

## Type 2 PRRS virus ORF5 divergence from VR2332 over time

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### Key Points:

- ORF5 sequencing for PRRSv monitoring have been increasing over time
- ORF5 nucleotide divergence from the reference VR2332 averaged at around 10-15% throughout 2000-2018.

Since the first North American PRRSv isolate in 1992 (VR2332)<sup>1</sup>, PRRS diagnosis has been rapidly evolving towards viral identification. Nucleotide sequencing of the ORF5 region of the viral genome has become a popular diagnosis method used to understand viral diversity. Genotypical differences between the European and North American first isolates in the early 1990's led to PRRSv classification into two distinct genotypes, type 1 (European) and type 2 (North American)<sup>2</sup>.

One of the Morrison Swine Health Monitoring Project's (MSHMP) primary objectives is to prospectively monitor PRRS virus (PRRSv) sequence evolution. As part of this goal PRRSv sequence data from participants has been periodically captured and analyzed. Type 1 PRRSv has been reported in our database since 2007, but the vast majority (95%) of MSHMP's sequence dataset is comprised of type 2 PRRSv and is the focus of this analysis. PRRSv is expected to have a relatively high mutation rate and rapid evolution<sup>3</sup>. Assuming the VR2332 as representative of the early outbreaks, we hypothesized a higher divergence of ORF5 nucleotide from VR2332 over time.

To assess this hypothesis we analyzed type 2 PRRSv sequences from 1998 to 2018 (N=21,694). The first appearance in time of each unique sequence was kept, resulting in a database of 8,837 sequences. These were aligned using VR2332 as a reference. We calculated the percent difference as the amount of nucleotide characters that did not exactly match the reference. This difference over time is shown on Figure 1. The sequences within each year are shown by a box with upper and lower whiskers. The box represents 50% (2<sup>nd</sup> and 3<sup>rd</sup> quartiles) of the sequences with each whisker representing 25% (1<sup>st</sup> and 4<sup>th</sup> quartiles). The points represent extreme upper or lower values also known as outliers.

The majority of sequences are around 10-15% different from VR2332. Although not statistically significant, the boxes appear to be gradually increasing in difference from the VR2332 sequence through time. It is important to note however, that the range of difference within each year is also increasing. Possible explanations for these trends are that PRRSv diversity is slowly increasing, or that ORF5 sequencing has become more accessible, making variability in recent years more representative of the field viral population now than it was in the previous decade.

We continue to observe a low frequency of sequences that are less than 5% different from VR2332. This could be partially explained by vaccine use since some vaccines were derived from VR2332 or closely related isolates. However, PRRS vaccination was only massively introduced in the early 2010's. In our database, the frequency of sequences less than 5% different from VR2332 before that period was also low.

Lastly, the range in nucleotide difference varies largely between years even considering only the last 10 years which represents 95% of the data. Further investigations are necessary in order to understand if these differences are due to changes in sampling methods or if there are specific factors influencing how diverse a viral population is within a specific year.

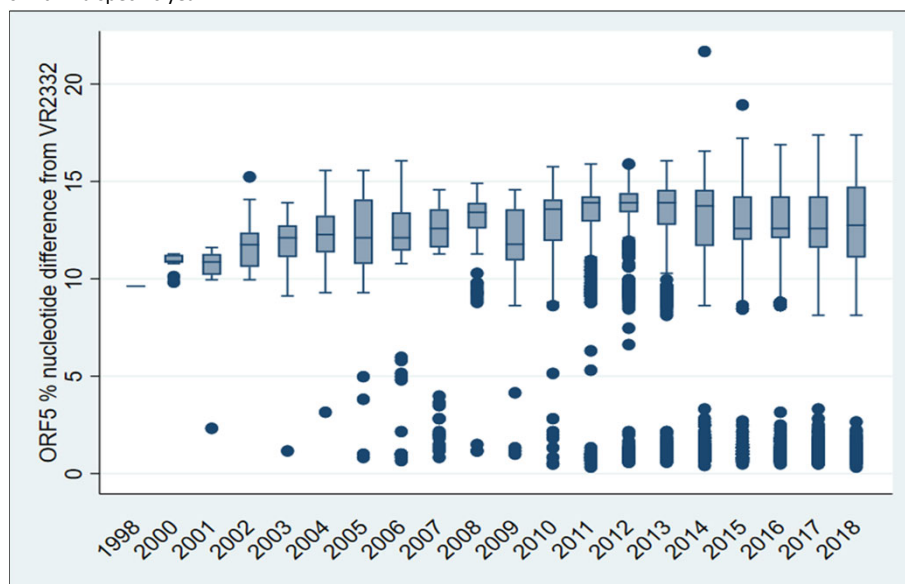


Figure 1. Percent of nucleotide difference from the first North American PRRSv isolate VR2332 over the years.

### Bibliography

1. Benfield D, Nelson E, Collins J, et al. Characterization of swine infertility and respiratory syndrome (SIRS) virus (isolate ATCC VR-2332). *J Vet Diagn Invest* 1992; **4**: 127-33.
2. Meng X, Paul P, Halbur P, Lum M. Phylogenetic analyses of the putative M (ORF 6) and N (ORF 7) genes of porcine reproductive and respiratory syndrome virus (PRRSV): implication for the existence of two genotypes of PRRSV in the U.S.A. and Europe. *Arch Virol* 1995; **140**: 745-55.
3. Cortey M, Diaz I, Martin-Valls G, Mateu E. Next-generation sequencing as a tool for the study of Porcine reproductive and respiratory syndrome virus (PRRSV) macro- and micro- molecular epidemiology. *Vet Microbiol* 2017; **209**: 5-12.

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