

## The changing disguises PRRSV might use to hide from a pig's immunity

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Poor cross-protection between different strains of PRRSV is one of the foremost challenges producers face when immunizing pigs to prevent and manage PRRSV outbreaks. Despite performing better than their unvaccinated counterparts, vaccinated animals still get infected and may show clinical signs and productive losses in a PRRSV outbreak, depending largely on the strain that infects them. Experimental studies show that changes in the virus's genetic sequence that result in the gain or loss of protein N-glycosylation sites have the potential to influence the virus's ability to evade the pig's immune system. However, the potential role of such genetic changes in driving the emergence of new PRRSV strains in the field is not known.

A frequent post-translational process known as glycosylation involves the addition of sugars to particular viral protein regions. These modifications can alter how these viral proteins are identified by the host and may have an effect on a number of biological functions of proteins, including their 3D structure.

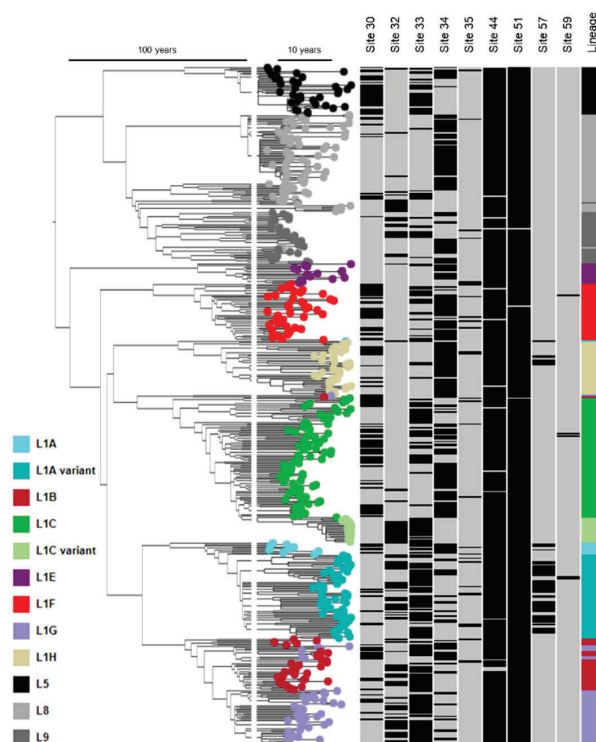
The genetic diversity of PRRSV-2 includes a large number of lineages and sub-lineages. The investigation of possible glycosylation occurring on the PRRSV genome is a pertinent subject because it is believed that immunologic pressure is largely responsible for the formation of various strains of PRRSV-2. Animals infected with different PRRSV strains that had varied glycosylation patterns showed varying neutralizing antibody production profiles, which suggests that the presence or lack of specific glycosylation could change the virus' immunogenicity.

In order to explore the occurrence of different glycosylation patterns of PRRSV in the U.S. over time, we analyzed 19,179 PRRSV ORF5 sequences from the University of Minnesota Veterinary Diagnostic Laboratory from 2004 to 2021. The ORF5 gene encodes the GP5 protein, which contains the Principle Neutralizing epitope for PRRSV. To visualize phylogenetic relationships, we constructed a time-scaled phylogenetic tree for 500 randomly selected sequences (see Figure).

We identified nine sites that are potentially glycosylated on the GP5 protein. Those are sites 30, 32, 33, 34, 35, 44, 51, 57, and 59. Different combinations of glycosylated sites were prevalent over time. Even though the glycosylation patterns found in different lineages were not necessarily lineage-defining, the emergence of certain combinations of glycosylations coincided with past PRRSV epidemics in the U.S. For example, the rapid expansion of sub-lineages L1A, L1C, and L1H were associated with a novel glycosylation pattern that emerged in those sublineages. For L1A, sequences glycosylated at sites 32, 33, 44, 51, and 57 first appeared in 2012, and by 2014 it represented more than 40% of all L1A sequences identified, reaching a peak of 62% of all L1A sequences identified in 2015. This coincides with the emergence of the L1A 1-7-4 virus, which was and still is a widely recognized event of clinical significance in the industry.

The emergence of the L1C 1-4-4 variant in 2020 can also be observed from a glycosylation pattern perspective. This virus's glycosylation pattern (at sites 32, 33, 44, and 51) was circulating since at least 2007, though represented only a small percentage of sequences. In 2020 and 2021, this pattern was observed in 44 and 47% of the L1C sequences identified in those years. Glycosylation patterns are not solely responsible for the emergence of new strains. However, in a dynamic landscape of cross-immunity elicited by diverse immunization practices and natural occurrence of PRRSV, the relative fitness of viruses with specific glycosylation patterns may change over time.

As an observational study, we cannot assess causality (do novel glycosylation patterns drive viral fitness for a given strain, or are these mutations just coincidental hitchhikers present in a successful strain?). However, studies have shown that the glycosylation of proteins is relevant to protein folding, immune recognition, neutralization, and immune evasion of viruses. This supports the hypothesis that the glycosylation pattern of a PRRSV sequence could be a relevant aspect when it comes to understanding the sequential dominance of different PRRSV strains in the field. Further studies are needed to explore how that can be leveraged to improve PRRSV control.



**Figure:** Time-scaled phylogenetic tree of 500 randomly selected sequences illustrating if each sequence was potentially N-glycosylated (black) or not (gray) at each residue site in which glycosylation was identified. Note that certain glycosylation at certain sites are more prevalent for certain sub-lineages: site 32 is more glycosylated on L1C variant than in non-variant L1C sequences; site 57 is almost exclusively glycosylated on L1A variant (1-7-4) viruses.

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