



# Implications of porcine reproductive and respiratory syndrome virus recombination and practices that may facilitate its occurrence under field conditions

Giovani Trevisan, Daniel Linhares, Andreia Arruda, Cesar Corzo, Mariana Kikuti, Igor Paploski, Kimberly VanderWaal, Paul Yeske, Joel Sparks, Attila Farkas, Jianqiang Zhang, Phillip Gauger, Christopher J. Rademacher, Derald Holtkamp

Porcine reproductive and respiratory syndrome virus (PRRSV) is an RNA virus<sup>1</sup> characterized into two distinct species present in the United States: a) *Betaarterivirus suis* 1 known as PRRSV-1 or the European type and b) *Betaarterivirus suis* 2 known as PRRSV-2 or the North American type.<sup>2,3</sup>

## Natural PRRSV genetic evolution

A natural characteristic of RNA viruses is their unique evolutionary capability of exhibiting mutations and frequent recombination during the replication process.<sup>4</sup> These include:

- Random substitutions occur when one or more nucleotides are replaced with other nucleotides on the newly assembled genome without changing the genome length. When occurring in the gene coding regions, a nucleotide substitution can be synonymous (also called a silent substitution) where the amino acid is not changed or nonsynonymous where the amino acid is altered.
- Random insertions or deletions are a special mutation event that occurs when one or more nucleotides are inserted or deleted in the genome changing its genome length.
- Recombination occurs when the new viral genome is generated from acquiring some genome regions from two or more parental PRRSV genomes.

Substitutions, insertions, and deletions occur as part of the replication process when a single replicating viral particle goes through a random substitution or has nucleotides inserted or deleted in the genome during the replication process. Apart from eradicating PRRSV, there are currently no known methods to reduce the likelihood of mutation events from occurring.

## PRRSV recombination

The co-infection and replication of two or more viruses in the same host cell is a prerequisite for the occurrence of a recombination event through the exchange of genetic material.<sup>5,6</sup> A

recombination event produces a hybrid viral genome and has been associated with increased PRRSV genetic evolution and variability<sup>7,8</sup> (Figure 1).

Recombination breakpoints of the PRRSV can occur in different genome regions (open reading frames) and can occur between wild-type and wild-type, wild-type and PRRSV modified-live virus (MLV) vaccine, or between PRRSV MLV vaccine genomes, with the three different recombination arrangements previously described in North America for PRRSV-2.<sup>9-15</sup> Recombination events for PRRSV-1 have also been reported in Europe.<sup>16-18</sup>

Recombination events are drivers for PRRSV genetic evolution over time; they have been reported to occur in the field and were associated with longer time to PRRSV stability and increased production losses in breeding herds.<sup>11</sup> Replication of PRRSV MLV vaccines can occur in the host and, whenever in contact with other replicating strains, they fulfill the required characteristics for recombination events. Additionally, PRRSV MLV vaccines have label recommendations to be used in *healthy* animals to stimulate the immune system of the pigs for development of some level of protective immunity to PRRSV without causing severe disease. Consultation with your veterinarian for guidelines on a PRRSV immunization program is recommended. There have been some reports on the virulence phenotypes of recombinant PRRSVs. For instance, in a controlled laboratory setting, a recombinant genome composed of wild-type and PRRSV MLV vaccine virus was produced and a bioassay study demonstrated that, even though the recombinant virus was not as aggressive as the parental wild-type virus, it was also not as mild as the PRRSV MLV vaccine virus.<sup>19</sup> Conversely, the wild-type recombinant viruses, such as the LIC variant RFLP 1-4-4 (currently classified as LIC.5) in the United States, and Rosalia in Europe,<sup>20-22</sup> have been shown to be clinically aggressive with increased occurrence of clinical signs.<sup>23-25</sup>

GT, DL, JZ, PG, CJR, DH: Iowa State University, Ames, Iowa.

AA: The Ohio State University, Columbus, Ohio.

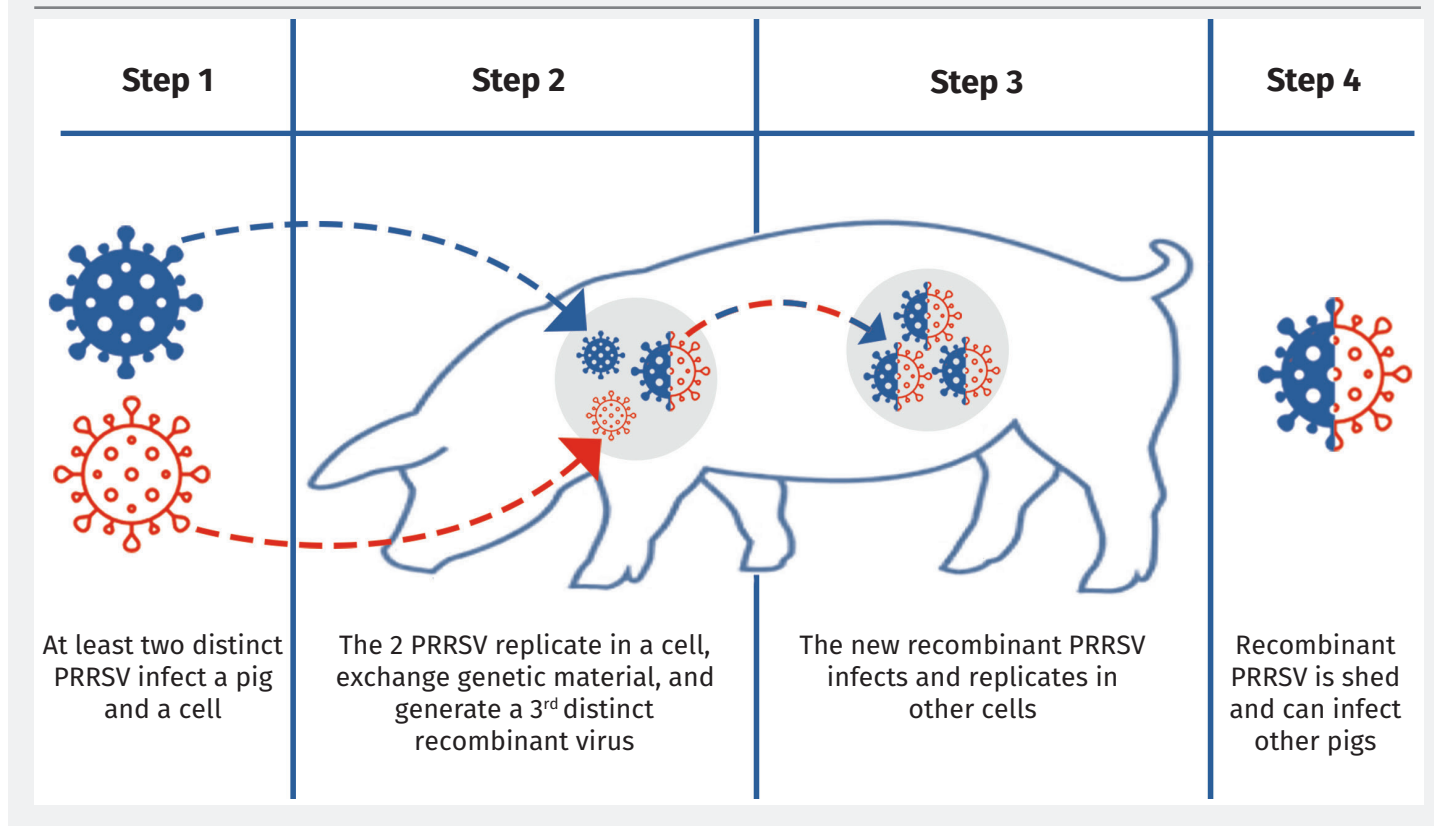
CC, MK, IP, KV: University of Minnesota, St. Paul, Minnesota.

PY: Swine Vet Center, St Peter, Minnesota.

JS: AMVC, Frankfort, Indiana.

AF: Carthage Veterinary Services, Carthage, Illinois.

**Figure 1: PRRSV infection, replication & recombination**



## Potential alternatives to reduce PRRSV recombination

Considering that viral co-infection is required for the occurrence of a recombination event, some field practices can be implemented or discontinued in efforts to reduce the probability of recombination events. These include:

- Seek PRRSV elimination from herds.
- Conduct routine testing of replacement gilts to avoid the introduction of PRRSV-infected gilts into breeding herds. If bringing positive gilts into the herd, avoid the introduction of gilts infected with distinct wild-type PRRSV strains unique to the farm.
- Avoid intentional exposure of animals with multiple wild-type strains including vaccinating PRRSV-infected pigs.
- Avoid commingling pigs infected with distinct PRRSV strains (eg, wild-type or vaccine) within the same barn.
- Test live virus inoculum (LVI) material using a next-generation sequencing approach to investigate the presence of single or multiple wild-type PRRSV strains or vaccine strains. Refer to the *When to use next-generation sequencing for clinical and epidemiological decisions related to porcine reproductive and respiratory syndrome virus* factsheet for more information on next-generation sequencing.
- Avoid using an LVI with multiple wild-type strains. This procedure helps to minimize the intentional exposure of pigs to multiple PRRSV strains (wild-type or MLV vaccine) and decreases the opportunity for co-infection of the same host and cell with distinct PRRSV strains.
- Do not rotate PRRSV MLV vaccines. Rather, keep a consistent vaccination program in replacement gilts and the breeding herd and within sow herds and downstream flows. If rotation

is needed in a breeding herd, wait for the current vaccine viremia to decline before injecting the new MLV vaccine.

- Immunizing weaned pigs with an MLV vaccine different than the one used in the breeding herd is not recommended. If needed, do vaccinate when pigs have been moved from the breeding herd to offsite facilities.
- Do not use two or more MLV vaccines in the same animal or herd.<sup>5</sup>

## References

1. Kappes MA, Faaberg KS. PRRSV structure, replication and recombination: Origin of phenotype and genotype diversity. *Virology*. 2015;479-480:475-86. <https://doi.org/10.1016/j.virol.2015.02.012>
2. Zimmerman JJ, Dee SA, Holtkamp DJ, Murtaugh MP, Stadejek T, Stevenson GW, Torremorell M, Yang H, Zhang J. Porcine reproductive and respiratory syndrome viruses (Porcine Arteriviruses). In: Zimmerman JJ, Karriker LA, Ramirez A, Schwartz KJ, Stevenson GW, Zhang J, eds. *Diseases of Swine*. 11<sup>th</sup> ed. Wiley Blackwell; 2019:685-708.
3. Trevisan G, Linhares LCM, Crim B, Dubey P, Schwartz KJ, Burrough ER, Main RG, Sundberg P, Thurn M, Lages PTF, Corzo CA, Torrison J, Henningson J, Herrman E, Hanzlicek GA, Raghaan R, Marthaler D, Greseth J, Clement T, Christopher-Hennings J, Linhares DCL. Macroepidemiological aspects of porcine reproductive and respiratory syndrome virus detection by major United States veterinary diagnostic laboratories over time, age group, and specimen. *PLoS One*. 2019;14(10):e0223544. <https://doi.org/10.1371/journal.pone.0223544>
4. Dolan PT, Whitfield ZJ, Andino R. Mechanisms and Concepts in RNA Virus Population Dynamics and Evolution. *Annu Rev Virol*. 2018;5(1):69-92. <https://doi.org/10.1146/annurev-virology-101416-041718>
5. Murtaugh MP, Yuan S, Nelson EA, Faaberg KS. Genetic interaction between porcine reproductive and respiratory syndrome virus (PRRSV) strains in cell culture and in animals. *J Swine Health Prod*. 2002;10(1):15-21.

6. Mötz M, Stadler J, Kreutzmann H, Ladinig A, Lamp B, Auer A, Riedel C, Rümenapf T. A conserved stem-loop structure within ORF5 is a frequent recombination hotspot for porcine reproductive and respiratory syndrome virus 1 (PRRSV-1) with a particular modified live virus (MLV) strain. *Viruses*. 2023;15(1):258. <https://doi.org/10.3390/v15010258>
7. Yuan S, Nelsen C, Murtaugh MP, Schmitt BV, Faaberg KS. Recombination between North American strains of porcine reproductive and respiratory syndrome virus. *Virus Res*. 1999;61(1):87-98. [https://doi.org/10.1016/S0168-1702\(99\)00029-5](https://doi.org/10.1016/S0168-1702(99)00029-5)
8. Guo J, Liu Z, Tong X, Wang Z, Xu S, Chen Q, Zhou J, Fang L, Wang D, Xiao S. Evolutionary dynamics of type 2 porcine reproductive and respiratory syndrome virus by whole-genome analysis. *Viruses*. 2021;13(12):2469. <https://doi.org/10.3390/v13122469>
9. Risser J, Ackerman M, Evelsizer R, Wu S, Kwon B, Hammer JM. Porcine reproductive and respiratory syndrome virus genetic variability a management and diagnostic dilemma. *Virol J*. 2021;18(1):206. <https://doi.org/10.1186/s12985-021-01675-0>
10. Trevisan G, Magstadt D, Woods A, Sparks J, Zeller M, Li G, Krueger KM, Saxena A, Zhang J, Gauger PC. A recombinant porcine reproductive and respiratory syndrome virus type 2 field strain derived from two PRRSV-2-modified live virus vaccines. *Front Vet Sci*. 2023;10:1149293. <https://doi.org/10.3389/fvets.2023.1149293>
11. Trevisan G, Zeller M, Li G, Zhang J, Gauger P, Linhares DCL. Implementing a user-friendly format to analyze PRRSV next-generation sequencing results and associating breeding herd production performance with number of PRRSV strains and recombination events. *Transbound Emerg Dis*. 2022;69:e2214-e2229. <https://doi.org/10.1111/tbed.14560>
12. Wang A, Chen Q, Wang L, Madson D, Harmon K, Gauger P, Zhang J, Li G. Recombination between vaccine and field strains of porcine reproductive and respiratory syndrome virus. *Emerg Infect Dis*. 2019;25(12):2335-2337. <https://doi.org/10.3201/eid2512.191111>
13. Lalonde C, Provost C, Gagnon CA. Whole-genome sequencing of porcine reproductive and respiratory syndrome virus from field clinical samples improves the genomic surveillance of the virus. *J Clin Microbiol*. 2020;58(11):e00097-20. <https://doi.org/10.1128/JCM.00097-20>
14. Shi M, Lemey P, Singh Brar M, Suchard MA, Murtaugh MP, Carman S, D'Allaire S, Delisle B, Lambert M-E, Gagnon CA, Ge L, Qu Y, Yoo D, Holmes EC, Leung F. The spread of type 2 porcine reproductive and respiratory syndrome virus (PRRSV) in North America: A phylogeographic approach. *Virology*. 2013;447(1-2):146-54. <https://doi.org/10.1016/j.virol.2013.08.028>
15. Rawal G, Krueger KM, Yim-Im W, Li G, Gauger PC, Almeida MN, Aljets EK, Zhang J. Development, evaluation, and clinical application of PRRSV-2 vaccine-like real-time RT-PCR assays. *Viruses*. 2023;15(11):2240. <https://doi.org/10.3390/v15112240>
16. Kvisgaard LK, Kristensen CS, Ryt-Hansen P, Pedersen K, Stadejek T, Trebbien R, Andresen LO, Larsen LE. A recombination between two type 1 porcine reproductive and respiratory syndrome virus (PRRSV-1) vaccine strains has caused severe outbreaks in Danish pigs. *Transbound Emerg Dis*. 2020;67:1786-1796. <https://doi.org/10.1111/tbed.13555>
17. Eclercy J, Renson P, Lebret A, Hirchaud E, Normand V, Andraud M, Paboeuf F, Blanchard Y, Rose N, Bourry O. A field recombinant strain derived from two type 1 porcine reproductive and respiratory syndrome virus (PRRSV-1) modified live vaccines shows increased viremia and transmission in SPF pigs. *Viruses*. 2019;11(3):296. <https://doi.org/10.3390/v11030296>
18. Renson P, Touzain F, Lebret A, Le Dimna M, Quenault H, Normand V, Claude J-B, Pez F, Rose N, Blanchard Y, Bourry O. Complete genome sequence of a recombinant porcine reproductive and respiratory syndrome virus strain from two genotype 1 modified live virus vaccine strains. *Genome Announc*. 2017;5(22):e00454-17. <https://doi.org/10.1128/genomeA.00454-17>
19. Wang Y, Liang Y, Han J, Burkhard KM, Vaughn EM, Roof MB, Faaberg KS. Attenuation of porcine reproductive and respiratory syndrome virus strain MN184 using chimeric construction with vaccine sequence. *Virology*. 2008;371(2):418-29. <https://doi.org/10.1016/j.virol.2007.09.032>
20. Yim-Im W, Anderson TK, Paploski IAD, VanderWaal K, Gauger P, Krueger K, Shi M, Main R, Zhang J. Refining PRRSV-2 genetic classification based on global ORF5 sequences and investigation of their geographic distributions and temporal changes. *Microbiol Spectr*. 2023;11(6):e0291623. <https://doi.org/10.1128/spectrum.02916-23>
21. Martín-Valls GE, Cortey M, Allepuz A, Illas F, Tello M, Mateu E. Introduction of a PRRSV-1 strain of increased virulence in a pig production structure in Spain: virus evolution and impact on production. *Porcine Health Manag*. 2023;9(1):1. <https://doi.org/10.1186/s40813-022-00298-3>
22. Martín-Valls GE, Cortey M, Allepuz A, Illas F, Tello M, Mateu E. Description of a new clade within subtype 1 of *Betaarterivirus suid 1* causing severe outbreaks in Spain. *Microbiol Resour Anounc*. 2022;11(7):e0030422. <https://doi.org/10.1128/mra.00304-22>
23. Pamornchainavakul N, Kikuti M, Paploski IAD, Makau DN, Rovira A, Corzo CA, VanderWaal K. Measuring how recombination re-shapes the evolutionary history of PRRSV-2: A genome-based phylodynamic analysis of the emergence of a novel PRRSV-2 variant. *Front Vet Sci*. 2022;9:846904. <https://doi.org/10.3389/fvets.2022.846904>
24. Trevisan G, Li G, Moura CAA, Coleman K, Thomas P, Zhang J, Gauger P, Zeller M, Linhares D. Complete coding genome sequence of a novel porcine reproductive and respiratory syndrome virus 2 restriction fragment length polymorphism 1-4-4 Lineage 1C variant identified in Iowa, USA. *Microbiol Resour Anounc*. 2021;10(21):e0044821. <https://doi.org/10.1128/MRA.00448-21>
25. Rawal G, Almeida MN, Gauger PC, Zimmerman JJ, Ye F, Rademacher CJ, Leyva BA, Minguia-Ramirez B, Tarasiuk G, Schumacher LL, Aljets EK, Thomas JT, Zhu J-H, Trexel JB, Zhang J. In vivo and in vitro characterization of the recently emergent PRRSV 1-4-4 L1C variant (L1C.5) in comparison with other PRRSV-2 Lineage 1 isolates. *Viruses*. 2023;15(11):2233. <https://doi.org/10.3390/v15112233>

