Phylogenetic analysis of PRRS virus strains

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Introduction

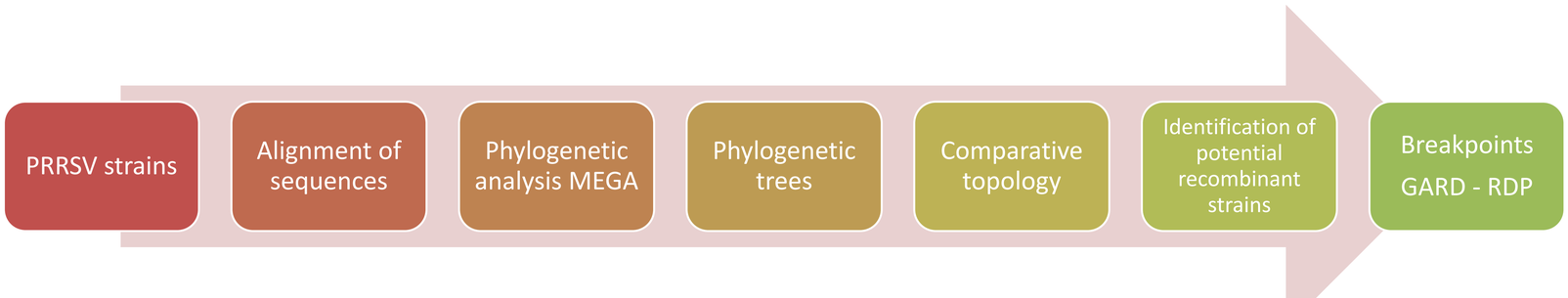
The porcine reproductive and respiratory syndrome virus (PRRSV) is one of the diseases with the highest economic impact affecting the swine industry. The virus comprises two genotypes designated as 1 (formerly European) and 2 (formerly North American). PRRSV is a positive-sense ssRNA enveloped virus classified within the Genus *Arterivirus* in the Order *Nidovirales* .

PRRSV genome is approximately 15 Kb in length and is organized in 11 open reading frames (ORFs). ORF1a i 1b, encode the replicase and the non-structural proteins (nsp). ORF 2a, 2b and 3-7 encode for the viral structural proteins. Within the proteins that compose the virus, GP5 protein (encoded by ORF5) is one of the proteins presenting the highest genetic variability. This is why ORF5 has been used in phylogenetic analysis because of the highly variable fragments that it contains along with other less variables. This allows significantly discrimination of groups of strains. However, there are strains which phylogeny cannot be established with certitude since the ORF5 group do not correspond to other genes groups such as ORF7.

The aim of this study is to define the existence of recombinant strains by constructing phylogenetic trees based on specific genes and entire sequences of PRRSV.

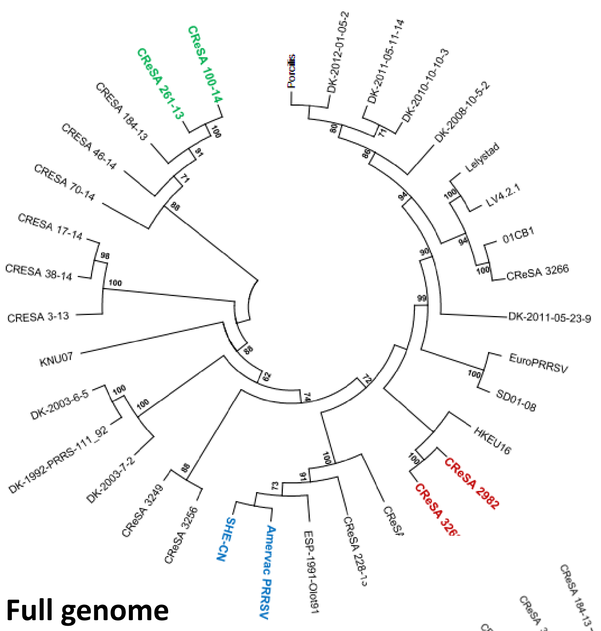
Materials & Methods

The present study was performed with a set of 34 PRRSV sequences representing isolates of genotype 1 subtype 1. Those sequences comprised ORFs 1 to 7 from the virus and the period of obtention lasted from 1991 to 2004. Nine of these sequences were acquired specifically for this purpose: CRESA_46-14; CRESA_228-13; CRESA_70-14; CRESA_3-13 CRESA_17-14; CRESA_38-14;CRESA_261-13;CRESA_100-14 CRESA_184-13. The phylogenetic analysis was performed with MEGA 6.0 followed by a comparative topology using ORF5, ORF7, nsp1, nsp2, nsp9 and nsp11. to identify the recombinant strains .

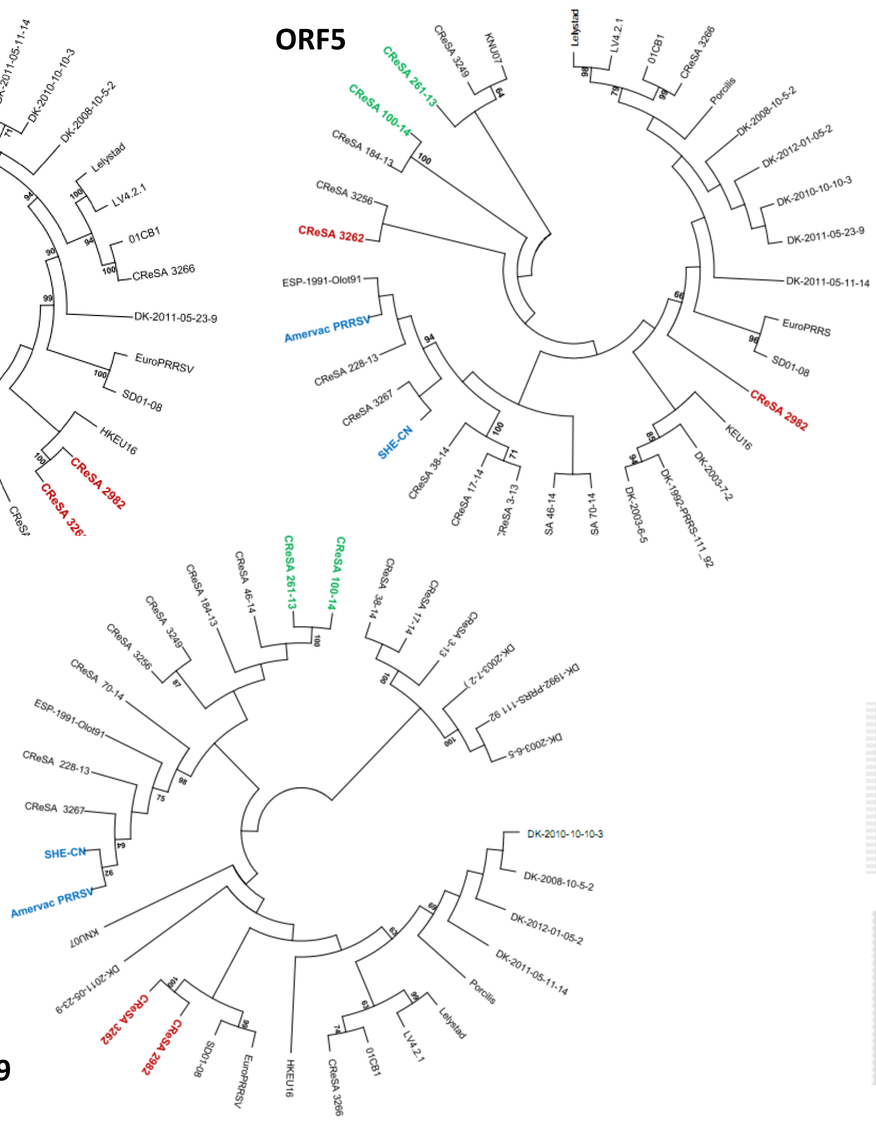


The complete genome sequences were split into two segments and the GARD program (available at http://www.datamonkey.org) was used to detect specific points of recombination. To recognize the similarity in different segments of the genome in relation to the closest sequences , RDP v4.01 program was employed.

Results



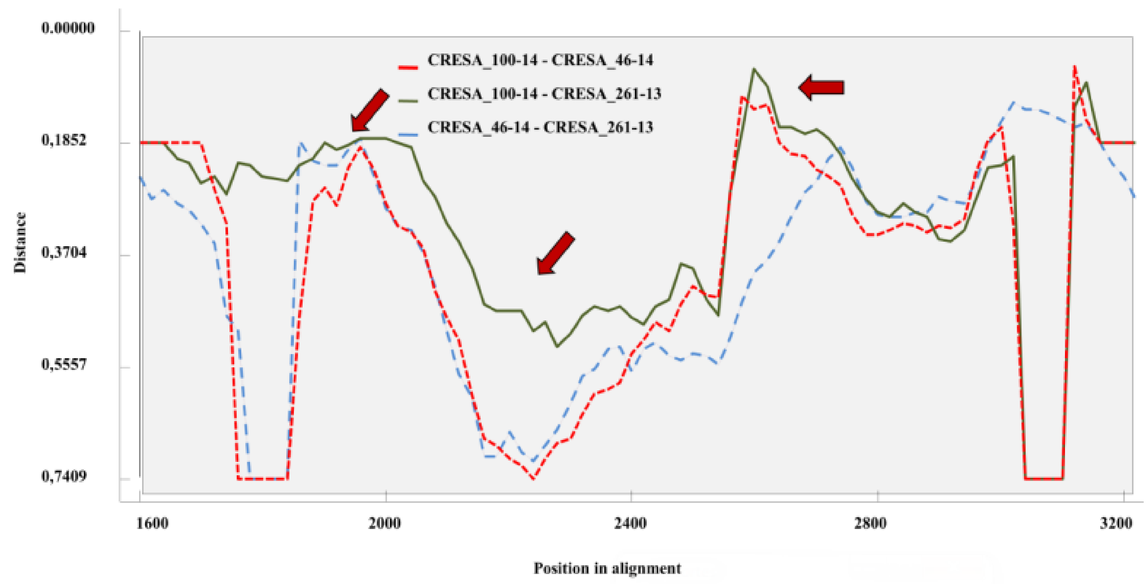
Full genome



nsp9

ORF5

Recombination was detected between strains: CReSA 100-14, CReSA_46-14 and CReSA_261-13 in the segment comprised between nucleotides 1600 and 3200.



Breakpoint	LHS p-value	RHS p-value
2004	0.00080	0.00080
2671	0.00400	0.00080
3448	0.00080	0.00080
4728	0.00080	0.00080

Potential breakpoints from the first segment corresponding to nsp2 and nsp3.

Breakpoint	LHS p-value	RHS p-value
4046	0.00080	0.00080
4791	0.00080	0.00080
6150	0.00080	0.00080
6765	0.00080	0.00080

Potential breakpoints from the second segment corresponding to nsp11, ORF3, ORF5 and ORF7.

Conclusion

In the present study six potential recombinant strains were identified. As previously reported, PRRSV presents frequent recombination phenomena. However, this fact is even more frequent that it had been thought since two of the nine strains specifically sequenced for this study were recognized as recombinants. Recombinant events were detected in CReSA 100-14, CReSA_46-14 and CReSA_261-13. The breakpoints were noticed in nsp2 , nsp3, nsp11, ORF3, ORF5 and ORF7.

References

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