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# **A porcine reproductive and respiratory syndrome virus candidate vaccine based on the synthetic attenuated virus engineering approach is attenuated and effective in protecting against homologous virus challenge**

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1 A porcine reproductive and respiratory syndrome virus candidate  
2 vaccine based on the synthetic attenuated virus engineering approach is  
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4 challenge

5

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14

15 Running title: Efficacy of the SAVE approach in protecting against PRRSV

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18

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## Efficacy of the SAVE approach in protecting against PRRSV

### 23 **Abstract**

24 Current porcine reproductive and respiratory syndrome virus (PRRSV) vaccines sometimes fail  
25 to provide adequate immunity to protect pigs from PRRSV-induced disease. This may be due to  
26 antigenic differences among PRRSV strains. Rapid production of attenuated farm-specific  
27 homologous vaccines is a feasible alternative to commercial vaccines. In this study, attenuation  
28 and efficacy of a codon-pair de-optimized candidate vaccine generated by synthetic attenuated  
29 virus engineering approach (SAVE5) were tested in a conventional growing pig model. Forty  
30 pigs were vaccinated intranasally or intramuscularly with SAVE5 at day 0 (D0). The remaining  
31 28 pigs were sham-vaccinated with saline. At D42, 30 vaccinated and 19 sham-vaccinated pigs  
32 were challenged with the homologous PRRSV strain VR2385. The experiment was terminated at  
33 D54. The SAVE5 virus was effectively attenuated as evidenced by a low magnitude of SAVE5  
34 viremia for 1-5 consecutive weeks in 35.9% (14/39) of the vaccinated pigs, lack of detectable  
35 nasal SAVE5 shedding and failure to transmit the vaccine virus from pig to pig. By D42, all  
36 vaccinated pigs with detectable SAVE5 viremia also had detectable anti-PRRSV IgG. Anti-IgG  
37 positive vaccinated pigs were protected from subsequent VR2385 challenge as evidenced by lack  
38 of VR2385 viremia and nasal shedding, significantly reduced macroscopic and microscopic lung  
39 lesions and significantly reduced amount of PRRSV antigen in lungs compared to the non-  
40 vaccinated VR2385-challenged positive control pigs. The nasal vaccination route appeared to be  
41 more effective in inducing protective immunity in a larger number of pigs compared to the  
42 intramuscular route. Vaccinated pigs without detectable SAVE5 viremia did not seroconvert and  
43 were fully susceptible to VR2385 challenge. Under the study conditions, the SAVE approach  
44 was successful in attenuating PRRSV strain VR2385 and protected against homologous virus

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- 45 challenge. Virus dosage likely needs to be adjusted to induce replication and protection in a  
46 higher percentage of vaccinated pigs.

47 **1. Introduction**

48 Porcine reproductive and respiratory syndrome virus (PRRSV) is widespread in the  
49 global pig population and associated with reproductive failure in adult pigs and respiratory  
50 disease in growing pigs [1] resulting in estimated annual losses of \$664 million to the U.S. swine  
51 industry [2]. PRRSV is an enveloped, positive-sense, single-stranded RNA virus [3;4] that  
52 belongs to the family *Arteriviridae* in the order *Nidovirales* [4]. The PRRSV genome contains  
53 eight structural protein open reading frames (ORFs) and a few non-structural protein ORFs [5]  
54 and isolates can be divided into two main genotypes: type 1 (European type), and type 2 (North  
55 American type) [6]. Mutations such as insertions or deletions occur frequently in the PRRSV  
56 genome making it one of the most genetically diverse pig viruses [7].

57 The enormous continual change and diversity of PRRSV strains has resulted in limited  
58 efficacy of current commercial vaccines and vaccination strategies [8]. Modified live-attenuated  
59 virus vaccines (MLVs) are the most effective option currently available to control clinical signs  
60 associated with PRRSV infection; however, while these vaccines in general protect pigs well  
61 against homologous challenge they are not always capable of eliciting protective immunity  
62 against heterologous field strains [9]. Due to limited vaccine homology with circulating field  
63 strains on some farms, pig producers often rely on planned exposure to the pathogenic farm  
64 strain [10] which is risky and may not always be economically beneficial or acceptable from an  
65 animal welfare point of view. Therefore, rapid attenuation of PRRSV would afford an  
66 opportunity to quickly generate farm-specific vaccines.

67 Recently the synthetic attenuated virus engineering (SAVE) approach was utilized to  
68 rapidly attenuate the wild-type PRRSV isolate VR2385 [11]. Specifically, the codon-pairs of the  
69 major envelope GP5 gene of PRRSV were deoptimized through a computer algorithm which

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70 resulted in a modified GP5 nucleotide sequence while retaining the original amino acid  
71 sequence. The resulting virus was designated SAVE5. When SAVE5 was tested *in vitro* it was  
72 genetically stable. Experimental infection of pigs resulted in lower levels of viremia and reduced  
73 macroscopic and microscopic lung lesions compared to the wild-type VR2385 virus [11]. The  
74 protective efficacy of the SAVE5 was unknown. In the present study, the immunogenicity and  
75 protective efficacy of SAVE5 in decreasing clinical signs, lesions and viremia associated with  
76 wild-type PRRSV challenge were assessed using a conventional pig model.

77

## 78 **2. Methods**

### 79 *2.1. Animals and housing*

80 The experimental protocol was approved by the Iowa State University Institutional  
81 Animal Care and Use Committee (14-D-0008-A). Sixty-eight, 2-week-old, commercial crossbred  
82 pigs from a PRRSV-free source herd were transported to the Livestock Infectious Disease  
83 Isolation Facility at Iowa State University, Ames, Iowa, USA. The study was done in two  
84 replicates 12 months apart. The same source herd was used for both replicates. Upon arrival, the  
85 pigs were randomly assigned to one of five groups. Initially and for each replicate, vaccinated  
86 pigs were housed in one room and the sham-vaccinated pigs were housed in another room. Prior  
87 to virus challenge, the pigs were further separated by treatment status into four (Replicate 1) or  
88 three (Replicate 2) rooms with one pen in each room. Pigs were given continuous access to age  
89 appropriate feed (Nature's Made, Heartland Co-op, Cambridge, Iowa, USA) and water.

90

### 91 *2.2. Experimental design*

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92           The experimental groups are outlined in [Table 1](#). At day 0 (D0) when the pigs were 3  
93 weeks old, VAC-IM-CONTROL, VAC-IM-PRRSV and VAC-IN-PRRSV were vaccinated with  
94 the SAVE5 candidate vaccine and the NEG-CONTROL and POS-CONTROL groups were  
95 sham-vaccinated with saline. The intramuscular (IM) route was used for the VAC-IM-  
96 CONTROL, VAC-IM-PRRSV, NEG-CONTROL and the POS-CONTROL groups and the  
97 intranasal (IN) route was used for the VAC-IN-PRRSV group. At D42, pigs were challenged  
98 with the homologous PRRSV strain VR2385 (VAC-IM-PRRSV, VAC-IN-PRRSV, POS-  
99 CONTROL) or were sham-inoculated with saline (VAC-IM-CONTROL, NEG-CONTROL). All  
100 pigs were necropsied at D54. Blood samples were collected weekly from D0 through D42 and on  
101 D44, D46, D48, D51 and D54. The blood was centrifuged at 3000 g for 10 min and the serum  
102 was stored at -80°C until testing. Nasal swabs were taken from each pig on D7, D14, D21, D28,  
103 D35, D42, D44, D46, D48, D50 and D53, placed into 1 ml of saline and stored at -80°C until  
104 testing. The pigs were weighed on D0, D42 and D54.

105

### 106 *2.3. Vaccination*

107           A previously described vaccine candidate, the SAVE5 virus, was utilized [\[11\]](#). At 3  
108 weeks of age, the VAC-IM-CONTROL and the VAC-IM-PRRSV groups received 3 ml of  
109 SAVE5 virus at a dose of  $10^{4.5}$  TCID<sub>50</sub>/ml intramuscularly into the right neck. The VAC-IN-  
110 PRRSV group received 3 ml of SAVE5 virus at a dose of  $10^{4.5}$  TCID<sub>50</sub>/ml intranasally by slowly  
111 dripping the inoculum into the nostrils. The NEG-CONTROL and the POS-CONTROL groups  
112 received 3 ml of saline intramuscularly into the right neck.

113

### 114 *2.4. Challenge*

## Efficacy of the SAVE approach in protecting against PRRSV

115 At 9 weeks of age (D42 post-vaccination), POS-CONTROL, VAC-IM-PRRSV and  
116 VAC-IN-PRRSV groups were challenged intranasally with  $10^{6.6}$  TCID<sub>50</sub> of PRRSV VR2385 by  
117 slowly dripping 1 ml of inoculum into each nostril. The NEG-CONTROL and the VAC-IM-  
118 CONTROL groups were similarly sham-challenged with 1 ml of saline.

119

### 120 *2.5. Clinical evaluation*

121 On D42, D44, D46, D48, D50 and D53, rectal temperatures were taken and respiratory  
122 scores were evaluated [12]. The respiratory scores included 0=normal, 1=mild  
123 dyspnea/tachypnea when stressed, 2= mild dyspnea/tachypnea at rest, 3= moderate  
124 dyspnea/tachypnea when stressed, 4= moderate dyspnea/tachypnea at rest, 5= severe  
125 dyspnea/tachypnea when stressed and 6= severe dyspnea/tachypnea at rest [12].

126

### 127 *2.6. Serology*

128 All serum samples were tested for PRRSV specific IgG antibodies using the IDEXX  
129 PRRS X3 Ab ELISA (IDEXX Laboratories, Inc.) according to the manufacturer's instructions. A  
130 sample-to-positive (S/P) ratio greater than 0.4 was considered positive. A fluorescent focus  
131 neutralization (FFN) assay for determination of amount of PRRSV-specific neutralizing  
132 antibodies was done on sera collected on D42 according to protocols routinely performed at the  
133 Veterinary Diagnostic Laboratory at Iowa State University. The PRRSV strain used was type 2  
134 PRRSV strain ISU-P. In addition, FFN on D42 samples were also done at the Animal Disease  
135 Research and Diagnostic Laboratory at South Dakota State University using the VR2332 PRRSV  
136 type 2 strain. These strains are routinely used for client submissions regardless of the PRRSV on  
137 farm.



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138

### 139 *2.7. RNA extraction and real-time PCR*

140 Total nucleic acids from serum and nasal swabs were extracted using the KingFisher Flex  
141 96-tip comb (Thermo Scientific) and the MagMax-96 viral RNA isolation kit (Life  
142 Technologies) [13]. Positive and negative controls were included on each plate. Extracted  
143 samples were tested by quantitative reverse transcriptase (RT) PCR for the presence and amount  
144 of PRRSV RNA [14]. The threshold was set at 0.05 with a cycle threshold ( $C_T$ ) of less than 37  
145 cycles considered positive.

146

### 147 *2.8. Characterization of the PRRSV strains detected by differential real-time RT-PCR*

148 To differentiate the vaccine candidate SAVE5 and the wild type PRRSV VR2385, a  
149 duplex differential real-time RT-PCR was established based on the alignment of GP5 gene. The  
150 VR2385 primers and probe were PRRS2385F: 5'-GTGCCCTGGCTGCGTTGAT-3',  
151 PRRS2385R: 5'-CAACGATAGAGTCTGCCCTTAGTGTC-3', PRRSprob2385: FAM-5'-  
152 CTTCGTCATTAGGCTTGCGAAGAATTGC-3'-BHQ1. The SAVE5 primers and probe were  
153 PRRSSAVEF: 5'-GCTGATTTACAACCTTGACGCTATGTG-3', PRRSSAVER: 5'-  
154 GACAGGAAAAATGACAAAGCACTCG-3', PRRSprobSAVE: CAL-Fluor®-Orange-560-5'-  
155 TAACGGTACCGACTGGCTTGCGAATAAG-3'-BHQ1. The real-time RT-PCR was carried  
156 out in 96-well plates, with each reaction consisting of a total volume of 25  $\mu$ l, containing 12.5  $\mu$ l  
157 TaqMan One-Step RT-PCR master mix reagent (Applied Biosystems), 6  $\mu$ l RNA, 0.625  $\mu$ l 40 $\times$   
158 MultiScribe and RNase Inhibitor, 1  $\mu$ l each of the two primers (10  $\mu$ M), 0.5  $\mu$ l probe (10  $\mu$ M)  
159 and 3.375  $\mu$ l RNase-free water. Amplification reactions were performed using an Applied  
160 Biosystems 7500 Fast Real-Time PCR System (Applied Biosystems) under universal conditions:

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161 30 min at 50°C, 10 min at 95°C, followed by 40 cycles of 15 s at 95°C and 1 min at 60°C. A  
162 sample was considered negative if no signal was detected during 40 amplification cycles.

163 PRRSV ORF5 was sequenced from two PRRSV RT-PCR positive pigs in each of the  
164 VAC-IM-PRRSV, VAC-IN-PRRSV and the POS-CONTROL groups on D54. In addition, the  
165 SAVE5 vaccine strain and the VR2385 challenge strain used for inoculation were also  
166 sequenced. PRRSV ORF5 amplification was performed using primers GP5F (5'-  
167 ATGTTGGGGAAATGCTTGACCG-3') and GP5R (5'-CTAAGGACGACTCCATTGTTCCG-  
168 3') [13]. The PCR products were sequenced using a 3730xl DNA Analyzer at the Iowa State  
169 University DNA Facility, Ames, Iowa, USA.

170

### 171 *2.9. Necropsy*

172 All pigs were humanely euthanized on D54 by intravenous injection of pentobarbital  
173 sodium overdose (Fatal Plus®, Vortech Pharmaceuticals, LTD, Dearborn, Michigan, USA). A  
174 veterinary pathologist blinded to the treatment groups of the pigs scored and recorded the  
175 severity of macroscopic lung lesions for each pig. Specifically, macroscopic lung lesions were  
176 assessed to determine the percentage of the lung affected by pneumonia [12]. Five sections of  
177 lungs, tonsil and tracheobronchial lymph nodes were fixed in 10% neutral buffered formalin and  
178 routinely processed for further histopathological analysis.

179

### 180 *2.10. Histopathology and immunohistochemistry*

181 Microscopic lesions were assessed by a pathologist blinded to the treatment groups. Lung  
182 lesions were scored for severity of PRRSV induced interstitial pneumonia lesions ranging from

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183 0=normal to 6=severe diffuse [15]. Each pig was scored by two different pathologists and the  
184 average score was determined and used for further analysis.

185 To assess presence and amount of PRRSV antigen, immunohistochemistry was  
186 conducted on sections of lung tissues [16]. Scores ranged from 0=no antigen detectable to  
187 3=abundant antigen diffusely distributed. All slides were independently evaluated by two  
188 pathologists and the mean score was utilized.

189

### 190 2.11. Statistical analysis

191 Summary statistics were calculated for groups to assess the distributional property.  
192 Quantitative RT-PCR data was log transformed prior to analysis. A generalized linear mixed  
193 model was used for all statistical comparisons with SAS version 9.3 (SAS Institute, Cary, NC)  
194 with group, time and their interaction were fixed effects and pig as random effect. A P-value of  
195 less than 0.05 was considered significant. Difference in mean response was assessed between  
196 groups. Protection was assessed by regrouping pigs in the VAC-IM-PRRSV and VAC-IN-  
197 PRRSV groups by seroconversion to PRRSV (S/P ratio greater than 0.4) at challenge (D42) into  
198 VAC-D42-POS (n=10) and VAC-D42-NEG (n=20) pigs (Table 2). These two groups were  
199 directly compared to the POS-CONTROLS. Area under the curve viremia (AUC) was calculated  
200 for each pig from D45 to D54. Correlation between AUC and antibody levels at challenge (D42)  
201 was assessed by Pearson's correlation test.

202

## 203 3. Results

### 204 3.1. Clinical signs and weight gain

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205 The NEG-CONTROL, the VAC-IM-PRRSV and the VAC-IN-PRRSV groups had no  
206 clinical signs of disease during the experiment. One POS-CONTROL pig had bilateral mucous  
207 discharge starting at D44 with a respiratory score of 2 by D53. Two other POS-CONTROL pigs  
208 had mucous discharge on D53. Statistical evaluation of the respiratory scores (data not shown) or  
209 the average daily weight gain (ADG, [Tables 1 and 2](#)) indicated no significant differences among  
210 the treatment groups. Differences in rectal temperatures were observed on D46 when the POS-  
211 CONTROL group had statistically ( $P < 0.05$ ) higher rectal temperatures compared to VAC-IN-  
212 PRRSV and NEG-CONTROL groups.

213

### 214 3.2. Seroconversion to PRRSV

215 Mean group anti-PRRSV IgG antibody levels are summarized in [Fig. 1](#). None of the pigs  
216 had detectable PRRSV antibodies on arrival and the NEG-CONTROL group remained  
217 seronegative until termination of the study. Vaccinated pigs developed anti-PRRSV IgG  
218 antibodies starting at D14 and VAC-IN-PRRSV pigs had significantly higher S/P ratios  
219 compared to pigs vaccinated intramuscularly at D28 ([Table 3](#)). Among vaccinated and  
220 subsequently challenged pigs only 10/30 pigs (4/20 VAC-IM-PRRSV and 6/10 VAC-IN-  
221 PRRSV) pigs had seroconverted by D42 (VAC-D42-POSITIVE). There was no evidence of  
222 seroconversion in the remaining 20/30 pigs (VAC-D42-NEGATIVE; 16/20 VAC-IM-PRRSV  
223 and 4/10 VAC-IN-PRRSV).

224 Neutralizing antibodies against PRRSV type 2 strains ISU-P or VR2332 were not  
225 detected in any of the serum samples collected at D42.

226

### 227 3.3. PRRSV viremia and shedding

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228 The NEG-CONTROL pigs remained PRRSV RNA negative throughout the study.  
229 PRRSV VR2385 was not detected prior to D42 in any vaccinated pig and PRRSV SAVE5 was  
230 never detected in POS-CONTROL pigs based on differential PCR and sequencing. In vaccinated  
231 pigs, SAVE5 RNA in serum samples was first detectable by D7 (Table 4). At the time of PRRSV  
232 challenge on D42, 22.5% (9/40) of the vaccinated pigs were SAVE5 viremic. After challenge,  
233 only VR2385 was detected in the VAC-IM-PRRSV group and in 3/5 viremic VAC-IN-PRRSV  
234 pigs; however, mixed SAVE5/VR2385 was detected in 2/5 viremic VAC-IN-PRRSV pigs.  
235 Prevalence rates and PRRSV loads were lower in VAC-IN-PRRSV pigs compared to POS-  
236 CONTROLS which was significant on D51 (Table 4).

237 When the D42 seropositive and seronegative subgroups were analyzed, VAC-D42-POS  
238 pigs had evidence of SAVE-replication prior to challenge whereas VAC-D42-NEG pigs did not  
239 (Fig. 2). Significant differences in amounts of VR2385 PRRSV viremia after challenge indicate  
240 that VAC-D42-POS pigs but not VAC-D42-NEG pigs were protected from VR2385 challenge  
241 (Fig. 2). There was a high correlation between AUC viremia and levels of antibody at challenge  
242 ( $r = -0.84$  [CI -0.90, -0.73],  $p < 0.0001$ ) indicating that the presence of antibodies at challenge  
243 was correlated with lack of viremia after challenge.

244 Nasal SAVE5 shedding was not detected in any of the vaccinated pigs prior to challenge.  
245 After challenge, nasal VR2385 shedding was observed in 25% (D46), 35% (D48) and 15%  
246 (D50) of the VAC-IM-PRRSV pigs; in 40% (D48) of the VAC-IN-PRRSV pigs; and in 47.3%  
247 (D46 and D48), 10.5% (D50) and 5.3% (D53) of the POS-CONTROLS. The group mean  $\log_{10}$   
248 PRRSV genomic copy numbers in nasal swabs ranged from 3.3 to 6.8. Nasal VR2385 shedding  
249 was not detected in any of the VAC-D42-POS pigs whereas it was observed in 55% (11/20) of

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250 the VAC-D42-NEG pigs for 1-4 consecutive days and in 47.3% (9/19) of the POS-CONTROL  
251 pigs for 1-7 consecutive days.

252

### 253 *3.4. Macroscopic lesions*

254 Gross lesion scores are summarized in [Tables 1](#) and [2](#). Multifocal mottled tan areas of  
255 consolidation were found throughout the lungs of selected pigs in all PRRSV VR2385-infected  
256 groups. Challenged groups had significantly more severe lesions than the non-challenged groups  
257 ( $P < 0.01$ ). The mean macroscopic lung lesions in VAC-D42-POS pigs were not significantly  
258 different from VAC-D42-NEG pigs but were significantly lower compared to the POS-  
259 CONTROL ([Table 2](#)).

260

### 261 *3.5. Microscopic lesions*

262 When observed, lung lesions were characterized by mild-to-moderate multifocal  
263 lymphohistiocytic interstitial pneumonia with type 2 pneumocyte hypertrophy and hyperplasia,  
264 and increased numbers of macrophages and necrotic cellular debris in the alveolar spaces. Group  
265 mean interstitial pneumonia scores are summarized in [Table 1](#). Overall, pigs in the VAC-IN-  
266 PRRSV group had significantly less severe microscopic lesions compared to the POS-PRRSV  
267 group. When the D42 seropositive and seronegative subgroups were analyzed, VAC-D42-POS  
268 pigs had significantly lower scores compared to VAC-D42-NEG pigs and POS-CONTROL pigs  
269 ([Table 2](#)).

270 PRRSV antigen was observed in alveolar and septal macrophages in several pigs in the  
271 PRRSV-infected groups. The amounts of detectable PRRSV antigen in VAC-IN-PRRSV pigs  
272 were significantly lower compared to the POS-CONTROL pigs ([Table 1](#)). Among VAC-D42-

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273 POS pigs, 3/10 were PRRSV IHC positive with significantly lower amounts of PRRSV antigen  
274 in lung tissues compared to VAC-D42-NEG pigs and POS-CONTROL pigs (Table 2).

275

### 276 4. Discussion

277 The SAVE approach to attenuate viruses requires significantly less time compared to the  
278 traditional cell culture attenuation, and most importantly the SAVE approach attenuates the virus  
279 without altering the antigenicity of the virus protein on the virion, since the protein sequence of  
280 the SAVE5 ORF5 is identical to the wild-type PRRSV ORF5. The attenuation is achieved by  
281 modification of the naturally optimized pairs of codons in a viral gene sequence without altering  
282 the codon bias or the amino acid sequence [17]. A potential drawback of the SAVE approach is  
283 over-attenuation which may affect the ability of the virus to replicate in the host. In contrast, the  
284 traditional cell culture back passage approach to attenuate viruses may introduce critical  
285 mutations in the virus genome during serial passages in cell culture, and these mutations, often  
286 only a few critical amino acid changes, contribute to virus attenuation but can also revert back to  
287 pathogenic phenotype. Therefore, there are pros and cons for both approaches.

288 In this study, the vaccine efficacy of the PRRSV vaccine candidate SAVE5 was tested *in*  
289 *vivo* using the homologous wild-type PRRSV strain VR2385. Similar to a previous pilot study,  
290 SAVE5 virus proved to be attenuated. After vaccination, SAVE5 viremia was sporadic (< 50%  
291 of the vaccinated pigs for 1-5 consecutive weeks) and of low magnitude, SAVE5 nasal shedding  
292 was not detectable and clinical signs were absent. This further documents that codon-pair de-  
293 optimization is an effective way to attenuate PRRSV. A farm-specific attenuated PRRSV  
294 vaccine could be produced via the SAVE approach in less than 2 months from PRRSV positive  
295 lung tissue or serum obtained from the farm.

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296 In vaccinated pigs in this study, seroconversion was initially observed by D14 similar to  
297 that previously reported [11]. At challenge at D42, 30% (9/30; intramuscular route) to 60%  
298 (6/10; intranasal route) of the vaccinated pigs had detectable anti-PRRSV-IgG levels, and 20%  
299 (6/30; intramuscularly route) to 30% (3/10; intranasal route) were SAVE5 viremic. It is possible  
300 that the SAVE5 vaccine virus was too attenuated to replicate and elicit an antibody response in  
301 all of the pigs or that the vaccine virus dose used for vaccination was too low. To account for  
302 this, vaccinated pigs were further divided into VAC-D42-POS and VAC-D42-NEG groups.

303 It is well accepted that antibodies detected in the serum shortly after infection do not  
304 necessarily correlate with protection, and that rapid induction of neutralizing antibody provides  
305 vaccine-based protection against infection [8;18]. However, a neutralizing antibody response was  
306 not detected in the present study. This may be due to antigenic differences between the viruses  
307 used in the FFN assay and the actual SAVE5 strain used. The percentage of amino acid identity  
308 of VR2385 was 92.5% for ISU-VDL strain ISU-P and 91% for the VR2332. It may also provide  
309 further evidence that detectable neutralizing antibodies may not be an ideal correlate of  
310 protection against PRRSV. Cell-mediated immunity is considered more important than  
311 neutralizing antibodies in conferring protection against PRRSV, and PRRS-specific T-cells can  
312 be observed as early as 2 weeks after infection [19]. In this pilot study cellular immunity was not  
313 assessed due to limited access to necessary reagents; however, cellular immunity needs to be  
314 addressed in future studies. In general, the best measurement of protective immunity triggered by  
315 an effective vaccine is in a challenge infection model [18]. Interestingly, VR2385 viremia and  
316 nasal shedding after challenge was not detectable in VAC-D42-POS pigs which all had  
317 seroconverted by the time of challenge. This could indicate that vaccine-induced seroconversion  
318 is associated with protection.



## Efficacy of the SAVE approach in protecting against PRRSV

319 Under the study conditions, the nasal vaccination route appeared to be more effective  
320 resulting in protective immunity in a larger number of pigs compared to the intramuscular route.  
321 The first pig with a SAVE5 viremia was detected by D7 in the VAC-IN-PRRSV group whereas  
322 the first SAVE5 viremic VAC-IM-PRRSV pig was detected by D14. At the day of challenge  
323 60% (6/10) of the VAC-IN-PRRSV pigs had seroconverted to PRRSV in contrast to 20% (4/20)  
324 of the VAC-IM-PRRSV pigs indicating an earlier and more efficient induction of a low grade  
325 SAVE5 viremia with subsequent development of an adaptive immune response after  
326 administration of the SAVE5 vaccine strain by the intranasal route. **Determination of possible**  
327 **differences between intramuscular and intranasal vaccination by assessing local mucosal**  
328 **immunity needs to be done in future.**

329 The VAC-D42-POS pigs were protected from subsequent VR2385 challenge as  
330 evidenced by lack of VR2385 viremia, VR2385 nasal shedding, significantly reduced  
331 macroscopic and microscopic lung lesions and significantly reduced amounts of PRRSV antigen  
332 in lungs compared to the non-vaccinated POS-CONTROL pigs. This indicates that the SAVE  
333 vaccine strategy may be a feasible alternative in rapidly producing a farm-specific autogenous  
334 vaccine if the immunogenicity of the SAVE vaccine can be improved. Additional experimental  
335 studies using larger number of pigs, a higher vaccine virus dose, different challenge time point  
336 post-vaccination, and heterologous PRRSV strains including concurrent infections are needed to  
337 better evaluate the benefits of this novel vaccine approach.

338

### 339 **5. Conclusions**

340 The present study further confirmed that the SAVE approach can effectively attenuate a  
341 PRRSV strain. Additional work needs to be done to further improve SAVE5 vaccine efficacy.

## Efficacy of the SAVE approach in protecting against PRRSV

342 The ability to utilize the SAVE technology to rapidly produce, safe and efficacious, farm-  
343 specific PRRSV vaccines is practical and could have a major impact on reducing the major  
344 economic losses associated with PRRSV.

345

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352

### 353 **Conflict of Interest**

354 The authors declare no financial and personal relationships with other people or organizations  
355 that could inappropriately influence this work.

356

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360

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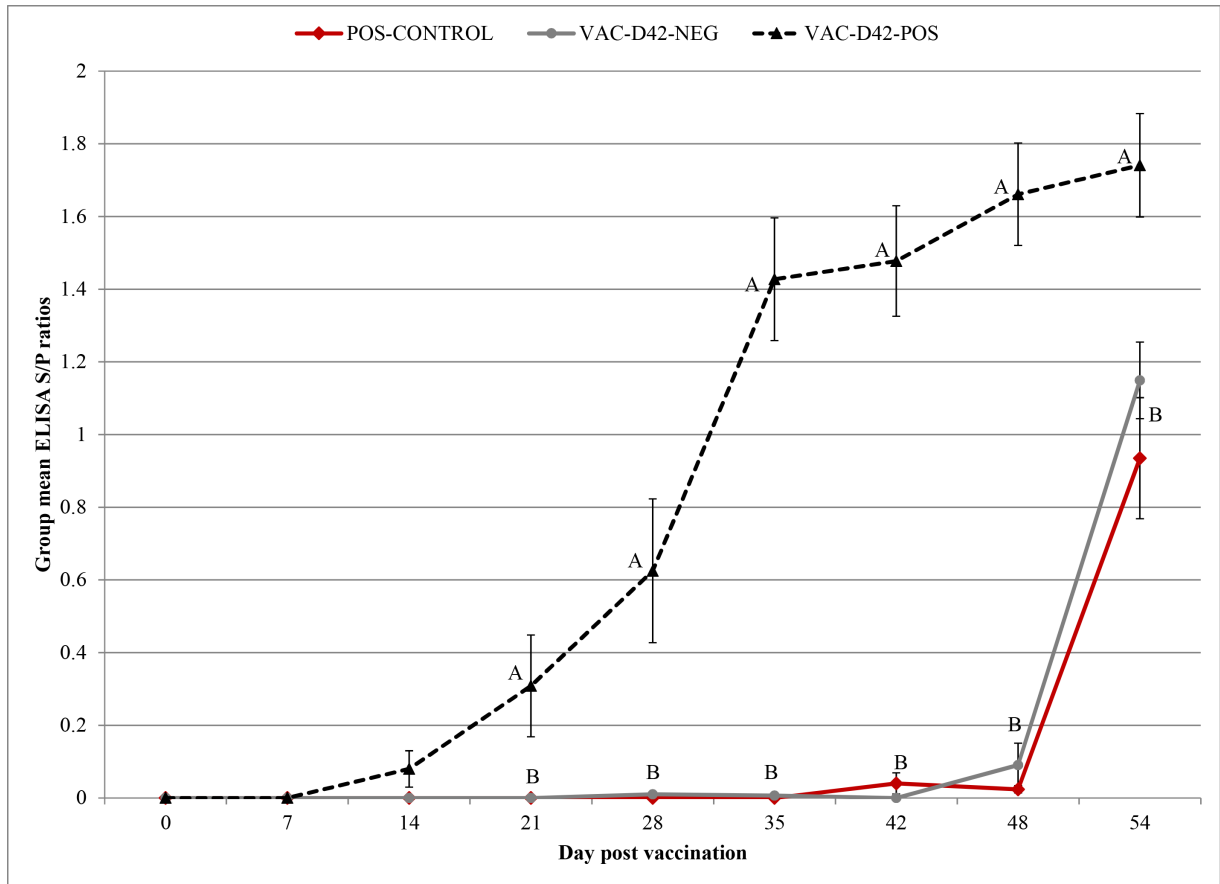
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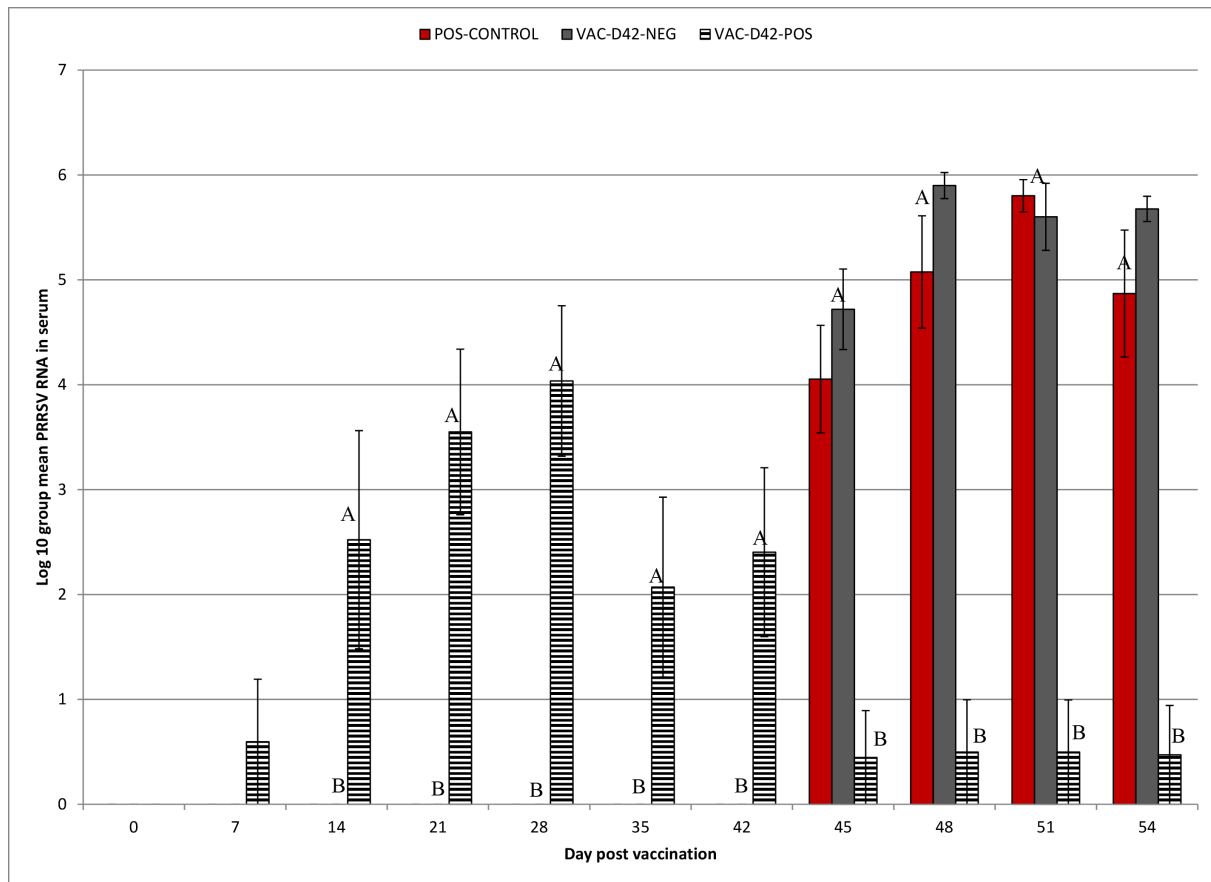
### 421 Figure Legends



422

423 **Fig. 1.** Group mean ELISA sample-to-positive (S/P) ratios over time in POS-CONTROL pigs  
424 (n=19), VAC-D42-NEG pigs (n=20) and in VAC-D42-POS pigs (n=10). Pigs were vaccinated at  
425 D0 and inoculated with PRRSV on D42 (arrow). An S/P ratio greater than 0.4 was considered  
426 positive. Different superscripts (<sup>A,B</sup>) indicate significantly ( $P < 0.05$ ) different group means for a  
427 certain day.

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428

429 **Fig. 2.** Group mean log<sub>10</sub> PRRSV genomic copies per ml serum over time in POS-CONTROL  
 430 pigs (n=19), VAC-D42-NEG pigs (n=20) and in VAC-D42-POS pigs (n=10). Pigs were  
 431 vaccinated at D0 and SAVE5-PRRSV and inoculated with PRRSV on D42. Different  
 432 superscripts (<sup>A,B</sup>) indicate significantly ( $P < 0.05$ ) different group means for a certain day.

433