Experimental safety and efficacy of a unique MLV PRRSV vaccine: PRRSGard®

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Introduction

PRRSV is the most economically important pathogen in US pigs due to significant losses in reproductive and growing pigs' performance.[1,2] PRRSV control and elimination is mainly based on herd closure and/or immunization with either live virus inoculation (LVI) or modified live virus (MLV) vaccines.[3-5] To aid in the control and elimination of PRRSV in the US, a unique MLV vaccine (PRRSGard®) has been developed. PRRSGard® is a chimeric PRRSV MLV with the backbone of a proprietary attenuated isolate within lineage 1 and the structural proteins from a high virulence isolate (MN184) within lineage 1 too.[6] MN184 is the ancestor of the current dominant 174 PRRSV circulating in US pigs.[7] PRRSGard® has a genetic marker that will allow the molecular detection and differentiation from wild-type viruses through RT-PCR and sequencing. The purpose of this publication is to summarize the experimental safety and efficacy studies that have been completed with PRRSGard® to inform US swine veterinarians and producers. This information may aid in US PRRSV control and elimination programs.

Materials and methods

Experimental efficacy studies

A total of 3 separate studies were conducted at different times to test PRRSGard® efficacy on viremia, fever, clinical signs and lung lesions. In each study a placebo was compared against a PRRSGard® vaccinated group. For all 3 studies, pigs were vaccinated at weaning with 1 mL of PRRSGard® and then challenged at either 5 (study 1) or 7 weeks post-weaning with a heterologous PRRSV strain (NADC-20/RFLP142/Lineage 8-9). The challenge virus was administered as 2 mL intranasally containing 2.5×10^4 virus particles/mL (study 1) and 1.5×10^4 virus particles/mL (studies 2 and 3). Viremic pigs after vaccination and challenge were detected weekly either with virus isolation. Viremia after challenge was measured using a $TCID_{50}$ assay at 1- and 2-weeks post challenge. Antibody response after vaccination and challenge was determined weekly with a commercial ELISA PRRSV kit (IDEXX PRRS X3 Ab Test, IDEXX Laboratories, Inc., Westbrook, Maine). Rectal temperatures were measured daily for 14 days after both vaccination and challenge (studies 1 and 3). Clinical signs were assessed daily in 3 categories: respiratory (0=normal, 1=panting/rapid and 2=dyspnea), cough (0=cough, 1=soft or mild intermittent cough and 2=harsh or severe and repetitive cough) and behavior (0=normal, 1=mild to moderately lethargic and 2=severely lethargic or recumbent). A pig was considered clinically affected if it had a total score of 1 or higher (studies 1 and 3). At the end of the study (2 weeks after challenge), pigs were necropsied to assess macroscopic lung lesions and collect lung tissue samples for microscopic lung lesion and immunohistochemistry testing and scoring (study 3). Body weight was also measured at vaccination, challenge and end of the study (study 3).

Experimental transmission and tissue tropism

A group of 16 PRRSGard® vaccinated pigs were housed together with 4 commingled and 4 adjacent sentinel pigs to assess horizontal PRRSV transmission for 4 weeks after vaccination. Blood samples were collected weekly from all pigs to detect viremia by qRT-PCR and virus isolation. Clinical signs were assessed daily similarly to the efficacy studies described above. Nasal and fecal swabs were also collected weekly, and 4 vaccinated pigs were necropsied weekly to detect PRRSGard® in different tissues and score macroscopic lung lesions. Serum samples were tested by ELISA to detect antibodies against PRRSV. All samples were tested by RT-PCR to detect PRRSGard® strain.

Experimental in-vivo genetic stability and safety in pregnant sows

To assess PRRSGard® genetic stability after multiple passages in PRRSV-negative pigs, a group of 3 pigs intramuscularly were injected with 1 mL of PRRSGard® (passage 1). Then, serum was collected from those piglets 14 days post-vaccination and used to intranasally inoculate (2 mL) another group of 3 pigs (passage 2). This process was repeated three additional times to reach passage 5. Clinical signs were recorded daily as described above in all passages. Sanger sequencing of ORF5 was performed to compare PRRSGard® and the isolate from passage 5. Viremia was measured with a TCID50 assay at 7- and 14-days post vaccination/inoculation in all 5 passages. Additionally, to assess the safety of passage 5, a group of 8 piglets were inoculated intranasally with 2 mL of serum (passage 6) from pigs infected in passage 5 with another 5 pigs as negative controls. Clinical signs and rectal temperature were recorded daily as described above. Body weight was also measured and pigs were necropsied at 21 days after inoculation to assess lung macroscopic lesions. Finally, a group of 8 pregnant sows were intranasally inoculated with 2 mL of serum from passage 5 at 3 weeks prefarrow to evaluate experimental safety in

pregnant sows. Five pregnant sows served as negative controls. Clinical signs in sows scored as explained above were recorded daily from inoculation to weaning. Sow rectal temperature was recorded daily during 14 days post inoculation, weekly sow serum samples were collected from inoculation to weaning, and sow reproductive performance and body weight of the suckling piglets were recorded too.

In-vitro genetic stability and recombination potential

A total of 15 passages in MARC-145 cells were completed in-vitro to assess PRRSGard® genetic stability. ORF5 of PRRSGard® strain (passage 0) and passage 15 were Sanger sequenced to assess nucleotide and amino acid differences between them. Finally, MARC-145 cells were co-infected once with PRRSGard® strain and an experimental infectious chimerato assess PRRSGard® recombination potential after 5 days in culture. RT-PCR primers were designed to specifically detect the genomic region between ORF3 to ORF7 of each strain.

Results

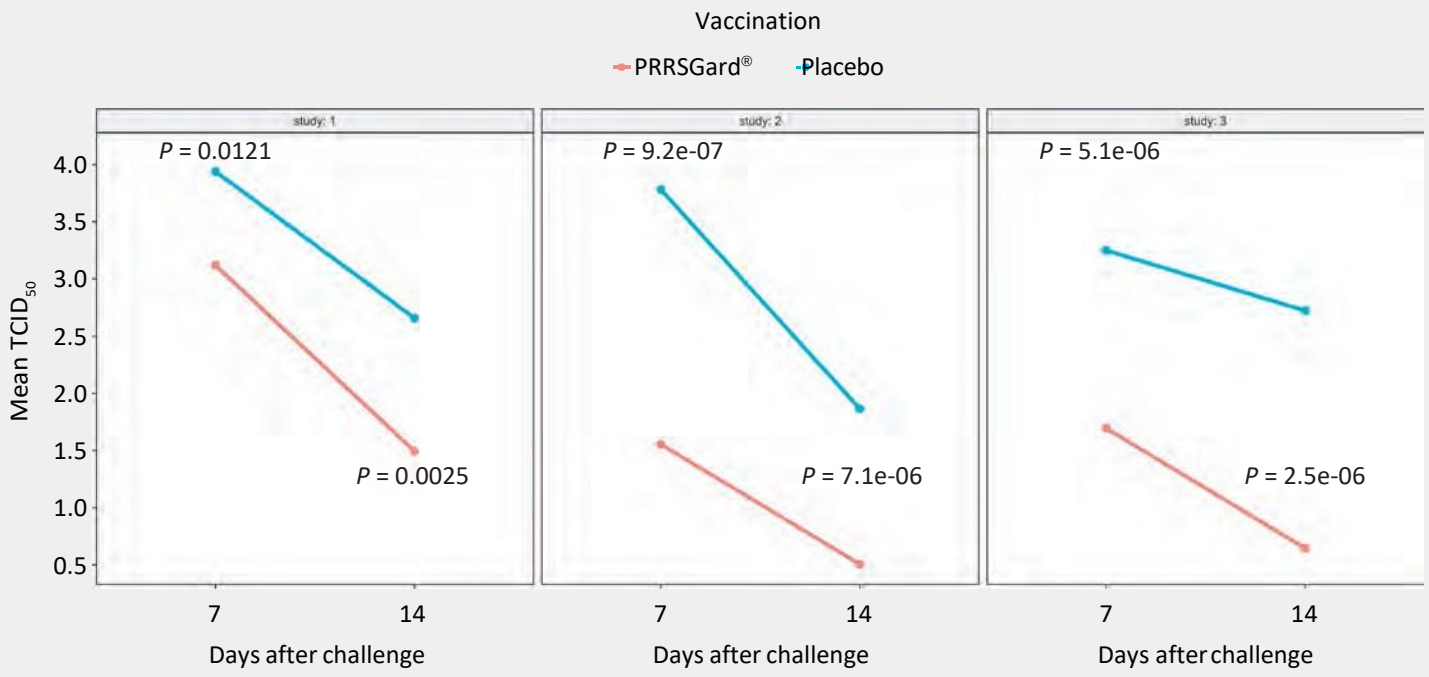
PRRSGard® was experimentally efficacious against a heterologous challenge

PRRSGard® significantly reduced PRRSV viremia after challenge with a heterologous strain. Figure 1 shows viremia levels as $TCID_{50}$ of infectious virus at 7 and 14 days after challenge. Moreover, PRRSGard® significantly reduced the number of PRRSV viremic pigs at 7 and 14 days after challenge in studies 2 and 3. Figure 2 summarizes the percentages of virus isolation positive pigs after vaccination and challenge. PRRSGard® significantly reduced the percentages of macroscopic lung lesions 2 weeks after challenge in studies 1 and 3. Figure 3 shows the mean percentage of macroscopic lung lesions 2 weeks after challenge. In addition, PRRSGard® significantly improved weight gain after challenge (study 3) and reduced the duration of viremia, fever (studies 1 and 3) and microscopic lung lesion scores (study 3). Percentage of ELISA PRRSV positive pigs were significantly higher in the PRRSGard® groups at 7 days post challenge and all pigs tested positive at 14 days post challenge. Table 1 summarizes the details of the evaluated parameters in each study.

PRRSGard® had limited experimental transmission and natural tissue tropism

Four weeks post-vaccination, one out of four commingled sentinels tested PRRSV VI and RT-PCR positive and none of the adjacent sentinels tested positive. All sentinels tested PRRSV ELISA negative on the weekly samplings including the sentinel that tested positive. All pigs vaccinated with PRRSGard® tested RT-PCR positive on the weekly blood samples, nasal and fecal swabs. PRRSGard® was detected in cervical lymph nodes, muscle, thymus, heart, large intestine, lung, liver, mandibular lymph node, spleen, stomach and kidney by RT-PCR. PRRSGard® was not detected in urine or small intestine. All sentinels were negative in all their tested tissues. The broad tissue detection of PRRSGard® confirmed its natural tropism similar to the wild type PRRSV parental strains. Finally, there were not observable clinical signs and lung lesions in any of the studied pigs.

Figure 1: PRRSGard® efficacy on viremia reduction after challenge.



PRRSGard® was experimentally stable (ORF5) in-vivo and safe in pregnant sows

PRRSGard® ORF5 passage 5 had just one amino acid change at position 23 [S23F] (GP5 signaling peptide) when compared to the PRRSGard® ORF5 master reference strain. At passage 6, pigs and pregnant sows did not show worse clinical signs than passage 5. There were no abortions in the inoculated pregnant sows. Piglet weight gain and sow reproductive performance was not affected by inoculation/vaccination with PRRSGard®. Table 2 summarizes the results from the PRRSGard® in-vivo passages and its safety in pregnant sows.

In-vitro, PRRSGard® was stable (ORF5) and had limited recombination

After 15 in-vitro cell passages, PRRSGard® ORF5 had one amino change at position 23 [S23C] (GP5 signaling peptide) in relation

Figure 2: PRRSGard® efficacy on reducing the number of viremic pigs after challenge.

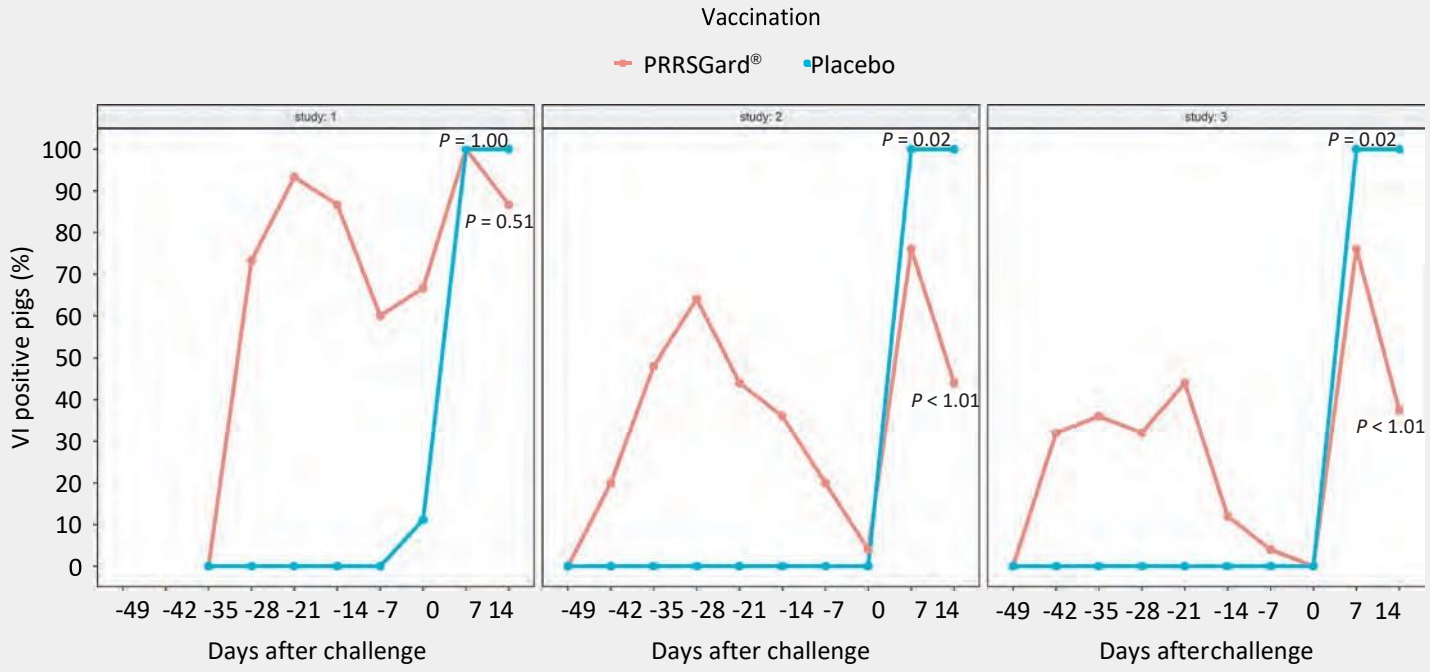


Figure 3: PRRSGard® efficacy on reducing macroscopic lung lesions 2 weeks after challenge.

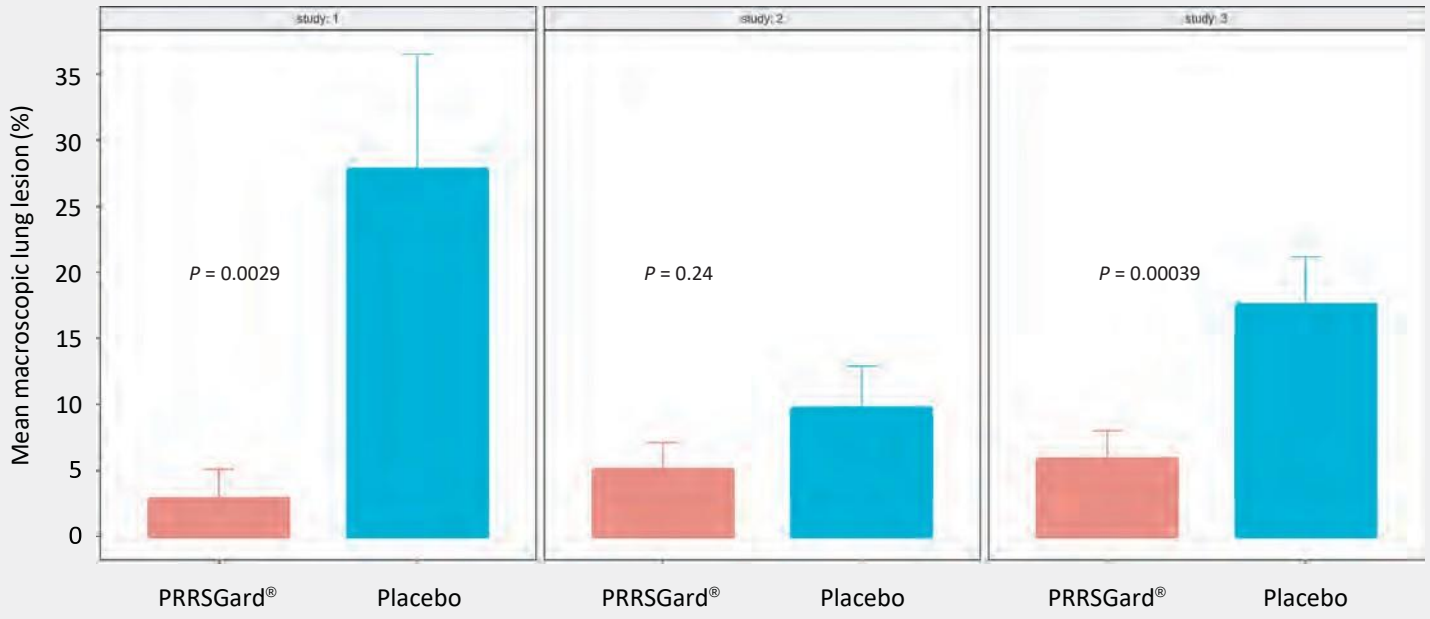


Table 1: PRRSGard® efficacy study results after challenge with a heterologous strain.

Parameter	Study 1			Study 2			Study 3		
Study group	**PRRSGard®**	**Placebo**	***P*-value**	**PRRSGard®**	**Placebo**	***P*-value**	**PRRSGard®**	**Placebo**	***P*-value**
No. pigs	15	11		25	25		25	25	
Duration of viremia, weeks, median	1.9	2.0	0.29	1.2	2.0	< .01	1.1	2.0	< .01
ELISA positives at 7 dpc, %	100	56	0.02	100	21	< .01	92	67	0.04
ELISA positives at 14 dpc, %	100	100		100	96	0.49	100	100	
Duration of fever, days, median	2.5	5.8	0.01				4.5	6.6	0.03
No. pigs with clinical signs	4/15	4/9	0.41				10/25	12/25	0.78
Duration of clinical signs, days, median	0.5	1.0	0.36				0.8	1.3	0.32
Micros. lung lesion score (0-4), median							2.3	2.8	0.08
IHC lung score (0-4), median							0.9	1.6	0.02
ADG, lb/day, median							0.72	0.43	0.03

Gray boxes mean that data was not collected or statistical comparisons were not made. IHC=Immunohistochemistry. ADG=Average daily weight gain. ELISA=Enzyme Linked Immunosorbent Assay. Micros.=Microscopic. dpc=days post challenge.

to the PRRSGard® ORF5 master reference strain. Recombination was not observed after one simultaneous infection with 2 PRRSV chimeras (PRRSGard® and one experimental PRRSV chimera).

Conclusions and discussion

Experimentally, PRRSGard® was efficacious against a heterologous challenge and safe in piglets and pregnant sows. Experimentally, PRRSGard® had limited spread to naïve commingled pigs and natural tissue tropism. Experimentally, PRRSGard® ORF5 was stable and had limited in-vitro recombination. New PRRSV vaccines are extremely important to aid in the control and elimination of this disease in US pigs. PRRSGard® needs to be tested under field conditions to understand its unique features.

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Table 2: Clinical findings after PRRSGard® inoculation in piglets and pregnant sows.

Parameter	Passage					Piglets			Pregnant sows		
Study group	**P1**	**P2**	**P3**	**P4**	**P5**	**P6**	**Control**	***P*-value**	**P6**	**Control**	***P*-value**
No. pigs	3	3	3	3	9	8	5		8	5	
No. viremic pigs	3/3	2/3	3/3	2/3	9/9	8/8	0/5	< .01	8/8	0/5	< .01
Weeks tested for viremia	2	2	2	2	2	3	3		6	6	
Duration of viremia, weeks, median	1	2	1	2	2	3	0	< .01	1.5	0	< .01
Viremia at 14 dpv, TCID50, median	2.8	4.9	2.5	4.1	4.8						
No. pigs with clinical signs	0/3	0/3	0/3	0/3	1/9	5/8	1/5	0.27	3/8	0/5	0.23
Duration of clinical signs, days, median	0	0	0	0	0	1.5	0	0.11	0	0	0.17
No. pigs with fever	3/3	2/3	3/3	3/3	9/9	8/8	2/5	0.04	4/8	0/5	0.11
Duration of fever, days, median	1	1	5	2	3	4.5	2	0.04	0.5	0	0.27
Weaning weight, lb, median						12.9	12.5	0.88			
Weight at 21 dpv, lb, median						29.0	26.0	0.22			
ADG at 21 dpv, lb/day, median						0.74	0.51	0.13			
Macro. lung lesion, %, median						0.1	0.0	0.09			
Total born, No. pigs, median									13	13	0.94
Born alive, No. pigs, median									11.5	11	1.00
Born dead, No. pigs, median									1	2	0.55
Weaned, No. pigs, median									10	10	0.71
Preweaning deaths, No. pigs, median									0	1	0.40
Birth weight, lb, median									3.4	3.5	0.72
Weaning weight, lb, median									12.4	14.9	0.42
Preweaning ADG, lb/day, median									0.45	0.54	0.22

Gray boxes mean that data was not collected or statistical comparisons were not made. dpv=days post vaccination. P =Passage. $TCID_{50}$=Tissue Culture Infectious Dose that infects 50% of the cells. lb=pounds. No.=number. ADG=Average Daily weight Gain. Macro.=Macroscopic. dpv=days post vaccination.