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Preparation of GP5-M Heterodimer Glycantype Specific Recombinant Protein and Replicon Particles

A.S. Leaflet R2381

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Summary and Implications
Porcine Reproductive and Respiratory Syndrome (PRRS) imposes a huge financial burden on the swine industry. Thus, there is a clear and immediate need for improved PRRS virus (PRRSV) vaccines. Our group has proposed a new classification scheme for PRRSV strains that allows for immunological differentiation based on level of GP5 glycosylation. This classification based on glycantype has allowed us to choose PRRSV strains that offer the best chance of protection against PRRS.

Introduction
The protective role of antibodies against PRRSV has been demonstrated by Osorio et al., who used hyperimmune serum to protect pregnant sows and their piglets from PRRSV infection. The importance of the GP5-M heterodimer for PRRSV has been demonstrated, as has equivalent protein heterodimers in other Arteriviruses. There are published studies which indicate that co-expression of both GP5 and M of PRRSV result in enhanced humoral and cell-mediated immune responses, and could therefore be effective at preventing PRRS. Similar studies with Equine Arteritis Virus (EAV) have also shown that the envelope proteins G and M, the equivalent of PRRSV GP5 and M respectively, do induce an antibody response, but only antibodies to the heterodimer were able to neutralize EAV and provided greater protection in vivo. These results indicate the necessity of the GP5-M heterodimer to induce a protective antibody response to protect against PRRSV.

Materials and Methods
Eight PRRSV negative gilts were inoculated with PRRSV strain HLV013 on Day 0 at 62 days of age, boosted with HLV013 on Day 52, and necropsied for serum collection on Day 86. Serum was then tested by fluorescent focus neutralization (FFN) on MARC-145 cells to determine virus neutralization titers. A second similar study was also done following the same infection schedule, except on Day 52 the eight gilts were boosted with PRRSV strain HLV093.

Preparation of GP5-M heterodimer glycantype specific recombinant protein and replicon particles
PRRSV strains HLV013 and HLV093 served as the parent strains for recombination. The PRRSV GP5 and M genes from each strain were cloned into replicon vectors that are based on a live attenuated strain of Venezuelan Equine Encephalitis Virus (VEEV). The GP5 and M replicon vectors were analyzed by both IFA and Western blot to confirm expression desired proteins. These replicons were then packaged into particles via co-electroporation with helper VEEV RNAs to produce RP. These same replicons were also co-electroporated without helper VEEV RNAs to produce replicon protein.

Results and Discussion
PRRSV strains with two N-linked glycans in the GP5 ectodomain, regardless of amino acid position, were designated NA2 strains. Strains with three N-linked glycans were designated NA3, and strains with 4 N-linked glycans were designated NA4. Although not used in this study, the glycantype scheme can easily be used for strains with additional glycans. Glycantype results are given in Table 1. Our data indicate that glycantype is the best predictor of cross neutralization when compared to ORF5 homology and RFLP patterns. As the number of N-linked glycans on GP5 increases so does resistance to neutralization by high titer sera. Several studies have shown that PRRSV strains with fewer glycans located in the GP5 ectodomain can induce neutralizing titers more rapidly and to a higher level than wild-type strains. The same results were seen in this study. When only one NA2 strain, HLV013, was used to infect the gilts, the highest homologous FFN titer observed was 140.4. However, when an additional NA2 strain, HLV093, was used to boost after HLV013 infection, there was a significant increase in FFN titers. High virus neutralizing titers were induced against all glycantypes when two NA2 strains were used. All titers seen after infection with two different NA2 strains are considered protective based on previous work. However, there are no current commercially available vaccines that fall into the NA2 group. This data supports the hypothesis that a vaccine which incorporates glycan mutant NA2 strains could be protective against PRRSV.

Recombinant proteins and replicon particles have been prepared with GP5 and M genes from 3 strains of PRRSV: HLV013 (NA2), HLV093 (NA2), and HLV349 (NA4). These preparations are being evaluated experimentally as vaccine candidates for prevention of PRRS.
## Table 1. Glycaltyp results. Strains are ordered highest to lowest by mean FFN results.

<table>
<thead>
<tr>
<th>PRRSV Strain tested in FFN assay</th>
<th>Glycaltyp</th>
<th>ORF5 Homology</th>
<th>RFLP</th>
<th>Mean FFN titer for each treatment group</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>HLV013, HLV013</td>
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<tr>
<td>HLV013</td>
<td>NA2 (44,51)</td>
<td>100</td>
<td>1-4-2</td>
<td>140.4</td>
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<td>ISU-P</td>
<td>NA2 (44,51)</td>
<td>90</td>
<td>1-5-2</td>
<td>91.2</td>
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<tr>
<td>NVSL 97-7895</td>
<td>NA3 (24,44,51)</td>
<td>99</td>
<td>1-4-2</td>
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<tr>
<td>SDSU 23983</td>
<td>NA3 (34,44,51)</td>
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<td>1-6-2</td>
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<tr>
<td>Prime Pac</td>
<td>NA3 (30,44,51)</td>
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<tr>
<td>VR2332</td>
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<td>HLV375</td>
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<tr>
<td>MN184b</td>
<td>NA4 (30,34,44,51)</td>
<td>86</td>
<td>1-8-4</td>
<td>3.7</td>
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