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# Compilation of experimental investigations of PRRS vaccine technologies using modified live vaccines, inactivated vaccines and immunomodulation

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**P**orcine Reproductive and Respiratory Syndrome Virus (PRRSv) continues to be a major pathogen that impacts the swine industry. Despite the recent emergence of Porcine Circovirus type 2, PRRS still remains the most significant economic swine disease globally.

Numerous vaccine and control tools have been introduced to the marketplace including licensed modified live PRRS vaccines (MLV), licensed inactivated PRRS vaccines (KV), autogenous KV vaccines, and use of virulent live virus exposure (LVI). Based on published data and controlled experimental studies, the licensed MLV clearly provide the current gold standard of protection in respiratory and reproductive models. Despite this, the protection is not “sterilizing” and further research is warranted to more fully investigate other potential tools for PRRS control.

This presentation will provide a summary of numerous controlled experimental studies investigating alternate PRRS vaccine prototypes. This will include internal efforts Boehringer Ingelheim Vetmedica, Inc.(BIVI), external published work, and collaborative efforts between BIVI and university or corporate partners. The efforts discussed will include:

1. Killed/Inactivated PRRS vaccines
2. Autogenous PRRS vaccines
3. PRRS “antigen extract” vaccines
4. Immunostimulation with adjuvants to enhance MLV immunity
5. DNA vaccines

## Autogenous vaccines

### Study 1

**Comparison of commercial vaccines in their ability to induce protection against current PRRSV strains of high virulence**

Osorio, F.A.; Zuckermann, F.; Wills R.; Meier, W.,  
Christian, S.; Galeota J.; Doster, A. – Leman 98

This study compared two commercially available MLV and an autogenous KV vaccine in a reproductive model. The MLV vaccines were each given once in the second

stage of gestation and the autogenous KV vaccine was administered three times prior to virulent challenge. The study investigated serology, cell-mediated immunity (CMI), clinical assessment, and virology in the reproductive model. The clinical results indicated that both MLV vaccines provided a significant level of protection and the autogenous KV vaccine (homologous to challenge virus and given as three doses) was not significantly different from non-vaccinated challenge controls. *“There is no statistical difference between the effect of infection (mean litter viability) on the control group that did not get vaccinated and the group vaccinated with the autogenous killed product.”* (Table 1)

**Table 1:** Results: Litter viability. Analysis of variance (Proc GLM, SAS )

Group	Number of litters	Mean litter viability* (%)	
		At birth	At weaning
NVC**	5	24.49 <sup>a</sup>	8.00 <sup>a</sup>
Autogen	3	20.49 <sup>a</sup>	8.00 <sup>a</sup>
MLV B	3	67.68 <sup>a,b</sup>	53.54 <sup>a,b</sup>
MLV C	3	61.11 <sup>a,b</sup>	61.11 <sup>a,b</sup>
NVNC***	3	90.48 <sup>b</sup>	90.48 <sup>b</sup>

\* **Litter viability** is defined as number of pigs alive divided by the total number of pigs born.

\*\* NVC = non-vaccinated, challenged controls

\*\*\* NVNC = non-vaccinated, non-challenged controls

<sup>a,b</sup> Numbers within a column with the same letter are not significantly different ( $P = 0.05$ ).

**Study 2**

**Efficacy of a killed and modified live PRRS virus vaccine when used alone and in combination in growing pigs.**

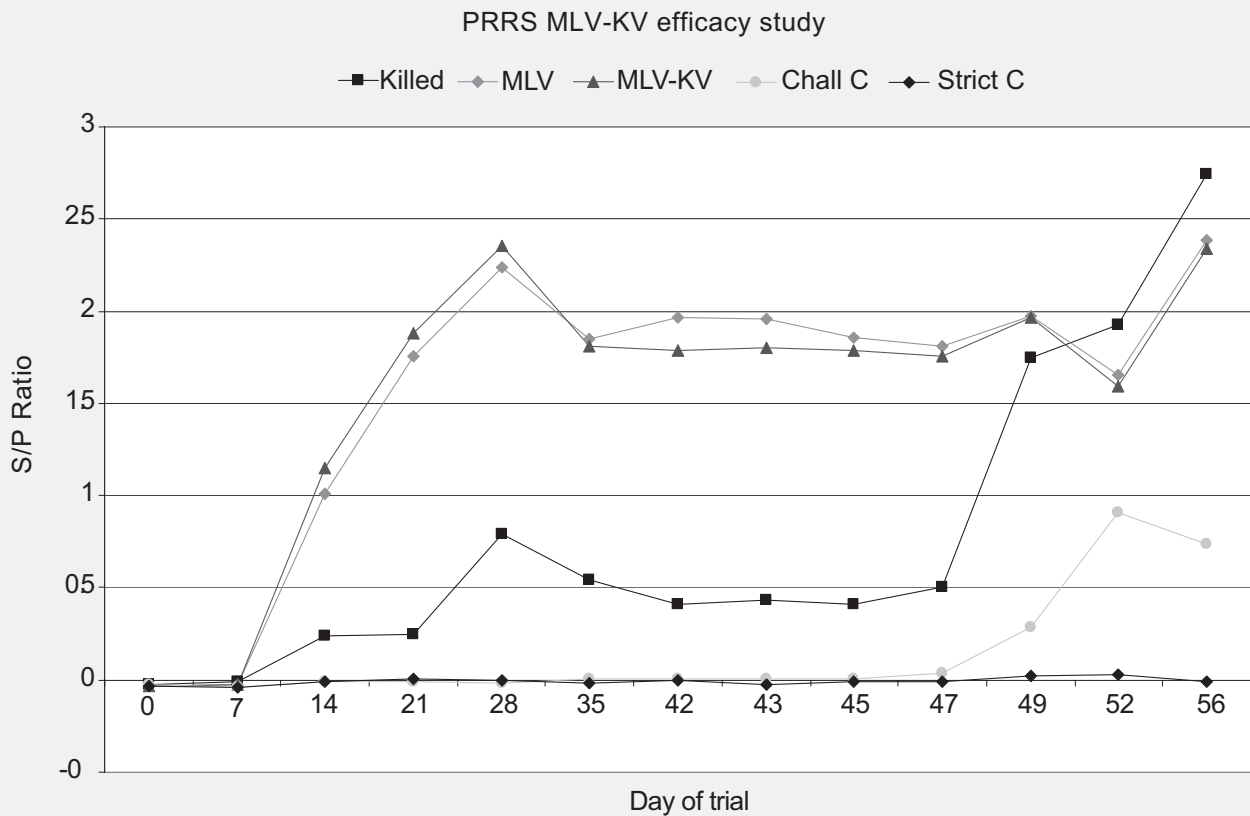
Pat Halbur, DVM, PhD; Kelly Burkhart; Eric Vaughn, PhD; and Mike Roof, PhD – Leman 1999

This study investigated the potential value of a MLV-KV vaccine combination in the control of PRRS using a respiratory challenge model. The MLV was administered a single time to pigs at 3 weeks of age. The KV vaccine was an autogenous vaccine homologous to the virulent challenge (SDSU 73) and was administered at 3 and 6 weeks of age in the KV-only group and at 6 weeks as a booster to MLV

group. Challenge occurred at 9 weeks of age. Study results included serology, lymph node histopathology, clinical disease assessment, virology and gross lung pathology.

The results of this trial confirmed that in the respiratory model, ANY treatment group that received MLV had clinical protection. The autogenous KV vaccine alone had no statistical or measurable positive impact nor did it improve upon the results achieved with MLV vaccine used alone when used in combination (as a booster vaccination) with MLV. (Figure 1 & Table 2)

**Figure 1**



**Table 2**

Treatment	% Pneumonia stats
KV	26% <sup>a</sup>
MLV	8% <sup>b</sup>
MLV-KV	6% <sup>b</sup>
Chall control	47% <sup>a</sup>
Strict control	1.5% <sup>b</sup>

Like letters are not statistically ( $P < 0.05$ ) different

## Commercial/licensed killed PRRS vaccines

### Study 3

#### Evaluation of a commercial killed PRRS vaccine alone and in combination with Ingelvac® PRRS MLV in a reproductive model.

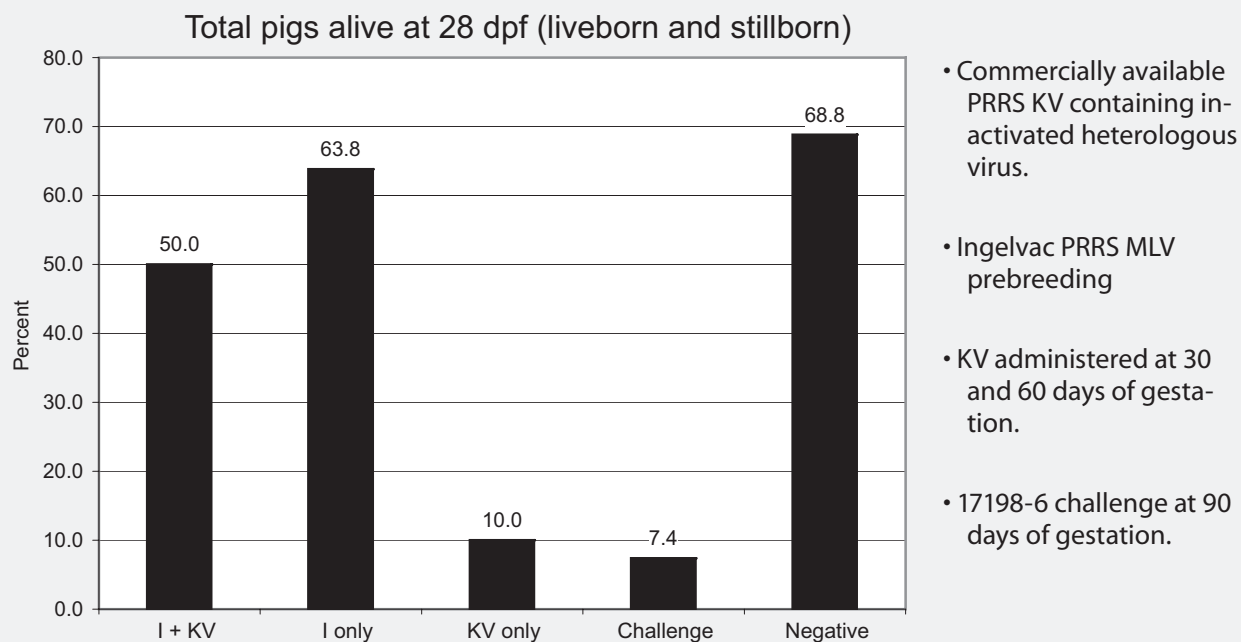
Dr. Eric Vaughn - Boehringer Ingelheim Vetmedica, Inc.

This study evaluated a commercial and licensed killed PRRS vaccine alone or as a booster to MLV vaccine in a reproductive model. The killed vaccine was administered two

times as directed on the label. The MLV was administered pre-breeding per label directions. A heterologous virulent challenge was administered at 90 days of gestation.

The results of this trial indicate that MLV alone was protective in the reproductive model following virulent challenge and that KV alone was not significantly different from challenge controls. Furthermore, the addition of KV as a booster to MLV did not provide any benefit in this controlled study. (Figure 2)

**Figure 2: Commercial PRRS KV evaluations-repro program**



**Study 4**

**Assessment of the efficacy of commercial PRRS vaccines based on measurement of serological response, frequency of gamma-INF producing cells and virological parameters of protection upon challenge.**

*that elicited the establishment of an effective protective immunity against wild-type (wt) PRRS infection was the attenuated modified live vaccine". The "killed vaccine tested did not evoke a measurable level of protective immunity"* (Table 3 & Figure 3)

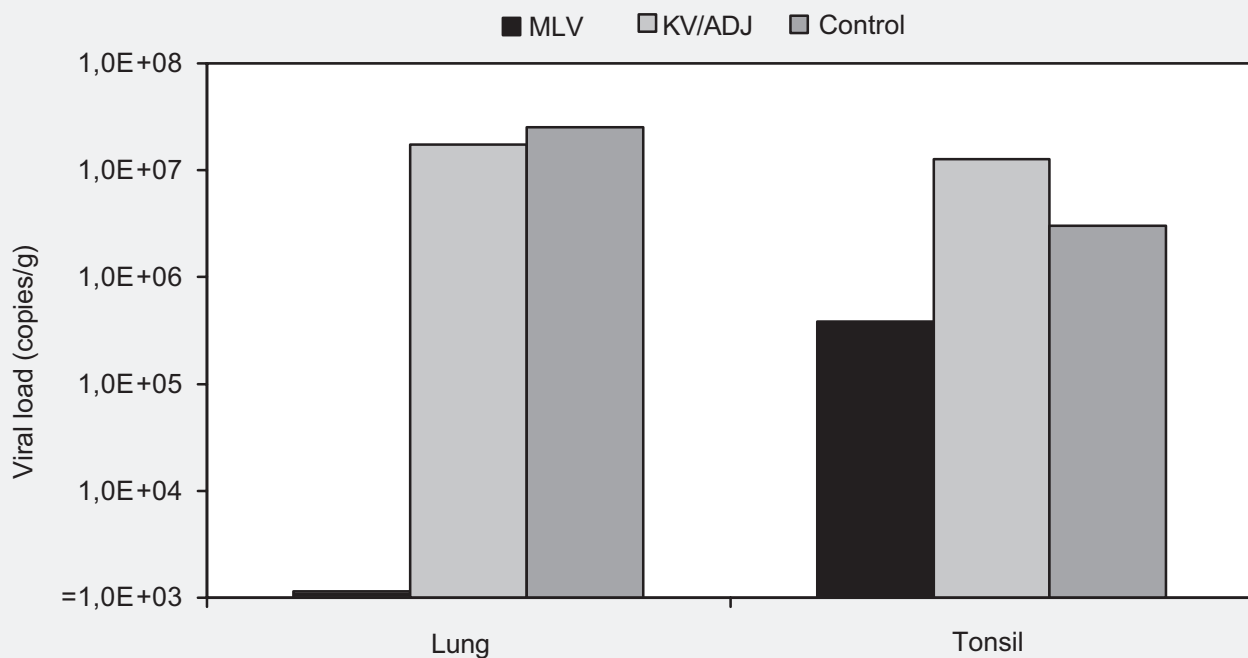
Zuckerman; Garcia; Luque; Christopher-Hennings; Doster; Brito; Osorio. Vet Micro 123 (2007)

This study compared two doses of a MLV to two doses of a commercial KV vaccine (3 weeks apart) in pigs at approximately five months of age. Animals were then challenged with virulent PRRS (Lelystad) one week after the second vaccination. The study evaluated serology, INF-gamma response via ELISPOT and viral load. The *"results clearly indicate that the only type of vaccine*

**Table 3**

Group	No.	Vaccine applied	Blood sample with anticoagulant	Serum sample	Challenge	Weighting of the animals	Temperature records	Necropsy
1	10	MLV 0,21	0,21,28,35, 42	0,21,28, 32,35,42	28	26 → 42	26 → 38	42
2	10	KV 0,21	0,21,28,35, 42	0,21,28, 32,35,42	28	26 → 42	26 → 38	42
3	5	None	0,21,28,35,42	0,21,28, 32,35,42	28	26 → 42	26 → 38	42

**Figure 3**



### Study 5

#### Failure of an inactivated vaccine against porcine reproductive and respiratory syndrome to protect gilts against a heterologous challenge with PRRSV.

Scotti M; Prieto C; Alvarez E; Simarro I; Castro JM. Vet Rec. 2007 Dec 15;161(24):809–13.

**Results – not shown, I think the title tells the story.**

#### PRRS “Antigen extract” vaccines

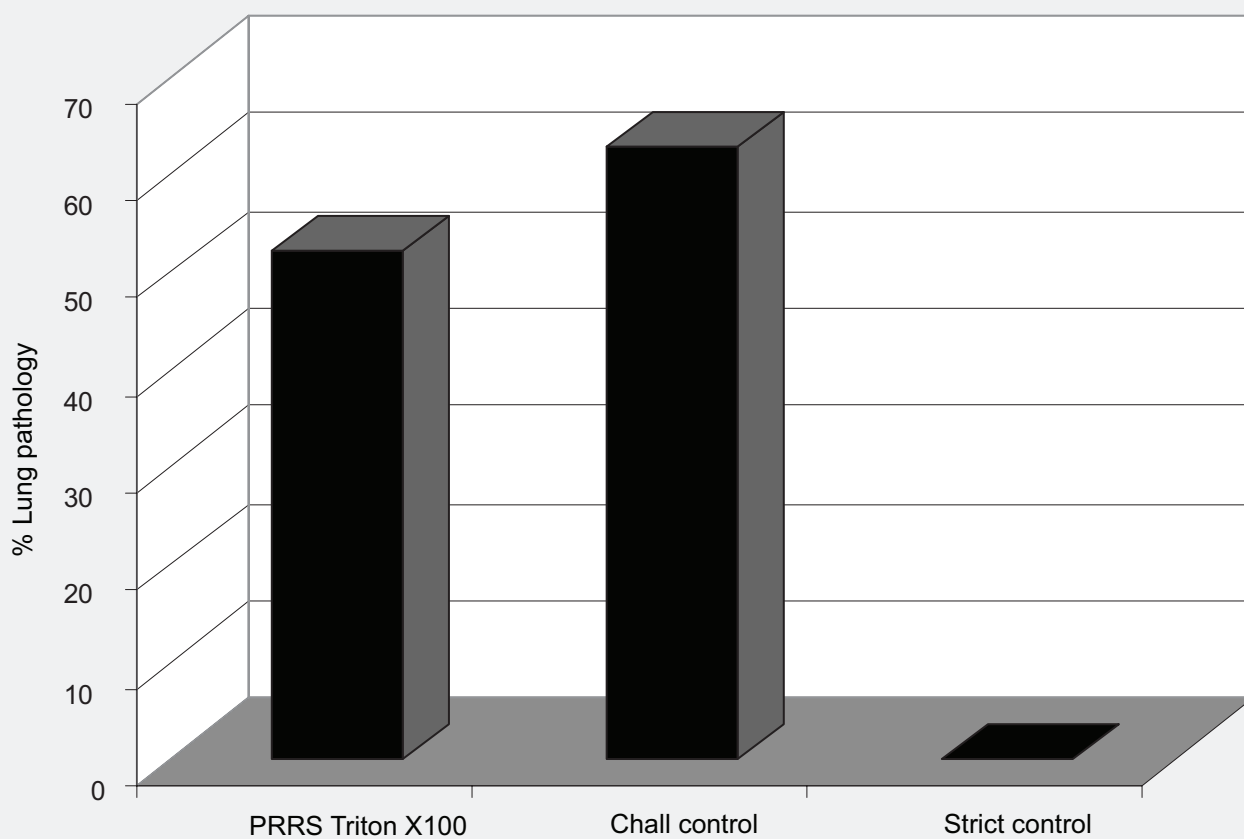
Dr. Han Soo Joo of the University of Minnesota had the hypothesis that harvest timing was key to an effective killed PRRS vaccine and a proprietary extraction process would lead to a superior immunogen. Dr. Joo and Dr. E. Vaughn evaluated this hypothesis in two controlled PRRS challenge trials. Both trials used antigen extracted early in the viral infection cycle.

### Study 6

Dr. Han Soo Joo and Dr. Eric Vaughn (BIVI – U. Minnesota Collaboration)

A respiratory study was conducted in 3 week old pigs using two doses of PRRS KV and antigen extract procedure. Following the second vaccination, a virulent exposure was conducted and animals were necropsied 14 days later and lungs evaluated for gross pathology. There was no significant difference between the vaccinated and challenge control treatment groups. (Figure 4)

**Figure 4**



### Study 7

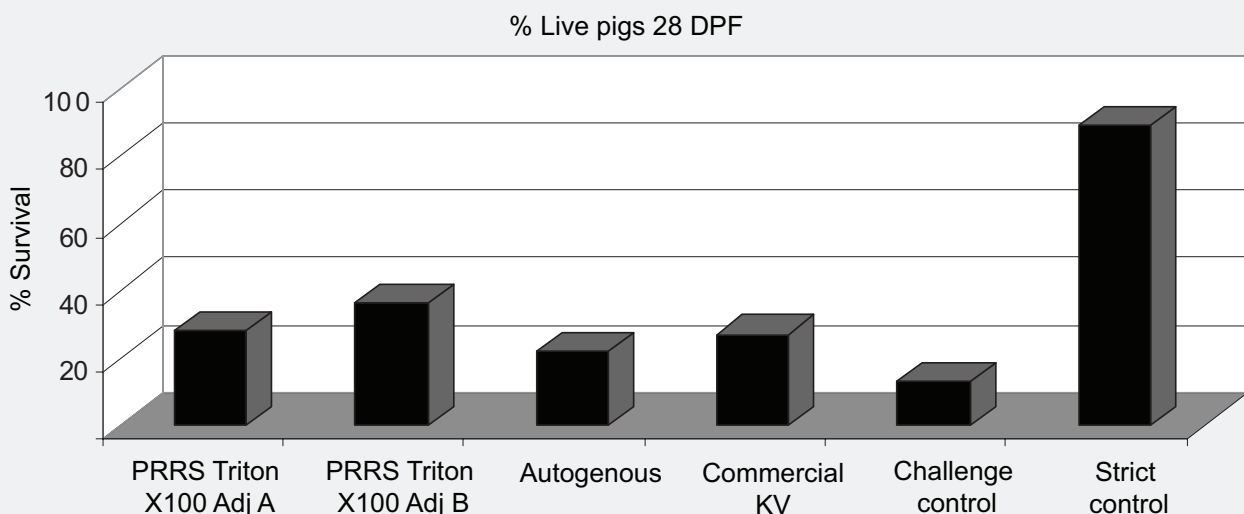
Dr. Eric Vaughn (BIVI internal)

A reproductive study was conducted using the PRRS antigen production and extract procedure (Dr. Han Soo Joo) which was then formulated with various adjuvants to try and improve protection. In addition, a commercial killed vaccine and an autogenous vaccine were also included. Sows were vaccinated twice after breeding and then challenged with a virulent PRRS isolate at 90 days of gestation. After challenge, severe reproductive failure occurred in all vaccinated groups and few piglets remained

alive at weaning (28 days post-farrow). The vaccinated groups were all significantly ( $P < 0.05$ ) different from the strict controls. The KV prototypes provided no benefit in the measured reproductive parameters as the vaccinated groups were not significantly ( $P < 0.05$ ) different from those of the challenge control group. (Figure 5)

### Adjuvants and immunostimulation to enhance PRRS immunity

**Figure 5**



### Study 8

#### Investigation and evaluation of alternate PRRS vaccine options to enhance immunostimulation and increased protection.

Investigators – Zuckerman; Roof; Vaughn; Wes Johnson (BIVI – U. Illinois Collaboration)

The objective of this study was to evaluate several adjuvants and/or immunomodulators in combination with Ingelvac® PRRS MLV and investigate potential clinical or immunological improvements *in vivo*. The study evaluated Ingelvac® PRRS MLV vaccine (USDA licensed) alone and in combination with various potential immunostimulators including:

- HS – adjuvant
- ORF 5 Pool – A pool of peptides representing the ORF 5 ectodomain region of five different PRRS virus isolates.
- INF alpha DNA vaccine provided by Dr. Zuckerman along with concurrent injections of purified and recombinant INF alpha from PBL Biomedical laboratories (100,000 units/dose).

- Poly ICLC (50 ug/kg) from Ribopharm, Inc. which has been reported to act as an interferon inducer and activates anti-viral immunity.
- IL-12 recombinant protein (2 ug/dose, 2 hours pre and post-vacc with MLV) from R&D Systems Inc. which has been shown to enhance T cell and NK cell activity.

The study also included an inactivated ORF 5 peptide (0.03 mg/ml × 2 ml dose) which was conjugated to cholera toxin beta subunit (CTB). The use of CTB has been reported to stimulate a better immune response due to enhanced receptor binding and antigen uptake. This was the only test article that did not include a PRRS MLV exposure.

This study included 90 healthy pigs at 2-3 weeks of age which were obtained from a herd free of PRRS virus that were randomly assigned to nine treatment groups. The study design is detailed in the table below in Table 4.

The results of this study confirmed that in a severe heterologous virulent challenge respiratory model, Ingelvac®

PRRS MLV provides significant levels of protection. Although treatment groups that received any adjuvant (HS) and IL-12 in combination with Ingelvac® PRRS MLV were also significantly different from challenge controls, they did not provide significant protection above and beyond MLV alone. It is interesting to note that MLV that had

ORF 5 protein supplemented to the vaccine in the diluent and the ORF 5 ectodomain peptides conjugated to cholera toxin were very similar if not numerically worse than the challenge controls.

**Table 4:**

Group	N=	Day 0	Day 55	Day 69	% Lung pathology
1	10	PRRS vaccine (MLV)	Challenge with PRRS 184	Necropsy	10.4 <sup>a</sup>
2	10	MLV +HS adjuvant	Challenge with PRRS 184	Necropsy	3.9 <sup>a</sup>
3	10	MLV+ORF5	Challenge with PRRS 184	Necropsy	30.3
4	10	MLV+INF alpha	Challenge with PRRS 184	Necropsy	16.2
5	10	MLV+Poly ICLC	Challenge with PRRS 184	Necropsy	13.8
6	10	MLV+IL-12	Challenge with PRRS 184	Necropsy	7.2 <sup>a</sup>
7	10	ORF 5 + Cholera toxin	Challenge with PRRS 184	Necropsy	45.3
8	10	Placebo	Challenge with PRRS 184	Necropsy	36.2
9	10	Strict controls	No treatment	Necropsy	0.42

<sup>a</sup> Indicates significant ( $P < 0.05$ ) differences between the indicated treatment groups and the challenge controls.

## DNA vaccines

### Study 9

#### Efficacy of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) Sequential expression immunization library (SELI) DNA vaccines in the respiratory model.

Wes Johnson and Eric Vaughn – Publication in progress (Internal BIVI study)

Sequential Expression Library Immunization (SELI) cDNA clones were generated that sequentially represent the open reading frame regions (ORFs) of the PRRSV genome. The cDNA clones comprising the ORF1a/1b region were designated A through M. Clone A utilizes the authentic ORF1a ATG start codon. The remaining ORF1a/1b clones B through M have had an ATG start codon added to their respective 5' coding regions. Additional cDNA clones representing the PRRSV structural protein ORFs 2a, 2b, 3, 4, 5, 6, and 7 were also generated.

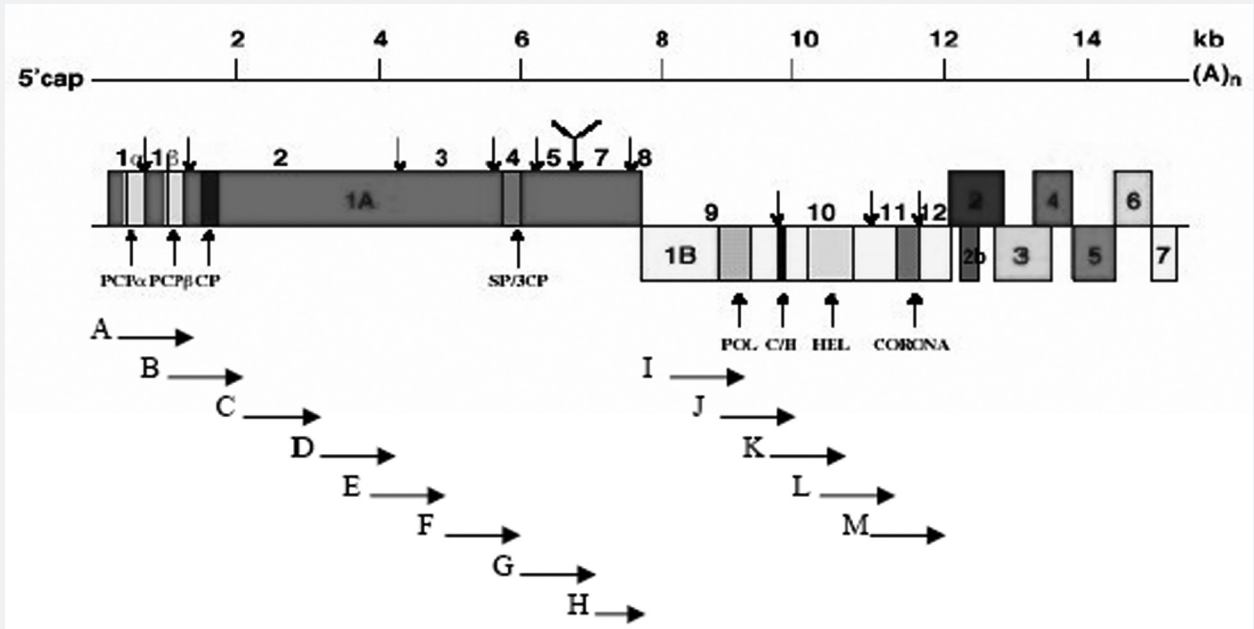
By utilizing combinations of these SELI clones, it is possible to vaccinate pigs with PRRSV DNA vaccines that encompass the whole PRRSV genome (i.e. ORF 1a/1b A through M and ORFs 2 through 7). (Figure 6)

This study consisted of six groups. Group 1 was treated with SELI “x” plasmids via electroporation on Day 0 and Day 21. Group 2 was treated with SELI “y” plasmids via electroporation on Day 0 and Day 21. Group 4 received Ingelvac® PRRS MLV on Day 0. Groups 5 and 6 were not treated with a vaccine prototype, and served as challenge and strict controls, respectively.

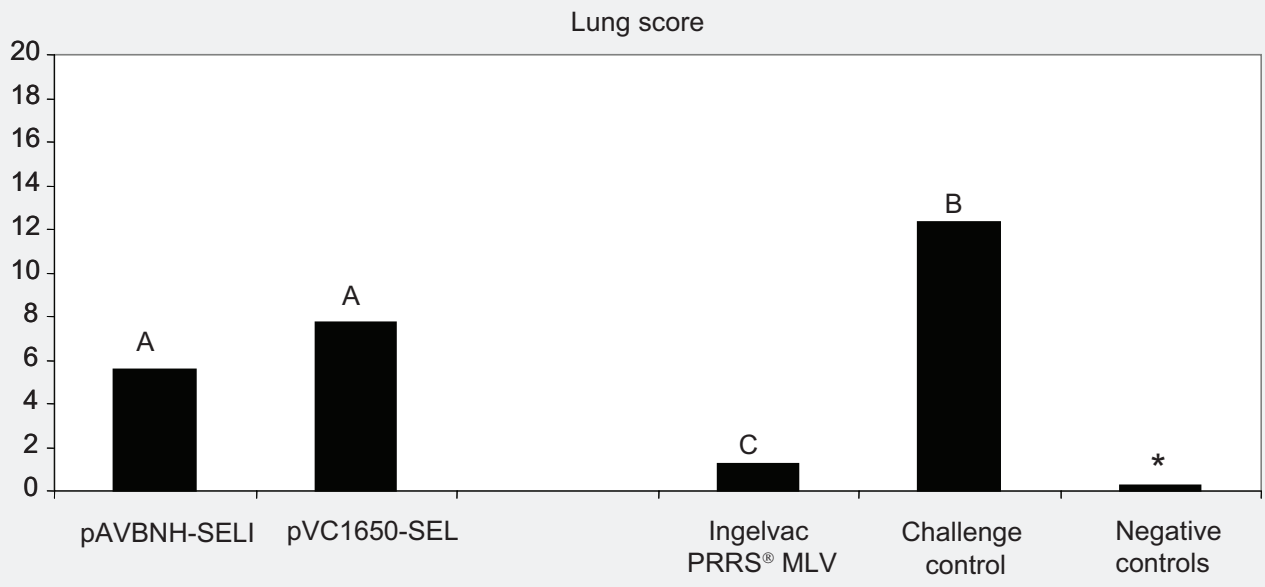
**Results** - The PRRS MLV-vaccinated group is clearly the best performing group in this study. The two DNA vaccine groups did have reduced lung scores at necropsy which were significantly better than those of both the challenge control group. However, the two DNA vaccine groups did not have any reduction in post challenge viremia as compared to the challenge controls. (Figure 7)



**Figure 6:** ORF1 regions A M plus ORFs 2-7 = 1-19 (i.e. represents entire PRRSV genome)



**Figure 7**



Disease I

## Modified live vaccines

### Study 10

#### Analysis of PRRS vaccination-challenge studies using a respiratory model

Investigators – Roof; Vaughn; Halbur; Burkhart and presented at Emerging Diseases Conference in Poland (2006)

The study evaluated 16 controlled respiratory trials using MLV and various heterologous challenge isolates. All

studies were done in 3 week old pigs with various times of challenge post-vaccination and utilize five different PRRSV challenge isolates. (Table 5 & Figure 8)

#### Results (% Gross lung pathology post-challenge)

The results of the above compilation of numerous controlled vaccination/challenge studies clearly indicates consistent reduction in lung lesions in the groups vaccinated with PRRSV MLV as compared to non-vaccinated/challenge control groups.

**Table 5: Summary of studies:**

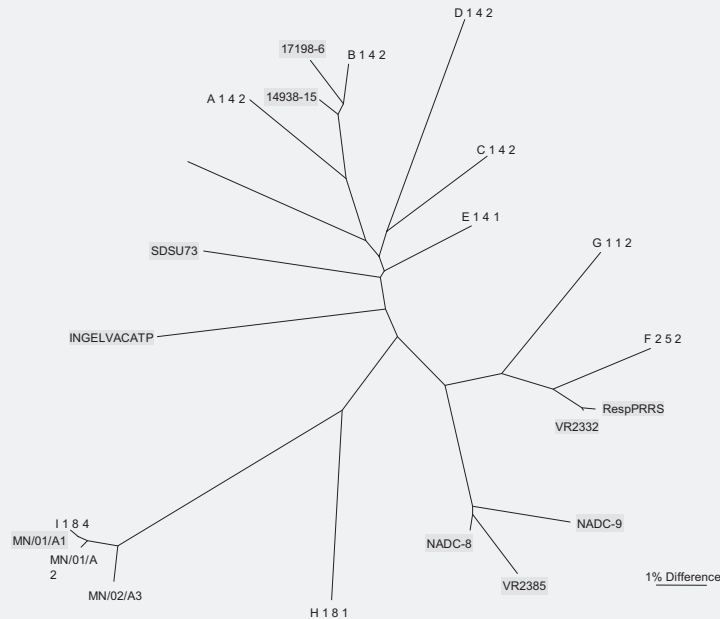
#	Vaccine	Chall day	Chall isolate
1	Ingelvac	35	NADC
2	Ingelvac	28	VR 2332
3	ATP	28	SDSU 73
4	ATP	120	SDSU 73
5	ATP	28	VR 2332
6	Ingelvac	42	17198-6
7	Ingelvac	56	SDSU 73
8	Ingelvac	56	17198-6
9	Ingelvac	28	MN 184
10	Ingelvac	55	MN 184
11	ATP	28	MN 184
12	Ingelvac	42	SDSU 73
13	Ingelvac	28	SDSU 73
14	Ingelvac	21	MN 184
15	ATP	28	SDSU 73
16	Ingelvac	28	MN 184

\*\* Group sizes range from 5-20 with most studies done with 15–20/group.

**Figure 8**



## Genetic Distribution of Isolates



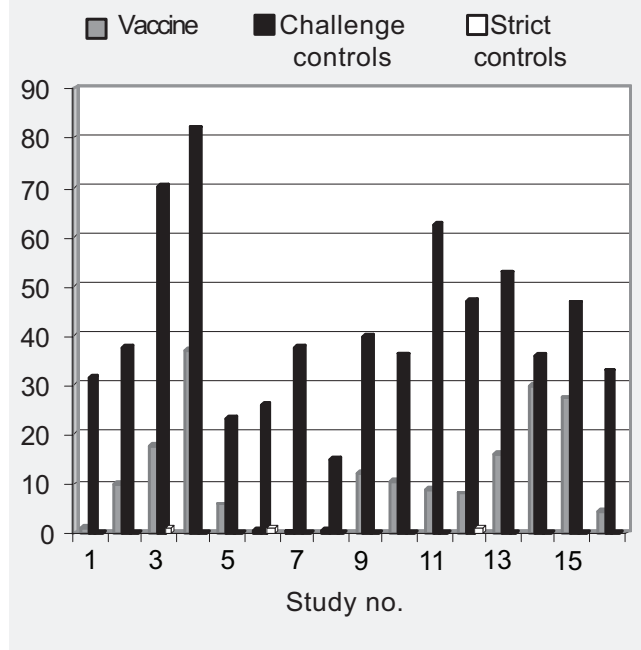
### Summary and conclusions

Based on numerous controlled studies and scientifically valid comparisons, modified live PRRS vaccines consistently provide statistically significant ( $P < 0.05$ ) levels of protection following heterologous challenge compared to challenge controls.

This is confirmed by numerous Boehringer Ingelheim Vetmedica, Inc. internal studies, collaborations with university experts, peer-reviewed publications, and external independent investigations.

In contrast, killed vaccines and inactivated antigens when investigated in controlled trials do not provide significant benefits compared to challenge control animals. To date, there are no published experimental data suggesting killed vaccines or inactivated antigens induce a protective immune response equal to or superior to that provided by MLV vaccines.

**Figure 9**



*Michael Roof*

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- Numerous Killed PRRS vaccine papers and presentations

Eric Vaughn – BIVI

- Numerous PRRS KV study reports
- PRRS SELI DNA vaccine report

Pat Halbur – ISU

- Lemman Abstract on PRRS autogenous

Federico Zuckerman – Illinois

- Immunomodulation- with IL 12 study

Wes Johnson – BIVI

- Numerous PRRS study reports

Han Soo Joo – University of Minnesota

- Collaboration on PRRS KV extract antigens

