

## Understanding whether PRRS viruses in a neighborhood are closely related

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### Key Points:

- 28 space-time clustering of PRRSV based on producers' routine molecular surveillance were identified in the course of 2010-2019.
- Being inside or outside a space-time cluster significantly explains the genetic variability of most, but not all cases.
- Assessing space, time, and genetic relatedness relationships in PRRSV transmission is complex, and overall trends might miss important case information.

Understanding disease occurrence is key to developing prevention and intervention strategies. Despite the large amount of knowledge about PRRS epidemiology, farms continue to break, especially those within high density regions. Through the Morrison Swine Health Monitoring Project (MSHMP), participating systems voluntarily share disease status and PRRS virus sequences obtained from their routine surveillance or outbreak investigations allowing a more comprehensive monitoring of disease trends. Thanks to these progressive producers and practitioners, the MSHMP PRRSV sequence database currently holds more than 30,000 sequences from 25 states. These sequences are linked to a location and time allowing us to further conduct analysis and return value to producers and practitioners. With this extensive dataset, we aimed to assess if there was PRRSV clustering in space and time. If clustering was detected, we then assessed whether sequences inside clusters were less diverse than sequences outside cluster. This could potentially indicate that a local component of transmission (e.g. through pig movement, fomite, or airborne) might help explain the agglomeration of cases in a specific time and space.

Before running the analysis, the database was simplified by removing sequences that had no available date or location information, or those that were identical (i.e. 100% nucleotide identity) and from the same farm up to 30 days apart, as this was considered repeated sampling. Sequences considered vaccine-like ( $\geq 98\%$  nucleotide identity with strain VR2332 – parent strain of the first modified-live vaccine) were also removed. Because sequences were mostly distributed in two different US regions, analysis was stratified per region as defined by the US Census. At the end, we worked with 6,959 PRRSV ORF5 sequences from 2010 to 2019, 1,382 from Region 1 and 5,197 from Region 2. To account for the fact that systems might monitor disease differently, and thus generate different amount of data, we included system in the clustering identification analysis. Three space-time clusters were found in Region 1 and 25 in Region 2. What this means is that we observed a higher number of cases than expected within each cluster's radius and time frame. Clusters ranged from 0.4 to 46.9 miles radius (approximately 0.6 to 75.5 km), from 6 to 286 days in duration and included between 4 and 57 sequences.

When clusters were identified, all sequences from clusters were considered cases and all sequences outside the clusters from the same time period ( $\pm 3$  months) and region were considered controls. We found that being categorized as a case or a control significantly explains the genetic variability in 17 of these clusters using analysis of molecular variance (AMOVA  $p$ -value $<0.05$ ). Among those, case and control sequences were compared regarding their internal percent nucleotide identity and restriction fragment length polymorphism (RFLP) diversity. Median percent identity was higher among cases than among controls for 14 of the 17 clusters and lower for the remaining 3 clusters, indicating that on average case sequences are more similar to each other than control sequences are to each other (Figure 1). For cases in which within cluster sequences showed higher similarity than within control sequences, this suggests that local processes of transmission might be playing an important role. On the other hand, for the remaining clusters in which this was not observed, it indicates that short distance processes might have less relevance in transmission. RFLP Shannon diversity index was higher among controls for all 17 clusters, indicating that on average controls have a higher variability of RFLP patterns than cases.

These results highlight the complex nature of assessing space, time, and genetic relatedness relationships when assessing PRRSV transmission. Unfortunately, we do not have movement data to add to the analysis, which would be very informative. However, we found that focusing on overall trends may lead to over conclusions as we might miss important case by case information. It also highlights the importance of maintaining a comprehensive up to date dataset that could eventually allow for prospective monitoring of new regional outbreak occurrences, allowing for interventions

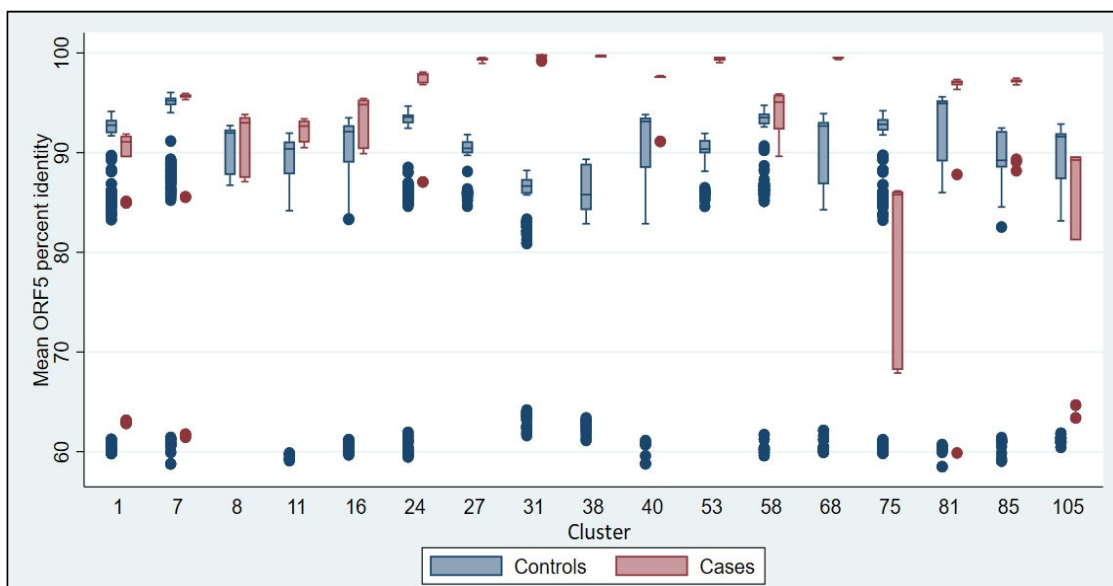


Figure 1. Distribution of mean PRRSV ORF5 percent identities of each sequence to others within its group by cluster.