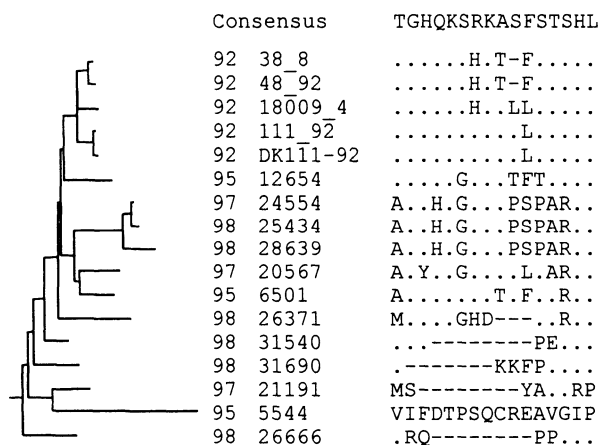


Figure 3. Amino acid sequence at residues 237-252 in ORF 3 of Danish PRRSV isolates. Phylogenetic relationships as determined by Clustal analysis, year of isolation, and isolate identification in Genbank are indicated. Dots represent the consensus sequence. Dashes represent amino acid sequence that is missing in the indicated isolates.

Five PRRSV samples isolated since 1997 contained deletions of 3-8 amino acids near the carboxyl terminus of the standard 265 residue protein. The deletion pattern is different for every isolate, indicating that each sample arose independently. In addition, isolate 5544, from 1995, had a unique sequence, due to an upstream fusion of ORF 3 and ORF 4, so that the

indicated residues actually are derived from ORF 4. These observations extend the findings of Drew et al. (1997) regarding the high degree of variation in this region. Deletions of this magnitude in PRRSV ORFs have not been reported previously, suggesting that nonhomologous recombination within or among viral strains is involved in the generation of genetic diversity.

#### 4. CONCLUSION

Reshuffling of genomic information due to recombination provides a high probability for rapid genotypic and phenotypic change in viruses due to the large scale introduction of new genetic information into a genome. Rapid evolution is a feature of PRRSV in all regions where pigs are intensively reared. Moreover, nucleotide and amino acid sequence differences between European and North American forms of the virus are found throughout the genome. We have demonstrated that recombination, occurring in the natural environment, has contributed to the genetic diversity of PRRSV. Local nucleotide changes due to spontaneous mutation and low fidelity of the arteriviral polymerase also occur at a relatively high rate. The combination of small local changes, genetic recombination, and geographic redistribution of European and North American genotypes are likely to result in continued rapid evolution of PRRSV and the further emergence of new viral phenotypes in the environment.

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