RESEARCH REPORT
SWINE HEALTH

Title: Epitope Inclusion: Enhancing porcine reproductive and respiratory syndrome virus (PRRSv) vaccine cross-protective efficacy - **NPB #14-217**

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Industry Summary: Producers need better vaccines for controlling disease and spread associated with PRRSv infection. This has been an extremely different task because the face of PRRSv is constantly changing making an escape from the host immune system, which is coupled with the ability to suppress the pig immune response. Until we have a better understanding of how the pig immune system “sees” PRRSv and thus, identify components of many different viral strains into a single vaccine the battle will continue. Our work used an objective classification scheme characterizing PRRSv’s based on what the host sees, or T cell epitopes. This information alone will be extremely valuable because it gives producers a better system for making informed choices about which commercial vaccine to select in order to provide maximal protection to the strain actually infecting the farm. For example, our computational results indicate that contemporary isolates are quite distant than three commercially available vaccines regardless of comparison method (full genome versus epitope content) but that using EpiCC comparison, one vaccine may be better than another. Our work to validate epitope predictions was limited given the ability of PRRSv to impact host immune responses, thus future work will need to consider another vaccination/disease system for validation. We performed a vaccine/challenge study using chimeric viruses or an attenuated strain of more contemporary isolate, but protection was minimal against a mildly virulent isolate of PRRSv174. Collectively, the approach can help with vaccine selection, but our work highlights that focus needs to shift away from using PRRSv vaccinated or infected pigs to identify T cell correlates of protection.

Keywords: PRRSV, T cell epitopes, vaccine, efficacy, immune

Scientific Abstract: Reproductive and Respiratory Syndrome virus (PRRSv) is a single-stranded RNA virus composed of nine open-reading frames (ORFs). The non-structural proteins are encoded in ORFs 1a and 1b and the structural proteins are encoded in ORFs 2a, 2b, and 3-7. PRRSv has been, and continues to be, an enormous economic burden to the swine industry. The genetic and antigenic variability between isolates, as well as the many mechanisms PRRSv has to alter pig immune responses, continues to make identification of correlates of protection and study of cellular immune responses quite difficult. Identification of T cell epitopes conserved across PRRSv isolates and vaccine strains, and thus comparison of T cell epitope content, provides a method to for vaccine strain selection. Our results indicate that using epitope comparison of isolates changes the phylogenetic relationship between strains, and that there is significant differences between commercially available vaccine strains and contemporary isolates of PRRSv. We used a vaccine/challenge study to validate epitope predictions, as well as capacity of epitope scores to be used in predicting protection. Vaccine immunogenicity was not optimal and difficulty in procuring pigs with specific SLA made final conclusions difficult. Our results do show some protection against viral replication of a contemporary PRRSv 174 isolate when vaccinated with a chimeric virus. Overall, validation of predicted epitopes will require a system in which PRRSv vaccination or infection is not used given the ability of PRRSv to hinder antigen presentation.

Introduction:

Reproductive and Respiratory Syndrome virus (PRRSv) is a single-stranded RNA virus composed of nine open-reading frames (ORFs). The non-structural proteins are encoded in ORFs 1a and 1b and the structural proteins are encoded in ORFs 2a, 2b, and 3-7. PRRSv has been, and continues to be, an enormous economic burden to the swine industry. The virus has considerable genetic and antigenic variability that has made the disease difficult to prevent with a vaccine and an efficacious, broadly cross-protective formulation has yet to be developed. While a great deal of research has described the

humoral and cell-mediated immune response elicited following vaccination and/or infection, a clear immune correlate of protection has not been identified. Thus, it is difficult to know which regions of the virus to direct an immune response to for maximal protection. In addition, little is known about the immunological relationship amongst PRRSv strains and only ORF5 alone is typically used to evaluate relatedness of different strains. While this approach can be useful, it may be misleading because the host and pathogen interface through host processing of viral proteins into short epitopes for loading into the major histocompatibility complex (MHC) for antigen presentation to T cells, a process that's required for both T cell activation and B cell production of antibody.

Currently there are two vaccine platforms commercially available for PRRSv – modified-live virus (MLV) vaccines or a killed virus (KV) vaccines. MLV vaccines are recognized for their protective efficacy against genetically homologous virus; however cross-protective efficacy and safety remain a concern. While MLV vaccine or PRRSv exposure primes an immune response against PRRSv that may provide protection against heterologous infection, MLV vaccines may recombine with circulating strains of the virus (1). KV vaccines are extremely safe; however, they confer very limited, if any, protection. Cell-mediated immunity, or T cell based immunity, is believed to play a role in an effective immune response to PRRSv because antigen-specific T cells are necessary to kill virally infected cells. The efficacy of MLV vaccines over KV vaccines provides additional evidence that T cells likely play a significant role in protection given that viral replication associated with MLV vaccines elicits stronger T cell responses. In addition, PRRSv is typically cleared from the pig prior to the appearance of neutralizing antibodies suggesting a significant role for T cells in viral clearance (2). There are currently three commercially available MLV vaccines, none of which utilize a strain of PRRSv isolated after 2000; thus, they may not be sufficiently antigenically related to contemporary isolates of the virus in which they are expected to provide protection. Recently there has been an increase in morbidity and mortality with a particular RFLP type of PRRSv (referred to as 174), though not all contemporary RFLP pattern 1-7-4 isolates are associated with increased disease. Those that are associated with disease seem to have evolved from the 1-8-4 family of PRRSv.

Recently, a number of research groups have embarked on identifying T cell epitopes present in PRRSv; however, the majority of these studies have been limited because they lack a robust bioinformatics approach that is swine specific. While T cell epitopes can be identified through a systematic approach of synthesizing and testing large sets of overlapping peptides with *in vitro* assays, this approach is very expensive and tedious. T cell epitope prediction informatics tools, which model the peptide-MHC interaction *in silico*, are widely used in human immunology. These algorithms, particularly when developed in a species-specific manner, significantly reduce time and effort required to identify putative T cell epitopes and decrease the number of proteins requiring *in vitro* testing for immunogenicity; applications include "vaccines-on-demand" for biodefense (3), vaccines against highly variable pathogens such as HIV (4, 5) and influenza (6). The tools are also used for large scale genome analysis and genome-to-vaccine design (7, 8); for immunology studies, such as understanding immunogenicity of existing proteins and selection of epitopes for further characterization; and for development of diagnostic tests such as TB-spot (ELISpot) and Quantiferon Gold (ELISA) (9, 10). Comparable T cell prediction tools are becoming available for pigs, and there is sufficient data available on swine MHC-restricted T cell epitopes to continue the process of compiling the algorithms. The continued development of these tools for swine will dramatically accelerate pig immunology research in a targeted, species-specific manner.

Although comparison of PRRSv genomes has led to a detailed characterization of the heterogenic nature of the virus, this approach has not identified differences in antigenicity to determine which viruses are considered homologous or heterologous as it relates to immune reactivity. While PRRSv genome sequence comparisons have been informative, they fail at estimating cross-reactivity because they do not take into consideration what the host immune systems 'sees'. Thus, a reliable objective method to compare the T cell epitope content and classify the immunological relationship between PRRSv will aid in accurately identifying predictors of protection and subsequently estimating cross-protection for the development of new safe and efficacious vaccines for the control of PRRS. As pig specific tools for identification of immunogenic T cell epitopes are being developed, a speedy and valid approach that incorporates T cell epitope prediction and classical immunization experiments can be utilized to classify PRRSv based on immunogenic T cell epitope content, identify epitopes in which immune reactivity correlates with viral clearance, and further methods to predict protection and cross-protection.

Stated Objectives from original proposal

Objective 1: Identify highly antigenic conserved and strain unique T cell epitopes in currently available commercial PRRS MLV vaccines and wild-type viruses using PigMatrix and EpiCC. While vaccine efficacy for PRRSv is limited, PRRS MLV vaccines are considered the most efficacious; however, a method to estimate cross-protection against variant viruses is lacking. Unfortunately, we know very little about what parts of the virus induce protective responses. Specific research priorities to be addressed in this objective include 1) Epitope identification for individual structural proteins, ORF1a and ORF1b for MLV vaccines and wild-type strains; 2) Comparison of PRRSv strains epitope content using EpiCC to assess PRRSv relatedness based on epitope makeup; and 3) Validation of conserved and unique predicted epitopes.

Objective 2: Determine if epitope identification and EpiCC estimates accurately assess protection using a vaccination/challenge experiment. It is difficult to predict what vaccine to use when a herd breaks with PRRSv – thus, testing of EpiCC between contemporary isolates and a commercially available MLV vaccine will be used to select two different wild-type strains for challenge of MLV vaccinated pigs. A vaccine/challenge study will be devised to validate

EpiCC predictions. As producers need immediately applicable solutions for the management of PRRSv, we will utilize a commercially available MLV vaccine. Work from this objective will provide in vivo validation of newly developed tools and a newly developed vaccine approach for both short-term and long-term solutions for PRRSv.

Objective 3: Correlate cell-mediated immune responses to specific PRRSv epitopes with viral clearance. Debate continues on the contribution of neutralizing antibody to viral clearance. The contribution of cell-mediated immunity to viral clearance is assumed; however, responses to specific PRRSv peptides, as it correlates to clearance, have not been well defined. Thus, we will evaluate cell-mediated immune responses to specific PRRSv peptides through the course of disease (up to 6 weeks post-infection) and correlate these responses with viral clearance from the sera. We will determine if responses to a specific peptide or if the magnitude in response correlates to resolution of viremia.

Materials and Methods:

Epitope analysis

Sequences: We initially analyzed 11 non-structural (NSP1a, NSP1b, NSP2, NSP3, NSP4, NSP5, NSP7, NSP9, NSP10, NSP11, and NSP12) and seven structural proteins (GP2a, GP2b, GP3, GP4, GP5, M, and N) from 3 vaccine strains (Fostera (P129), Ingelvac MLV and Ingelvac ATP) and 7 PRRSV strains (VR2332, SDSU73, NADC31, NADC30, JA142, MN184, and VR2385). NSP6 and NSP8 were not analyzed. NSP6 was short (16 aa); NSP8 was a subsequence of NSP9. Sequences were retrieved from GenBank.

In a second analysis, we analyzed the same non-structural and structural proteins from a challenge virus (PRRSV174) and vaccine viruses (two chimeric viruses and an attenuated virus). Genome sequences of the viruses in this analysis were provided under confidentiality agreement by BIVI.

Epitope prediction: Using PigMatrix, a T cell epitope prediction algorithm based on the pocket profile method (11), proteins were parsed into overlapping 9mer frames and scored for binding likelihood to class I (SLA-1*0801, 1*1201, 1*1301, 2*0501, 2*1201, 3*0501, 3*0601, and 3*0701) and class II (SLA-DRB1*0201, 0402, 0602, 0701, and 1001) SLA alleles. Peptides scoring above 1.64 on the PigMatrix Z-score were considered putative SLA binders. SLA alleles used for prediction were frequently expressed in a cohort tested in a previous study (12).

Epitope selection for synthesis: We identified class I and II peptides that were not only predicted to bind to at least two SLA alleles, but also were conserved across multiple strains in our analysis. Peptides were synthesized using 9-fluoronylmethoxycarbonyl (Fmoc) chemistry by 21st Century Biochemicals (Marlboro, MA). Peptide purity was >80% as ascertained by analytical reversed phase HPLC. Peptide mass was confirmed by tandem mass spectrometry.

T cell Epitope Content Comparison (EpiCC): To evaluate the T cell epitope-based relatedness between challenge and vaccine viruses, we compared their predicted T cell epitope content. The epitope content depends on the epitope density and the binding likelihood of the predicted epitopes. Class I and II predicted T cell epitope content of each protein of the challenge virus or the field viruses was compared to that of the vaccine viruses and relatedness scores (EpiCC scores) were calculated. EpiCC calculation included scores determined for shared epitopes and strain or vaccine unique epitopes. The identity of the T cell receptor (TCR)-facing residues was considered to determine shared epitopes. EpiCC scores were normalized by the number of SLA alleles and compared 9mers. The higher the EpiCC score, the higher the T cell epitope content relatedness between strains.

Vaccination and challenge study

Animal selection and viruses: In order to best test our hypothesis, which was based on specific SLA alleles, we elected to screen pregnant sows for SLA haplotype for subsequent selection of piglets to include in the study. Piglets were also SLA typed. For SLA haplotyping, blood was collected from pigs and DNA isolated from peripheral blood mononuclear cells (PBMC). DNA was sent to Dr. Chak-Sum (Sam) Ho at Gift of Life Michigan for SLA typing (13, 14). Piglets from 5 sows were selected and piglets from each litter were distributed into one of five groups, with 8 pigs per treatment group (Table 1). At least 2 pigs from each litter were represented in each treatment group, and pigs were distributed in rooms according to vaccine type. Pigs were allowed to acclimate for approximately 1 week prior vaccination. Pigs were vaccinated by the intramuscular route with approximately 2x10^4 $TCID_{50}$ of the indicated vaccine virus, which were used in a previous vaccine study (15). The viruses used as vaccine included one of two chimeric viruses constructed from combinations of a licensed modified live vaccine (Ingelvac MLV, attenuated VR2332) and a virulent isolate with RFLP pattern 1-8-4 (referred to as MN184), and a high cell-culture passaged MN184 isolate (passage 105. MN184-p105). The chimeric vaccine viruses encoded ORF1 of MLV with ORF 2-7 from MN184 (MLV/MN184:2-7) or ORF1-4 and 7 of MLV and ORF 5-6 from MN184 (MLV/MN184:5-6). The challenge virus was a contemporary PRRSV isolated with RFLP pattern 1-7-4. All viruses were propagated on MARC145 cells.

TABLE 1: EXPERIMENTAL GROUPS

GROUP	VACCINE	CHALLENGE PRRSV-174	PIG#	ROOM
1	None (nonvaccinated, NV)	None	50-51,70-72,81,89-90	8
2	MN184-p105	Yes	52-53, 73-74, 82-83, 91-92	10
3	MLV/MN184:2-7	Yes	54-55, 75-76, 84-85,93-94	12
4	MLV/MN184:5-6	Yes	56-57, 77-78, 86-87, 95-96	14
5	Cell culture media (NV)	Yes	58-59, 69, 79-80, 88, 97-98	16

Sample collection and assays: Blood was collected by venipuncture from pigs at day 0, 14, 21, 29, and 42 relative to the day of vaccination, and day 3, 7, and 10 after challenge (pigs were challenged on day 42 post-vaccination). Serum and/or peripheral blood mononuclear cells (PBMC) were isolated from blood and used for virus isolation/titration and PRRSV-specific antibody measurement, and evaluation of PRRSV-specific IFN-γ T cell responses, respectively. Serum was used for virus isolation by plating on MARC145 monolayers and evaluating cytopathic effect (CPE) 5-7 days later. PRRSV-specific antibody was evaluated using the HerdCheck Idexx ELISA according to manufacturer's recommendations. PBMC were isolated and used in IFN-γ ELISpot assays as previously described (12). PRRS viruses used as recall antigen included VR2332 (parental strain for MLV), MN184, and challenge virus (PRRSV174). Peptides predicted as T cell epitopes were synthesized as used as recall antigen (individual peptides) on a single day post-vaccination (day 42) or in pools at days indicated in the respective figures.
Weight gain, lung lesions and lung viral titers were measured to evaluate vaccine efficacy. Pigs were weighed at day 0, 3, 7 and 10 relative to challenge to determine the impact of challenge on weight gain. Pigs were necropsied at day 10 post-challenge to evaluate lung lesions and collection of broncho-alveolar lavage fluid to evaluate virus load. Lung lesions were scored as previously described (16). BALF was serially diluted and added to MARC145 monolayers in triplicate. CPE was evaluated 5-7 days later and titers calculated by the Spearman & Karber algorithm (17).

Results

Objective 1

Epitope prediction for whole PRRSV genome: We identified 56 epitopes that are conserved across all or most of the analyzed PRRSV strains - 3 vaccine strains [Fostera (P129), Ingelvac MLV and Ingelvac ATP] and 7 PRRSV strains VR2332, SDSU73, NADC31, NADC30, JA142, MN184, and VR2385 (Table 2).

Table 2. Number of predicted T cell epitopes conserved across 10 PRRSV strains

Structural proteins

Protein	Class I	Class II
GP2a	4	3
GP2b	2	1
GP3	2	1
GP4	1	2
GP5	1	3
M	2	3
N	1	2
Total	**13**	**15**

Non-structural proteins

Protein	Class I	Class II
NSP1a		1
NSP1b		2
NSP2	2	3
NSP3	1	1
NSP5	1	
NSP7		1
NSP9	3	4
NSP10	4	1
NSP11	2	
NSP12	2	
Total	**15**	**13**

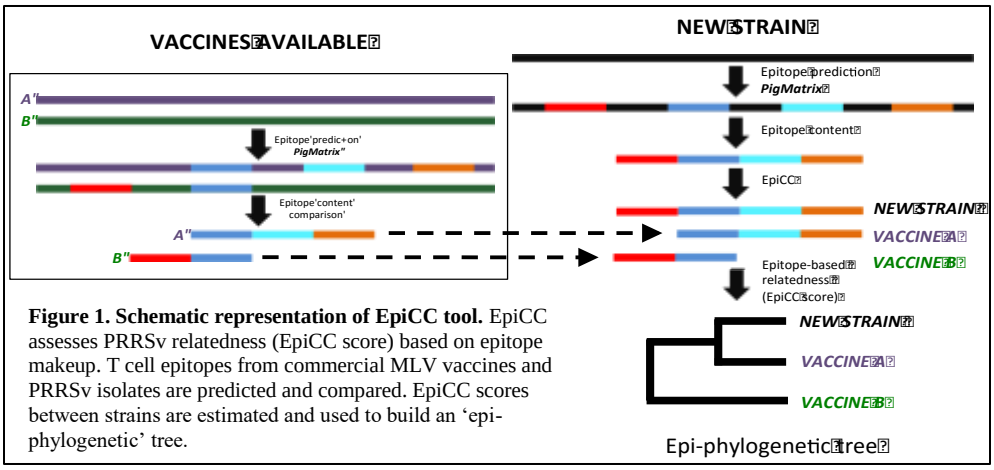

Figure 1. Schematic representation of EpiCC tool. EpiCC assesses PRRSv relatedness (EpiCC score) based on epitope makeup. T cell epitopes from commercial MLV vaccines and PRRSv isolates are predicted and compared. EpiCC scores between strains are estimated and used to build an 'epi-phylogenetic' tree.

Epitope content comparison (EpiCC): We compared individual viral proteins from 7 PRRSV strains to those of three commercial vaccines using computation approach outlined graphically in Figure 1. For each protein we compared class I and class II T cell epitope content of each strain to that of the vaccine viruses. The calculation of the T cell epitope-based relatedness considers scores of shared and unique epitopes. Scores of shared class I and II epitopes and EpiCC scores are shown in Figure 2. A higher score is indicative of the relatedness between the sequences. The three commercial vaccine viruses shared T cell epitopes with all analyzed strains (Figure 2A). The T cell epitope contents of the vaccine strains were less related to those of NADC31, NADC30, and MN184C when compared to the other viruses analyzed (Figure 2B). The negative EpiCC scores indicated that the scores of unique epitopes were higher than the scores of shared epitopes. Collectively, these results indicate that the T cell epitope content between vaccine viruses and these 3 viruses (NADC31, NADC30, and MN184C) have fewer shared epitopes and more unique epitopes, which may indicate less cross-protective efficacy against those strains, regardless of the commercial vaccine used.
The differences observed in the analysis of individual proteins were less noticeable when class I and II EpiCC scores were summed up, normalized and transformed to a distance matrix. This matrix was used to build an EpiCC-based tree, which was compared to a phylogenetic tree created using complete strain genomes (Figure 3). In the phylogenetic tree, Fostera (P129) is grouped with ATP, JA142 and SDSU73. But in the EpiCC-based distance tree, P129 is in the same branch as MLV, VR2332 and VR2385. Thus, in terms of T cell epitope content, P129 is more similar to VR2385, which differs to the result obtained by the comparison of the whole genome (Figure 3).

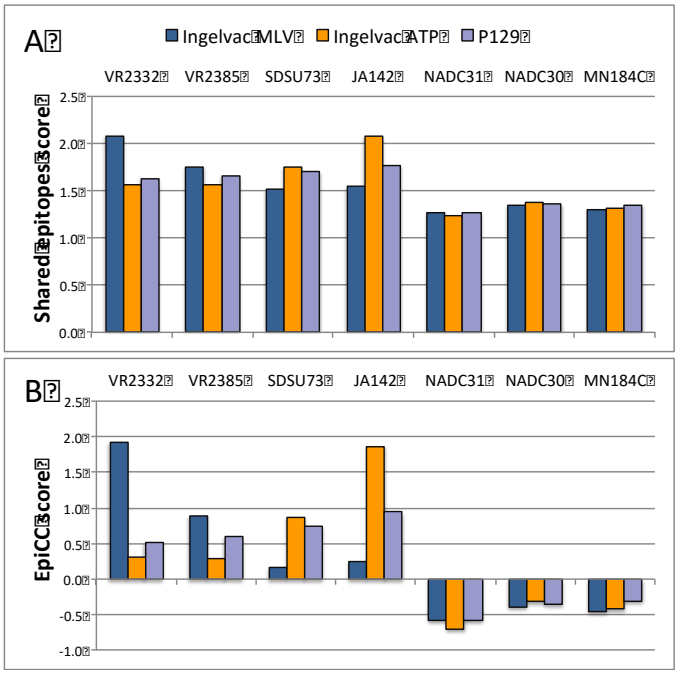

Figure 2: T cell epitope content comparison between a range of PRRSV strains and commercial vaccines. (A) Scores of shared T cell epitopes and (B) EpiCC scores

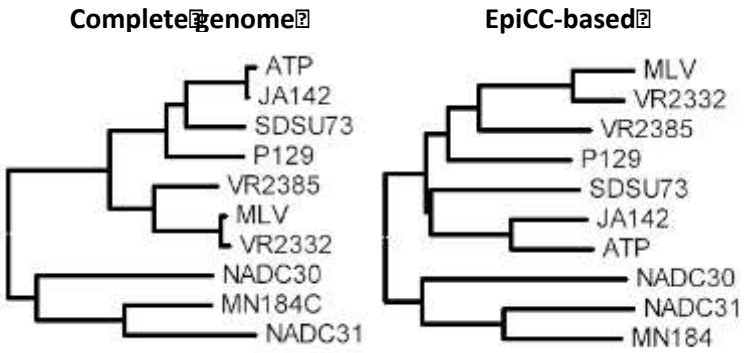

Figure 3. Comparison between phylogenetic tree generated using the whole genome and the EpiCC-based tree. Trees were generated using the Neighbor-joining algorithm.

Objective 2 and 3

Using the same approach as shown for objective 1, we compared the epitope content of individual proteins of three experimental vaccine viruses (Table 1) and the challenge strain (PRRSv174) used in the efficacy study. Shared epitope and EpiCC scores are shown in Figure 4. MLV/MN184:2-7 and MLV/MN184:5-6 had highly similar scores for NSPs, which was expected given that ORF1 was the same for both of these chimeric viruses. Small differences observed may be explained by nucleotide and amino acid changes in the chimeric viruses that result from the cloning and rescuing of the chimeric viruses (15). Some of the changed residues were within predicted epitopes. This demonstrates that few amino acid differences might have an impact in the T cell epitope content for a PRRSV isolate. The three experimental vaccine viruses shared T cell epitopes with PRRSv174. However, for 13 out of 18 analyzed proteins, the EpiCC scores were negative, indicating that the scores for unique epitopes were higher than scores of shared epitopes. NSP1a, NSP4, NSP9, NSP10, and NSP11 epitope content of the vaccines were more related to that of the challenge virus. Overall, the T cell epitope content of MN184-p105 was the most related that of PRRSV174 challenge virus (EpiCC score = -0.65).

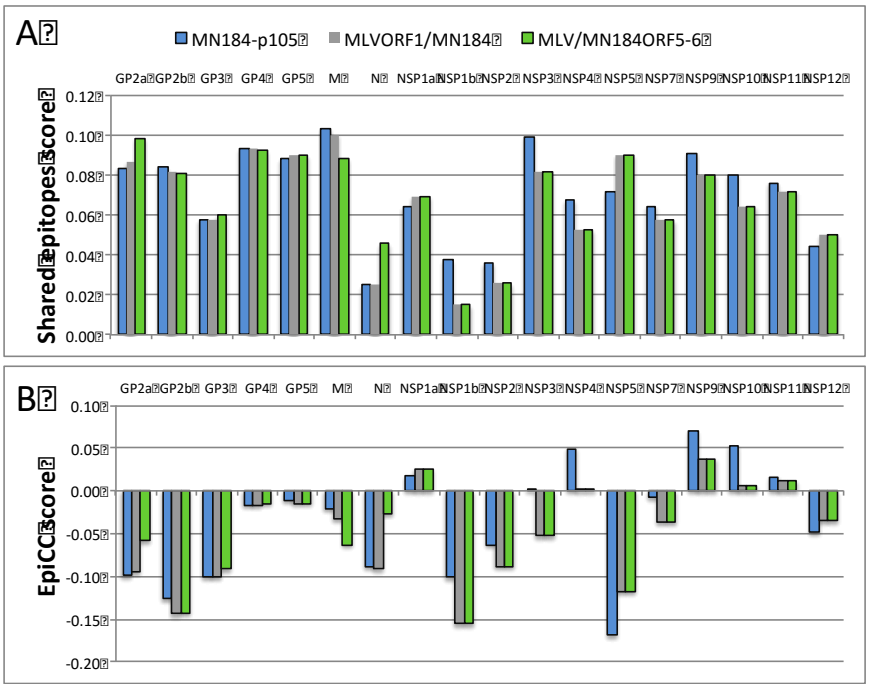

Figure 4. T cell epitope content comparison between PRRSv174 and experimental vaccines used in vaccine/challenge study. (A) Scores of shared T cell epitopes and (B) EpiCC scores of individual proteins.

Table 3: Percentage of pigs with virus in the serum at the indicated day relative to vaccination

	Day relative to vaccination			
Vaccine Virus	**14**	**21**	**29**	**42**
MN184-p105	50% (4/8)	37.5% (3/8)	37.5% (3/8)	50% (4/8)
MLV/MN184:2-7	37.5% (3/8)	25% (2/8)	25% (2/8)	50% (4/8)
MLV/MN184:5-6	12.5% (1/8)	75% (6/8)	50% (4/8)	12.5% (1/8)

Vaccine Immunogenicity: Pigs were vaccinated by the intramuscular route with 2ml of 10^4 $TCID_{50}$/ml of the indicated virus (p105, MLV/MN184:2-7, MLV/MN184:5-6) (Table 1). Blood was collected for isolation of serum and peripheral blood mononuclear cells (PBMC) weekly, beginning on day 14 post-vaccination. Serum was used for evaluating seroconversion by Idexx Herdcheck ELISA and virus isolation to evaluate replication of the vaccine virus. Table 3 indicates the percentage of pigs with virus in the serum at the indicated day relative to vaccination. While not all pigs were viremic on a given day relative to vaccination, pigs did seroconvert to PRRSV, as indicated by antibody reactivity against the nucleoprotein of PRRSV using a commercially available kit (Figure 3). Seroconversion in the group of pigs vaccinated with MN184-p105 was delayed when compared to seroconversion in pigs vaccinated with the other two vaccine viruses. However, by the time of challenge (day 42 after vaccination), all pigs had seroconverted with a single exception – one pig in the MLV/MN184:5-6 group did not seroconvert, nor was vaccine virus recovered from the serum of that animal.

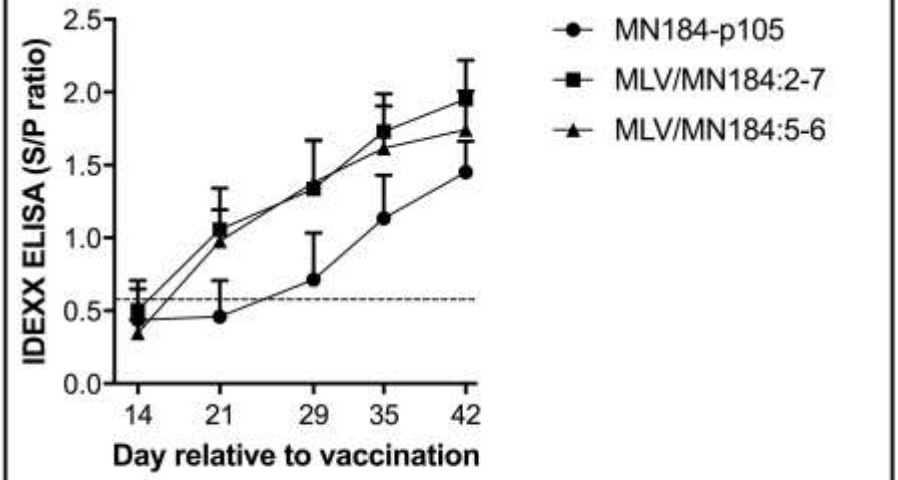

Figure 5: Seroconversion to PRRSV after vaccination. Pigs were vaccinated with the indicated vaccine virus and blood collected at the indicated day relative to vaccination to evaluate seroconversion using the Idexx Herdcheck ELISA.

IFN-γ ELISpot results

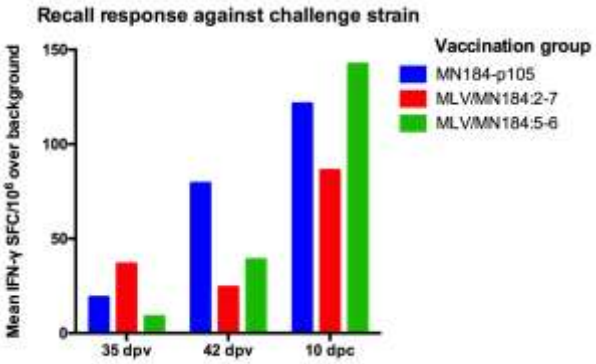

Figure 6: Number of PRRSv174-specific IFN-γ secreting cells in peripheral blood of pigs administered the indicated vaccine virus at 0 day post-vaccination (dpv) or 10 days post-challenge (dpc). Pigs were challenged on 42 dpv with PRRSv174, which was used as recall antigen in the assay.

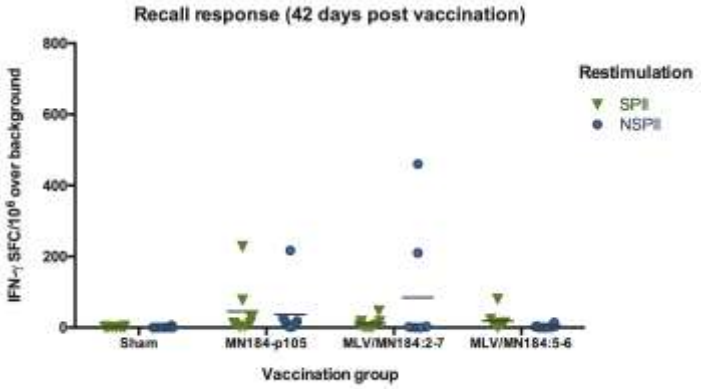

Figure 7: Number of IFN-γ secreting cells specific to predicted PRRSv specific T cell epitopes in peripheral blood of pigs administered the indicated vaccine virus. Two SLA class II predicted epitopes (SP and NSP) were used as recall antigen and the number of IFN-γ SC enumerated.

Protection against PRRSV challenge

Weight gain: On day 42 after vaccination, pigs were challenged by the intranasal route with a contemporary isolate of PRRSV with a RFLP pattern 1-7-4 (referred to as PRRSV174) at approximately 10^5 $TCID_{50}$/ml at 2ml per pig. Pigs were weighed on the day of challenge and again at days 3, 7 and 10 post-challenge. Change in weight relative to the day of challenge was calculated and pigs challenged with PRRSV174 gained less weight than non-challenged pigs, though statistical differences were only noted between the non-vaccinated/non-challenged group and the MN184-p105 group (Figure 8). This difference was also noted at day 7 post-infection, with a significant different in change in weight between the non-vaccinated/non-challenged group and MN184-p105 group as well as the MLV/MN184:5-6 group. Interestingly, all pigs compensated for the decreased weight gain by day 10, with all pigs gaining the same amount of weight by day 10 post-infection as the non-challenge controls (NV/NCh).

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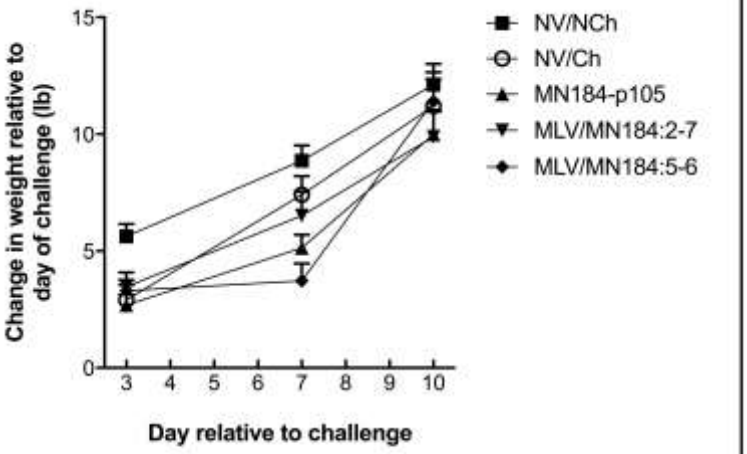

Figure 8: Change in weight relative to day of challenge with PRRSV in vaccinated pigs. Pigs were weighed on the day of challenge (day 0) and again at day 3, 7 and 10. Data is expressed as the average change in weight compared to day 0 at the indicated day relative to challenge. Data is expressed as the average + SEM for indcated group.

Replication of challenge virus: Blood was collected on days 0, 3, 7 and 10 relative to PRRSV174 challenge to evaluate protection against replication of challenge virus. Results Table 4 show that virus was recovered from 100% of non-vaccinated/challenged controls by 10 post-infection. However, not all of the pigs the vaccinated groups were viremic after challenge. The vaccine virus was not completely cleared from the periphery of pigs prior to challenge, but after challenge the percentage of pigs with virus in the blood did increase, which was likely due to replication of challenge virus. On day 10 post-challenge, the amount of virus in the serum of pigs in which virus was recovered was at the limit of detection; however, in the non-vaccinated/challenge control group five of eight pigs had titers greater than the limit of detection (data not shown). These data indicate that while the number of pigs with virus recovered may not be different across vaccine groups, the amount of virus recovered was different. These data indicate some protection against virus replication in pigs previously vaccinated with PRRSV. On day 10 post-challenge pigs were necropsied and broncho-alveolar lavage (BAL) was performed to evaluate virus titer in the lungs. There was significantly less PRRSV in the BAL fluid of pigs previously vaccinated with MLV/MN184:2-7 or MLV/MN184:5-6 when compared to non-vaccinated/challenge pigs (Figure 9).

Table 4: Percentage of pigs with virus in the serum at the indicated day relative to challenge

	Day relative to challenge			
Vaccine Virus	**0**	**3**	**7**	**10**
NV/Ch	0% (0/8)	75% (6/8)	87.5% (7/8)	100% (8/8)
MN184-p105	50% (4/8)	75% (6/8)	62.5% (5/8)	37.5% (3/8)
MLV/MN184:2-7	50% (4/8)	50% (4/8)	62.5% (5/8)	87.5% (7/8)
MLV/MN184:5-6	12.5% (1/8)	87.5% (7/8)	75% (6/8)	37.5% (3/8)

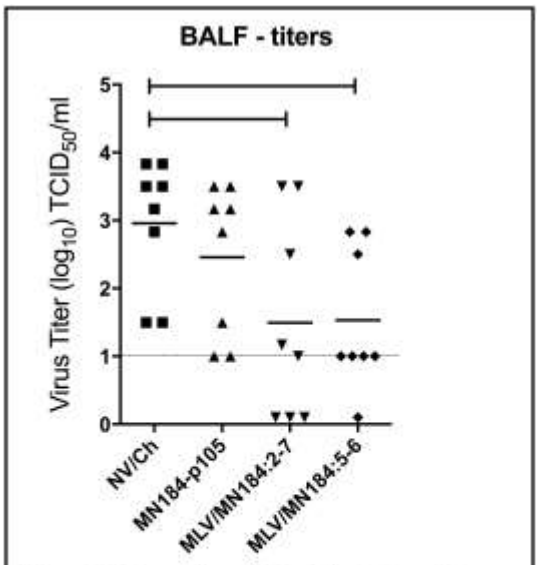

Figure 9: Change in weight relative to day of challenge with PRRSV in vaccinated pigs. Pigs were weighed on the day of challenge (day 0) and again at day 3, 7 and 10. Data is expressed as the average change in weight compared to day 0 at the indicated day relative to challenge. Each dot represents a single pig in the indicated group. Dotted line indicates limit of detection.

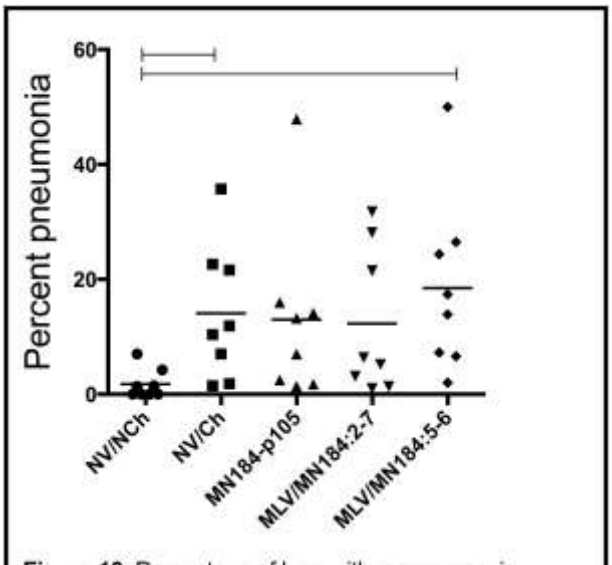

Figure 10: Percentage of lung with macropscopic lesions on day 10 following challenge with PRRSV174. Groups of pigs were vaccinated with the indicated virus and subsequently challenge with PRRSV174 on day 42 post-vaccination. On day 10 post-challenge the weighted percentage of lung lesions was determined for each pig. Each dot represents a pig in that group with the group average indicated with a line.

Lung lesions: Pigs were necropsied on day 10 following challenge for evaluation of lung lesions as a measure of vaccine efficacy (Figure 10). There was a significant increase in lung lesions in non-vaccinated/challenged pigs. While there was a numerical trend for increased lesion severity in pigs challenged with PRRSV174 that were previously vaccinated with MN184-p105 or MLV/MN184:2-7, there was no statistical difference when compared to non-vaccinated/non-challenged pigs. In addition, there was not a significant difference in lesion severity between any vaccinated group to the non-vaccinated/challenged group. There was significant variability within a group, ranging from pigs with minimal lesions to nearly 20% of the lungs affected with lesions. Collectively, it's difficult to make conclusions on the ability of vaccination to protect against lesions following challenge.

Discussion

PRRS continues to be a major problem for the swine industry worldwide. While there are commercially available MLV vaccines that prime for some level of protection against infection, protection is limited. And while MLV vaccination may limit morbidity and mortality, it does not necessarily limit virus evolution, transmission, or recombination. Thus, identification of clear immune correlates of protection is critical for improvement of tools for reduction of PRRSv circulation and burden on the industry. To this end, we set out to characterize PRRSv based on predicted T cell epitopes as a mechanism to better identify relatedness between viruses. We incorporated entire genome sequence into the analysis, not just a single gene. We tested the approach using a vaccination/challenge study to validate T cell epitope predictions and protection predictions. Unfortunately the experiments were met with a series of hurdles, but with careful interpretation, provided important information for consideration in future work.

The computational analysis for epitope prediction was based specifically on SLA allele of the pig, and to validate predictions, the antigen-presenting cell used for validation would need to express the specific SLA. Pigs are outbred animals with significant diversity in SLA alleles. While our analysis was based on prior work (12) and subsequent selection of sows/piglets with the at least some of the SLA's used in the predictions, we still had a limited number of animals with the optimal SLA alleles in the study. Thus, it was difficult to validate all of our predictions because of this. In addition, a number of other experimental factors may have contributed to the minimal response to peptides and PRRSv recall antigen in the IFN-γ ELISpot assay (Fig. 6 and 7). Thus, it is difficult to ascertain at this point whether the predicted epitopes are actually recognized by porcine T cells during vaccination and further work is warranted to complete the validation process. In addition to the mismatch in SLA and peptides, there were a number of other factors that may have contributed to the less than optimal responses that are described below.

First, the minimal PRRSv-specific IFN-γ T cell response detected after vaccination may be related to suboptimal vaccination regimen. Specifically, the vaccination dose used in the study was less than optimal as evidenced by the slow progression to seropositive status following vaccination (Fig. 5). In addition, vaccine virus was not recovered from all of the

pigs on the same day relative to vaccination suggesting an asynchronous response overall (Table 3). This may impact the immunogenicity of the vaccine and subsequent immune response. In addition, these data raise an extremely important point in that PRRSv is known to alter function of antigen-presenting cells, and to impair immune responses in cells of myeloid lineage. In an IFN-γ recall assay using PBMC, the assay relies on monocytes to serve as antigen-presenting cells to T cells for induction of effector function, including IFN-γ production. It's quite possible that the monocytes in culture were impaired by the vaccination and thus, it was difficult to draw major conclusions from the results of the IFN-γ ELISpot assay. Research is this area is need to accurately identify correlates of protection, particularly those related to T cell responses that require validation using cells from pigs vaccinated with MLV or live virus. To improve predictive indices of the EpiCC analysis and porcine T cell epitopes it may be better to validate with a vaccine or infection that has not been shown to impact host immunity as dramatically as PRRSv.

The PRRSv174 isolate used as challenge virus in the current study did not have the same clinical impact as other 174 isolates have been shown to induce, which was confirmed with a pathogenesis study of weanling pigs at BIVI. Thus, while we did want to evaluate the ability of a more related virus (MN184) and chimeric viruses between MLV and MN184 to provide protection against PRRSv174 we were unable to make significant conclusions based on our results. While all non-vaccinated pigs challenged with PRRSv174 did have a reduction in weight gain for the first three days after challenge, it was also observed in pigs administered a vaccine virus. Thus, vaccination did not limit the impact on performance associated with infection. However, vaccination with the two chimeric viruses did limit viral load in the lungs on day 10 after challenge. Predictions suggested that the greatest protection would be in pigs vaccinated with MN184-p105 given the similarity in epitope content between this virus and the challenge PRRSv174. Vaccination with MN184-p105 did not limit virus replication in the lungs after challenge and this could be due to the poor response to MN184-p105 vaccination, as evidenced by delayed seroconversion relative to other vaccine groups. Lung lesion scores provided little assistance in evaluating protection because for most comparisons scores were not signficiantly different than non-challenged or non-vaccinated/challenge groups. For future work it is worth noting that the mechanism by which PRRSv-specific T cells provide protection during viral infection is through killing of virally infected cells. Thus, it is possible that when T cells play a significant role in protection lung lesions may not be reduced but viral titers are decreased.

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