RESEARCH REPORT
SWINE HEALTH

Title: Rational Design of a New Generation of PRRSV Differential (Marker) Vaccines – **NPB # 05-159**

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Date Submitted: July 13, 2007

Abstract

This project is based on two main premises: 1) the conviction that the use of vaccines will always be a cost-efficient method and the preferred approach to control PRRSV infections, and 2) the notion that the best type of immunogen against PRRSV has been shown to be, so far, a live, replicating vaccine. Live vaccines present the antigens to the pig's immune system in a similar way as wild-type PRRSV. Therefore our ultimate goal is to develop a live, replicating vaccine that would be safe, efficacious and compatible with eradication. Our overall objectives are addressed to find answers to the following specific questions**: 1. What is the molecular basis of attenuation of virulence in PRRSV? 2. Can we molecularly attenuate PRRSV and obtain a replicating vaccine of unprecedented efficacy and safety? 3. Can we engineer this product to be a "marker" vaccine so that we can integrate robust vaccination together with efficient cleansing of PRRSV infection?** To achieve these goals, we are successfully applying the technology of reverse genetics [infectious clone, IC] developed in our laboratories to identify the genes responsible for virulence in PRRSV and precisely engineer an attenuated vaccine candidate. In this particular year of the project we have been able to show that two of the major PRRSV structural genes that are major determinants of virulence are ORF 5 (coding for GP5) and ORF2 (coding for GP2) . Likewise we have conducted a primary identification of small segments of PRRSV proteins (epitopes) that can be deleted from the vaccine so that could then be used as serologic diagnostic markers of infection (DIVA). These studies should continue with: 1) the identification of a major cluster of virulence detected in non-structural proteins that will permit to obtain full molecular attenuation of PRRSV 2) identification of the single aminoacids that determine the virulence and that should be targeted for mutations and 3) the establishment of a marker deletion and test for DIVA differentiation.

Introduction

The significance of a new generation of PRRSV differential vaccines is well exemplified by the prominent rank always given by industry to this topic. Since the launching of the PRRSV Initiative in 2003, the topic of new PRRS vaccines has been high in the list of research priorities for this NPB PRRSV research grant program. Furthermore, a joint review of the PRRSV Initiative components (NPB and CAP-USDA programs) conducted in 2006 has confirmed such priority and defined that, within the general area of "PRRSV vaccines", the four major priorities identified by industry are: **1. To develop (a) vaccine(s) that produce(s) complete heterologous protection. 2 Evaluate candidate vaccines using the sow model. 3 (..Determine…) what are the alternative strategies for vaccine development, and 4. (..Determine..) What are the genetic determinants for virulence attenuation.** More recently, on June 1st, 2007, a meeting (Title: **Colloquium on Prospects for Development of an Effective PRRS Virus Vaccine**) was held at the University of Illinois College of Veterinary Medicine to discuss the state of current knowledge about PRRS vaccination. The meeting was attended by 26 invited experts in PRRS, virology, immunology and vaccinology and included clinical veterinarians, academics and vaccine industry scientists. The main conclusions of this comprehensive meeting on PRRSV vaccines were:

- PRRS vaccines are effective against homologous challenge.
- Current vaccines are not adequate for producer needs.
- Important research questions that need to be addressed to improve PRRSV vaccines include: 1) to define all of the PRRSV components which have a role on induction of protective immunity 2) to pursue the mapping of T and B cell epitopes significant for the induction of protective immunity 3) fully understand the mechanisms of both homologous and broad (syn: heterologous) protection 4) identify the genetic determinants /phenotypes of virulence/attenuation, host range and immune evasion and how they relate to vaccine.
- Improved PRRSV vaccines should be available in 5-10 years.

In summary, vaccine improvement is a reachable goal within the technical capabilities currently available. In full agreement with the goals defined by the experts' symposium, this project represents efforts directed to the betterment of current modified live vaccines by generation of live PRRSV vaccines that have been attenuated in a molecular way, with increased immunogenicity obtained by molecular modification, and counting with the addition of molecular markers for DIVA differentiation through a simple serologic test.

Objectives

No.	Objective
1	**To construct molecularly defined PRRSV attenuated mutants and characterize them biologically**
2	**To define non-essential regions of the PRRSV genome that could be used as serologic indicators of infection**
3	**To test immunogenic efficacy of candidate vaccine in vivo (pregnant sows and pigs)**

Objectives involve two years of work. Funding was requested for one year only.

Materials and Methods:

Construction of full-length chimeric cDNA clones

We used chimeric constructs made between two (one virulent and another attenuated) strains of PRRSV to map the virulence markers of PRRSV. Full-length FL12 infectious clone (wt) and PP18 infectious clone (Primepac, PP, attenuated strain infectious clone) were utilized as backbones to construct chimeric clones. First, to make chimeric clones covering multiple genes derived from the PP vaccine virus strain, a series of PCR products, which contain naturally present or deliberately introduced restriction endonuclease (RE) sites, RsrII, SpeI, MluI, PmeI, SgfI, EcoRV, BssHII, BstBI and PacI, were used to replace the corresponding regions of FL12 clone as described in more detail in our paper (Kwon et al. 2006) . In addition, the construction of single gene chimeric clones comprising individual structural protein encoding genes from either the PP vaccine virus or vFL12, additional RE sites, BsrGI, BstEII and NruI were incorporated to facilitate the fragment exchange in a similar manner. The target genes were either directly cloned into full-length FL12 or into an intermediate plasmid encompassing the majority of ORF2, complete ORFs 3 to 7, and the entire 3' UTR derived from the FL12 as described In our paper (Ansari et al. 2006). Likewise the reciprocal chimeric clones containing either ORF2 or ORF5, and ORFs 2 and 5 of FL12 were prepared in the genetic background of PP18 clone (Fig. 2) by using the same (BssHII and BstBI) and alternative (SacII) RE sites. All generated chimeric cDNA clones were confirmed by sequence analyses of the corresponding fragments.

Rescue and analysis of chimeric viruses

In vitro transcription, RNA electroporation, assessment of virus rescue by immunofluorescence assay, and characterization of viral growth properties and kinetics in MARC 145 cells is fully described in our published papers (De Lima et al., 2006, Ansari et al. 2006, Kwon et al., 2006)

Animal experiments

Pregnant sows were purchased from a specific-pathogen-free herd with a certified record of absence of PRRSV infection. Their negative PRRSV infection status was confirmed upon arrival by a commercial ELISA serology test (IDEXX Labs, Portland, ME). Pregnant sows were inoculated at 90 days of gestation and the viability scores of off-springs at birth and weaning (at 15 days of age) were evaluated. All animals were tested for anti-PRRSV antibodies by ELISA (IDEXX Labs, Portland, ME). Two sows per chimera in each separate experiment were infected with parental and chimeric viruses. For gain-of-function experiments, six sows per chimera were tested (table 2) In all the cases, a total of 2 ml ($10^{5.2}$ $TCID_{50}$) of virus was administered intra-nasally with 1ml into each nostril. The rectal temperature and clinical signs of the inoculated animals were monitored daily from 3 days pre-inoculation through farrowing and to weaning. Viremia was measured by regular titration on MARC-145 cells and by nested RT-PCR , using the sera collected between 0-14 PI days PI. In some cases, copy numbers of viral RNA from the sera of sows were measured using a PRRSV real-time quantitative PCR kit (Tetracore, USA). Weaned piglets (~3 weeks old) were also purchased from a specific-pathogen-free herd with a certified record of absence of PRRSV infection. Six piglets per group were inoculated intramuscularly with total of 2 ml of virus ($10^{5.2}$ $TCID_{50}$)to confirm the effect of individual ORF2 and ORF5 on regaining/enhancing virulence of PP18 backbone, by comparison of viremia profiles. Blood samples were collected at days 0 to 10 PI, and the serum samples were tested for viremia by infectivity titration, as previously mentioned for the sow experiment.

Results:

Objective 1:

Using the FL12 full-length infectious clone, which contributes the genomic backbone of a highly virulent PRRSV, a series of full-length chimeric cDNA clones that would scan the entire genome of PP vaccine strain were generated. These clones, shown on figure 1 , comprised: 1) 5' UTR and NSP1 and part of NSP2 (cP5U.NSP1.2), 2) part of NSP2 and part of NSP3 (cPNSP2.3), 3) part of NSP3 to NSP8 (cPNSP3.8), 4) NSP9 (cPNSP9), 5) NSP10 to NSP12 (cPNSP10.12), 6) ORF3 to 7 and 3'UTR (cPORF3.3U) genes, and 7) the entire region spanning all the structural genes including 3'UTR (cPORF2.3U) of the PP vaccine virus genome (Fig. 1). Likewise, additional chimeric viruses containing individual structural genes of PP vaccine within the context of the FL12 genomic backbone were also constructed. In theses cases a full-length infectious clone (PP18) derived from PP vaccine virus described previously (31) was used to provide genomic components of PP vaccine virus

to the FL12 backbone in order to generate chimeric clones containing individual structural genes of PP vaccine virus as shown in figure 2. This approach generated a series of chimeric strains, which represent respectively: ORF2 (cPORF2), ORF3 (cPORF3), ORF4 (cPORF4), ORF5 (cPORF5), ORF6 (cPORF6), and ORF7 gene and 3' UTR (cPORF7.3U) of PP vaccine virus genome (Fig. 2). Finally, the PP18 clone was also used as a genetic background or backbone to generate, in this case, reciprocal chimeric viruses containing ORF2 and ORF5 of FL12 to confirm gain-of-function phenotype (restoration or enhancement of virulence) by the genes ORF2 and ORF5. For this, we generated three chimeric clones in which the following genes of FL12 replaced their PP18 counterpart: ORF2 (cFORF2) or ORF5 (cFORF5) alone, and ORFs 2 and 5 (cFORF25) simultaneously (Fig 2).

All the chimeric constructs described above and shown in figures 1 and 2 resulted in viable viruses. The growth kinetics of the chimeras were compared with the parental viruses: vFL12, PP vaccine strain and/or vPP18, using MARC-145 cells as previously described . The single- step growth curves show that the range of the growth kinetics of

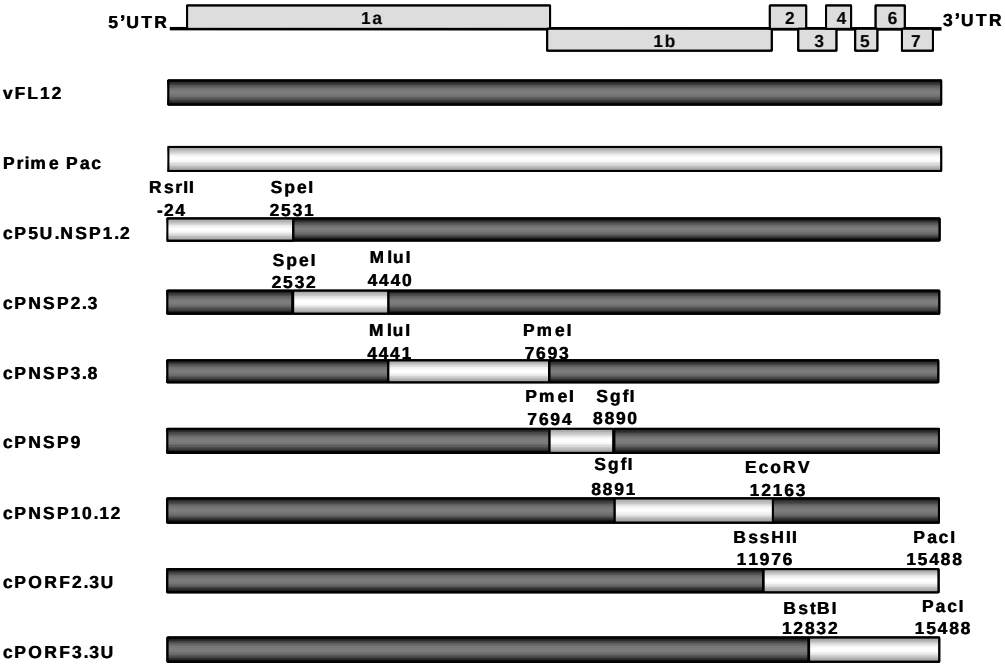

FIGURE 1. Construction of chimeric PRRS viruses for primary genome-wide scanning.

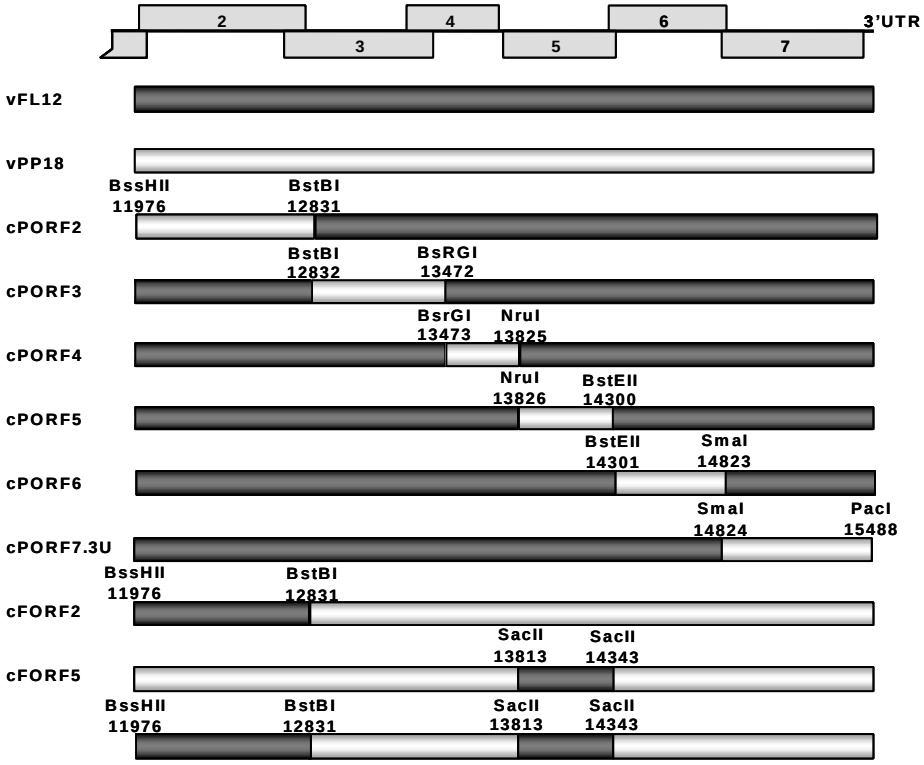

FIGURE 2. Construction of single gene or reciprocal chimeric PRRS viruses within structural regions.

the chimeras was quite diverse and that in most cases showed impaired growth characteristics, especially in the case of cPNSP9, cPNSP10.12 and cPORF4,

Measurement of virulence of the mutants by inoculation in pregnant sows:

(i) Primary genome-wide scanning of PP vaccine strain indicates that major attenuation of virulence may be based in certain non-structural (NSP3-8) and structural (ORF2-3'UTR) regions

Upon inoculation of sows at 90th day of gestation, the number of viable offspring at birth and at weaning at 15 days of age was evaluated. Other parameters, including daily clinical signs and rectal temperatures, total antibody response and viremia in the sows were also evaluated. As shown in table 1, the in vivo phenotypes of chimeric viruses were more unambiguous than what could be inferred from the in vitro growth properties. As typical for the swine reproductive failure model when using the FL 12 infectious clone or its parental strain, no specific clinical signs other than lack of appetite, lethargy and slight fever shortly after infection and prior to reproductive failure, which typically takes place at a later time PI, shortly before or at normal farrowing time were noticed in any group of sows inoculated with all of the different strains, throughout the experimental period. The vFL12-inoculated sows confirmed a fully pathogenic phenotype for our infectious clone, with 100 % mortality of the piglets and the PP vaccine virus inoculated-sows showed normal range of survival rate with 77% (Table 1). The sows inoculated with the chimeric viruses cPNSP3.8, cPORF2.3U and cPORF3.3U exhibited significant piglet survival rates: 75%, 69% and 71%, (97%, 90% and 92% when taking as 100 % base the PP performance) respectively. These findings strongly suggest that these genomic regions contribute the most to PRRSV virulence. During the primary screening, only NSP9 gene (cPNSP9) could be ruled out as an important virulence determinant (Table 1). It should be noted, thought, that most of NSP-encoding regions also seemed to play a relative role in virulence, as exhibited by cP5U.NSP1.2 (51%), cPNSP2.3 (56%) and cPNSP9.12 (57%) (66% - 74% when taking as 100 % base the PP performance) chimeric viruses (table 1).

(ii) PRRSV structural genes ORF5 and ORF2 contribute primarily to PRRSV virulence:

As a result of this initial genome-wide scanning for PRRSV virulence markers, we concluded that, besides NSP3-8, the structural protein-coding area was the other main genomic region where possible major virulence determinants seem to be located. Due to our availability of an intermediate plasmid encompassing the majority of structural ORFs and the entire 3' UTR derived from the FL12 , we decided to focus first on ascertaining which individual structural gene(s) contribute(s) most significantly to virulence, by analyzing the virulence phenotypes of the single structural genes chimeras. As shown in Table 1, ORF5 (cPORF5) (reaching 45% survival) gene primarily contributes to virulence and ORF2 (cPORF2) (reaching 25% survival) also play a role in the attenuation of highly virulent vFL12 strain. However, ORFs 3, 4, 6, 7 and 3'UTR did not contribute significantly to virulence , with all of them reaching negligible survival rates of just single-digit % (Table 1).

(iii) Reciprocal experiments (in vivo) to confirm the role of single structural genes ORF5 and ORF2 in PRRSV virulence

Animal experiments with the reciprocal chimeric viruses were performed to validate the role of ORF5 and ORF2 genes in virulence. However, this gain-of-function experiment was not as dramatic as described in the context of FL12 in terms of viability of off-springs. Compared to the vPP18 (87%) the reciprocal chimeric viruses showed less

Table 1. Viability scores of offspring born from sows infected with parental or chimeric viruses

Viruses	Genetic background	Sow No[a]	Viability at			
			Birth	15 days of age		
			Dead	Live	Live	Survival[b] (%)
vFL12	FL12	1	12	3	0	0
		2	13	1	0	
Prime Pac	Prime Pac	1	0	11	9	77(100)
		2	0	14	10	
cP5U.NSP1.2	FL12	1	1	12	9	51(66)
		2	6	6	4	
cPNSP2.3	FL12	1	4	12	9	56(73)
		2	1	15	9	
cPNSP3.8	FL12	1	0	15	12	75(97)
		2	3	10	9	
cPNSP9	FL12	1	13	3	0	3(4)
		2	9	11	1	
cPNSP9.12	FL12	1	5	11	10	57(74)
		2	5	5	5	
cPORF2.3U	FL12	1	0	15	9	69(90)
		2	2	7	7	
cPORF3.3U	FL12	1	0	10	10	71(92)
		2	3	9	5	
cPORF2	FL12	1	9	6	4	25(32)
		2	7	2	2	
cPORF3	FL12	1	15	0	0	7(9)
		2	9	5	2	
cPORF4	FL12	1	9	1	0	4(5)
		2	12	3	1	
cPORF5	FL12	1	7	7	4	45(58)
		2	3	7	6	
cPORF6	FL12	1	8	4	0	7(9)
		2	11	4	2	
cPORF7.3U	FL12	1	13	4	1	7(9)
		2	13	2	1	

[a]Two sows each per group. Arbitrary numbers. [b]Least squares means of each group. The values in parenthesis represent the transformed values by the survival rates of Prime Pac (77%) as 100%.

Table 2. Viability scores of offsprings born from sows infected with parental vPP18 and reciprocal chimeric viruses[a]

Viruses	Genetic background	Sow No[b]	Viability at			
			Birth	15 days of age		
			Dead	Live	Live	Survival[c] (%)
vPP18	PP18	1	0	16	16	87 (100)
		2	3	10	10	
		3	1	9	9	
		4	2	13	13	
		5	2	7	6	
		6	0	9	9	
cFORF2	PP18	1	0	12	10	74(85)
		2	0	10	10	
		3	1	5	4	
		4	0	2	2	
		5	3	7	7	
		6	2	10	3	
cFORF5	PP18	1	2	7	7	62(71)
		2	2	10	9	
		3	3	12	10	
		5	14	4	4	
		6	0	12	9	
cFORF25	PP18	1	1	16	14	86(99)
		2	1	9	9	

[a]One (cFORF25) or 3 (cFORF2 and cFORF5) and 4 (vPP18) experiment(s) were combined. Two (vPP18) and 1 (cFORF5) sow(s) each were not pregnant, and were not included.

[b]Two sows each per group per each experiment. Arbitrary numbers.

[c]Least squares means of each group. The values in parenthesis represent the transformed values by the survival rates of vPP18 (87%) as 100%.

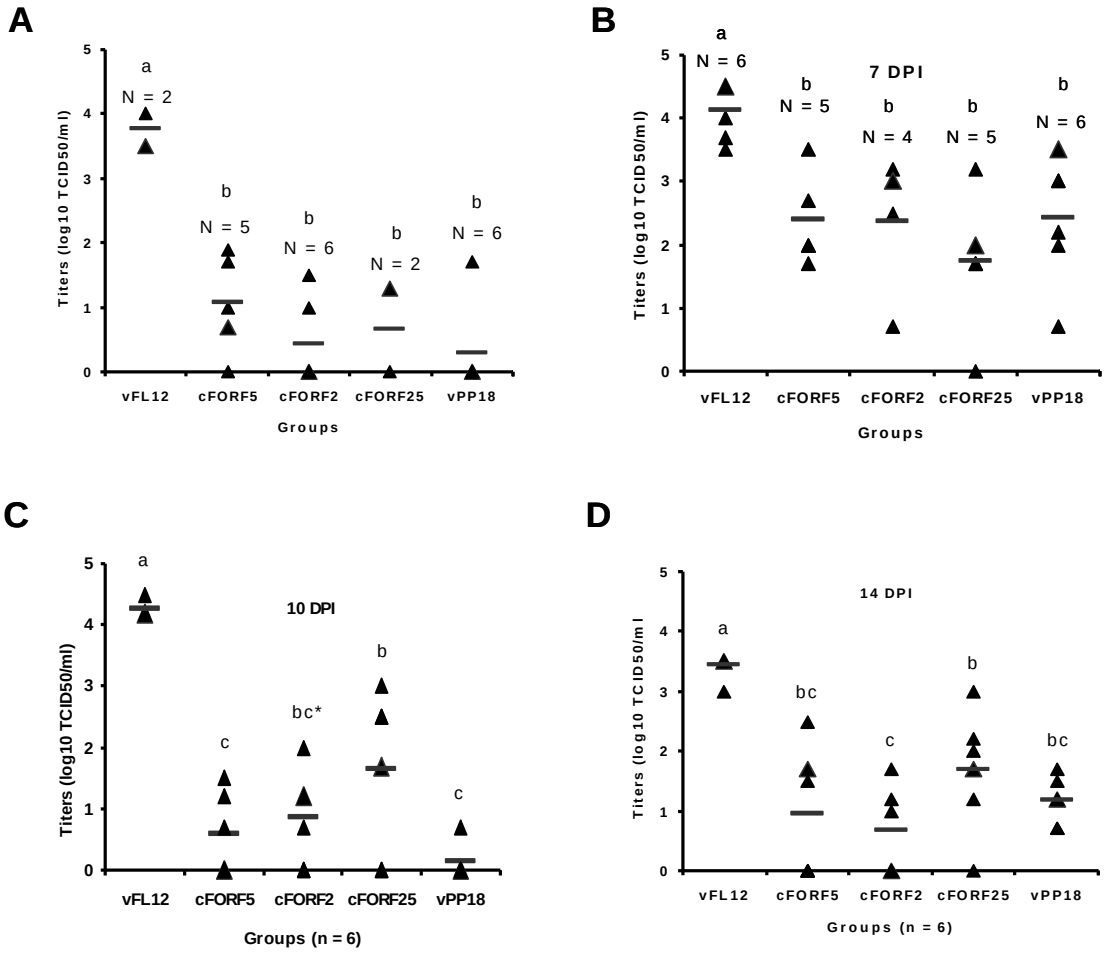

FIGURE 3. Viral concentrations in serum of sows and piglets infected with parental and reciprocal chimeric viruses. Results shown are mean values determined using the sera of sows (A) and young pigs (B-D) infected with the reciprocal chimeric viruses together with vPP18 and vFL12. Viral titers were measured with the sera collected at 7, 14 and 21 days PI for sows and at 7, 10 and 14 days PI for pigs. Viral infectivity (A-D) is expressed as log10 TCID50/ml of serum titers in MARC-145 cells. The results at 14 and 21 days PI in sows were not included since no viruses were detectable with a detection limit of 0.7. The horizontal bar is the mean of each group. Means with the same letter are not significantly different ($P \leq 0.05$). The cFORF2 (asterisk) in Fig. 3-C was different ($P \leq 0.1$) than cFORF25 group less significantly.

significant change in case of cFORF5 (62%) and cFORF2 (74%) or no change in case of cFORF25 (86%) in offspring viability at 15 days of age (Table 2). In spite of these apparently contrasting results, it should be noted, that, often times,

gain-of-function experiments based on direct rescue of full virulence by the mere reconstitution of a single gene is difficult to occur or assess due mostly to the fact that full virulence is generally multi-genic, with PRRSV not being the exception to this notion, as appreciated in our model through the results of our first genome-wide scanning for virulence (NSP3-8, plus structural contributions). In order to evidence gain-of-function by traits other than full fledged offspring mortality, we compared the kinetics of infection of the different mutants in their ability to establish viremia levels. Viremia levels have been described to closely correlate with the level of virulence of PRRSV strains (Johnson et al. , Vet Immunology Immununopat 102:233-47,2004). To this end we compared viremia levels established by the parental viruses and the reciprocal chimeric viruses in the inoculated sows and also in young weaned pigs inoculated with each of the strains (figure 3). The viral titers in MARC-145 cells were distinct between vFL12-infected group and other chimeric viruses-inoculated groups including vPP18. The vFL12-infected group showed higher viremia up to 14 days PI compared to others and cPORF5-infected group showed least viremia all through the infection period (Fig. 3 A-D) . Importantly we found that ORFs 2 and 5 genes showed a synergistic effect as evidenced by the fact that the viral load of dual chimeric cFORF25 virus-infected pigs was higher than cFORFs 2 and 5 alone or vPP18, especially at 10 days PI at which time this chimera showed a statistically significant difference with its pp 18 parental strain (figure 3C), Likewise, the single reciprocal chimeric mutants ORF2 and ORF5 show a trend to mimic the viremic phenotype of wt PRRSV FL12, although not at statistically significant level. We conclude that the analysis of viremic load showed these reciprocal chimeras mimicking the phenotype of the virulent donor strain FL12 instead of PP18, suggesting that regain of virulent function takes place in these chimeric strains, although not evidenced by the full recovery of ability to produce offspring mortality typical of the multi-genic virulence of the wt PRRSV.

Objective 2

As proposed in the Objective 2, we pursued, during this project NPB project # 05-159, serologic marker candidates that could be used in our future live vaccine for DIVA differentiatiion . We completed the mapping of B-cell linear epitopes detected by Pepscan in the Nsp2 and all of the structural proteins of FL12 IC strain, using sera of 15 pigs experimentally infected with this strain (de Lima et al., 2006). The Nsp2 was found to contain the highest frequency of immunodominant epitopes (n=18) when compared to structural proteins (figure 1) .Ten of these 18 Nsp2 peptides were reactive with 80 to 100% of the examined sera. In the structural proteins, epitopes consistently recognized by immune sera were located at ORF2 (n=2), ORF3 (n=4), ORF5 (n=3), ORF6 (n=2) and ORF7 (n=2).(figure 4 and 5)

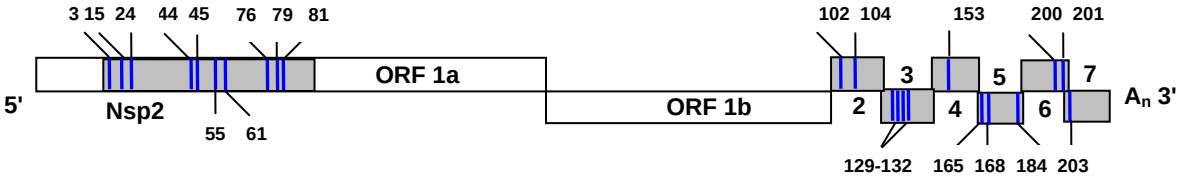

Fig. 4. Position of the immunodominant B-cell linear epitopes identified on the Nsp2 and structural proteins of PRRSV. The locations with the respective ORFs and identity number of the major synthetic peptides identified as B-cell epitopes in each protein are indicated.

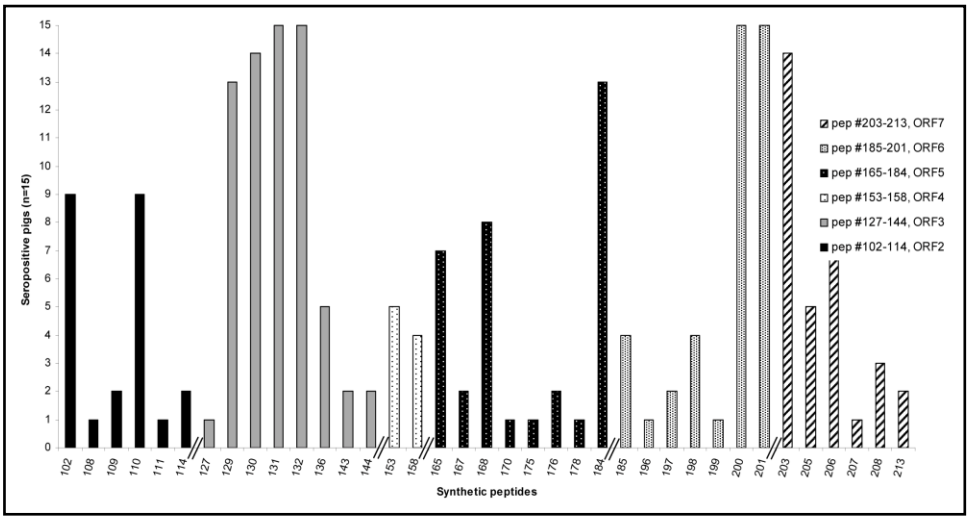

Fig. 5 B-cell linear epitopes identified along the structural proteins (ORFs 2-7) of FL12 by Pepscan analysis. The numbers of the corresponding peptides in each ORF are indicated. Sera were considered positive when the OD values were above the cutoff point (the mean OD of absorbance at 405nm of the negative sera plus 3 standard deviations).

The two epitopes identified in the C-terminal end of the M protein (ORF6) (peptide 202 and 201, figure 5) exhibited a unique combination of immunogenicity and sequence conservation among isolates from both PRRSV genotypes. Further studies revealed reactivity of field and reference antisera raised to other US-type of PRRSV with both M protein epitopes (n= @ 100 including diagnostic samples from diverse regons in Nebraska, Iowa and South Dakota) . In addition, one of those two epitopes was also recognized by sera against an EU-type strain. Seroconversion kinetics demonstrated that the antibodies recognizing the immunodominant epitopes appeared regularly between days 7 and 15 pi, remaining detectable until at least day 90 pi.

Objective 3:

The objective 3, as originally proposed in the project NPB project # 05-159, is not achievable yet. In order to consider a construct to be a rationally attenuated vaccine candidate we need to essentially find out more information about full attenuation of the PRRSV. We anticipate that elucidation of the virulence markers contained in the non-structural genes (i.e. NSP3-8, as identified in objective 1) will shed light for a more rational and complete attenuation of PRRSV for development of vaccines .

Discussion:

Although several previous studies suggested that several genes seem to be responsible for the attenuation of virulence of vaccine strains, these previous approaches provided only limited information because the analyses simply relied on sequence comparisons between wild type and vaccine virus or revertant viruses for the most cases. Furthermore the mutations which may support those speculations are located throughout the genome.

In this research project, by systemically exchanging genomic regions and generating a series of chimeric viruses containing the whole genome of PRRSV, specific regions associated with virulence were confirmed. Recently a similar approach was reasonably successful in mapping of virulence genes in several viruses (swine vesicular disease virus, foot- and mouth disease virus,, classical swine fever virus, porcine circovirus type 2 and type 1, simian immunodeficiency virus and vaccinia virus).

Based primarily on the sow reproductive failure model, we conclude that the ORF5 structural gene plays a major role in determining virulence of PRRSV. Likewise, the ORF2 gene would also have a measurable but lesser role in virulence. The ORF5 of the viral genome encodes the major viral envelope glycoprotein 5 (GP5), which is a glycosylated trans-membrane protein of approximately 25 kDa . Like EAV and LDV, the PRRSV GP5 and matrix (M) protein interact and form heterodimers, which may play a critical role in virus assembly but whose mode of interaction has not been

demonstrated yet. The GP5 is believed to be involved in the entry of virus into the host cells, presumably by interacting with the host cell receptor, especially for macrophages. The GP5 is considered to be important in the infection process because the presence of a major neutralization epitope in the N-terminal ectodomain may be involved in receptor recognition. Moreover, our results provide evidence that glycosylation of GP5 of PRRSV plays an important role for immune evasion and persistence to escape or minimize virus-neutralizing antibody response by the glycan-shielding mechanism. This glycan shielding mechanism is also important for LDV, HBV, SIV, Influenza, and HIV . The ORF2 of the PRRSV genome encode 2 different envelope proteins, one minor glycosylated glycoprotein 2a (GP2a) of 29~30kDa and the other non-glycosylated small membrane protein, 2b of 10kDa. Compared to GP2a, 2b is predominantly expressed in infected cells. In EAV, GP2b (GP2a of PRRSV) interacts with other glycoproteins GP3 and GP4 by forming intra- and inter-molecular disulfide bonds and the correct association is important for their efficient incorporation into viral particles and for virus infectivity and the protein E (2b of PRRSV) is required for the production of infectious virions. However the protein 2b of PRRSV has been shown not to interact with other proteins and the cysteine residues are not essential for virus replication .

Future work will continue to uncover the precise role of single residue(s) for virulence within ORFs 2 and 5 genes by site-directed mutagenesis followed by in vitro and in vivo characterization. In addition, more virulence determinants will be identified within NSP3 to 8 regions, which appears to represent a very strong cluster of virulence genes.

Pursuit of serologic markers to be deleted from future live vaccines:

We have been able to identify immuno-dominant epitopes that vary in the range of conservation among US PRRSV strains. Based on their prominence, we have pre-selected the most imunodominant epitopes in the structural region (two in ORF 6 and four in ORF3, figure 2) and with these we should start studies assessing their dispensability from the genome without altering the biological properties of the mutant. To study such potential function we should introduce point mutations, amino acid switches, or deletions of those B-cell epitopes on the genome of FL12 IC using site directed mutagenesis. If the mutants are viable, the rescued viruses will be sequenced to further confirm the presence of the mutations and unaltered status of the rest of the genome. If we are unable to obtain viable mutants deprived of one of these single epitopes in their structural proteins, we will proceed with NPS2, which, while rich in immunodominant epitopes, has precedents of dispensability as this NSP2 may carry naturally occurring deletions in wt PRRSV strains. In the latter case it will be extremely important to select one of the NSP2 epitopes that is well conserved, as judged by serology of field samples using appropriate Peptide ELISA. The goal is to ensure that we do not select the marker in one of the NSp2 areas that frequently presents deletions. These B-cell epitope-mutated viruses (either in structural gene or NSP2 regions) should be used to design future vaccine candidates whose compatibility with differential DIVA tests should be tested in field populations.

Support from NPB # 05-159 has been acknowledged in the following refereed publications:

De Lima M, Pattnaik A, Flores EF, and Osorio FA 2006. Serologic marker candidates identified amongst B-cell linear epitopes of Nsp2 and structural proteins of a North American strain of Porcine Reproductive and Respiratory Syndrome virus Virology 353: 410-421

Kwon, B. J., Ansari, I. H., Osorio, F. A., and Pattnaik, A. K. 2006.Infectious clone-derived viruses from virulent and vaccine strains of porcine reproductive and respiratory syndrome virus mimic biological properties of their parental viruses in a pregnant sow model. Vaccine. 2006 Nov 30;24(49-50):7071-80.

Ansari I H, Kwon B J , Osorio FA , and Pattnaik A K 2006. Influence of N-Linked Glycosylation of Porcine Reproductive and Respiratory Syndrome Virus GP5 on Virus Infectivity, Antigenicity, and Ability to Induce Neutralizing Antibodies, J Virol. 2006 Apr;80(8):3994-4004.

Lay Interpretation:

This project is based on two main premises: 1) the conviction that the use of vaccines will always be a cost-efficient method and the preferred approach to control PRRSV infections, and 2) the notion that the best type of vaccine against PRRSV has proved to be the live, attenuated vaccines. In all likelihood, the live vaccines are most effective because their components that are determinants of protection (a.k.a as antigens or immunogenic epitopes) are "seen" by the pig in a similar way as the animal "sees" those of live wild-type (fully infectious) PRRSV. Our ultimate goal is to develop a live vaccine of safety and efficacy that would be compatible with the ability of cleansing the PRRSV infection, that is, compatible with the ability of differentiating, through a simple test, the vaccinated/protected animals from those that have suffered infection by wild-type PRRSV. Our technical approach to the improvement of PRRS live vaccines is based on the notion that mapping the genes causing virulence in PRRSV should provide information for the development of a differential PRRSV vaccine of unprecedented safety and efficacy. The capacity of PRRSV to cause serious pathologic changes is called virulence. In our system we measure viral virulence in relation to the virus' ability of producing abortion in pregnant sows. This virulence is caused by the different genes and its proteins composing the PRRSV. The main expected outcome of this research is the alteration of the genes of the PRRS viruses to develop live attenuated/marker vaccine strains. Engineering of new live-attenuated PRRSV marker vaccines requires knowledge of the genetic make-up of PRRS virulence and identifying small areas of the proteins which can be eliminated from the vaccine without affecting the virus' ability to multiply in the pig. This concept is the same principle applied in the case of Pseudorabies marker vaccines. The differential vaccines, which, like in the example of Pseudorabies, were originally called "marker vaccines" are now also identified as DIVA vaccines (which stands for "Differentiating Infected from Vaccinated Animals"). This year we report the identification of two major genes (ORF5 and ORF2) that are base of PRRSV virulence. Likewise we have discovered a large series of small segments of PRRSV proteins (epitopes) that could be deleted from the live vaccine so that could then be used as serologic diagnostic markers of infection (DIVA markers).