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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

D. Evenson [a], P.F. Gerber [b], C.T. Xiao [a], P.G. Halbur [a], C. Wang [a], D. Tian [c], Y.Y. Ni [c], X.J. Meng [c], T. Opriessnig [a,b,*]

[a] Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA, USA
[b] The Roslin Institute and The Royal (Dick) School of Veterinary Studies, University of Edinburgh, Midlothian, UK
[c] Department of Biomedical Sciences and Pathobiology, College of Veterinary Medicine, Virginia Polytechnic Institute and State University, Blacksburg, VA, USA

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ABSTRACT

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

1. Introduction

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

The enormous continual change and diversity of PRRSV strains has resulted in limited efficacy of current commercial vaccines and vaccination strategies [8]. Modified live-attenuated virus

* Corresponding author at: The Roslin Institute and The Royal (Dick) School of Veterinary Studies, University of Edinburgh, Midlothian, UK.
E-mail address: Tanja.Opriessnig@roslin.ed.ac.uk (T. Opriessnig).

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vaccines (MLVs) are the most effective option currently available to control clinical signs associated with PRRSV infection; however, while these vaccines in general protect pigs well against homologous challenge they are not always capable of eliciting protective immunity against heterologous field strains [9]. Due to limited vaccine homology with circulating field strains on some farms, pig producers often rely on planned exposure to the pathogenic farm strain [10] which is risky and may not always be economically beneficial or acceptable from an animal welfare point of view. Therefore, rapid attenuation of PRRSV would afford an opportunity to quickly generate farm-specific vaccines.

Recently the synthetic attenuated virus engineering (SAVE) approach was utilized to rapidly attenuate the wild-type PRRSV isolate VR2385 [11]. Specifically, the codon-pairs of the major envelope GP5 gene of PRRSV were deoptimized through a computer algorithm which resulted in a modified GP5 nucleotide sequence while retaining the original amino acid sequence. The resulting virus was designated SAVE5. When SAVE5 was tested *in vitro* it was genetically stable. Experimental infection of pigs resulted in lower levels of viremia and reduced macroscopic and microscopic lung lesions compared to the wild-type VR2385 virus [11]. The protective efficacy of the SAVE5 was unknown. In the present study, the immunogenicity and protective efficacy of SAVE5 in decreasing clinical signs, lesions and viremia associated with wild-type PRRSV challenge were assessed using a conventional pig model.

2. Methods

2.1. Animals and housing

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

2.2. Experimental design

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

Table 1
Experimental design including number of pigs per group included in each replicate (R1 and R2), average daily weight gain (ADG), macroscopic and microscopic lung lesions, and PRRSV antigen load in lung tissues at D54 (12 days post PRRSV challenge). Different caps letters (A, B, C) within each column indicate significantly different group mean values.

Group	Number of pigs		Vaccination D0		Challenge D42		ADG[a] D42 to D54	Macroscopic lung lesions[b]	Interstitial pneumonia[c]	PRRSV antigen[d]
	R1	R2	Vaccine	Route	Challenge	Route				
NEG-CONTROL	9		Saline	Intramuscular	Saline	Intranasal	632.8 ± 35.3	0.0 ± 0.0^{A} (0)	0.4 ± 0.8^{A} (0, 0, 0.8)	$0/9\ (0.0 \pm 0.0)^{A}$
VAC-IM-CONTROL	10		SAVE5	Intramuscular	Saline	Intranasal	640.4 ± 42.1	1.6 ± 1.3^{A} (0; −1.5, 4.7)	$1.1 \pm 0.8^{A,B}$ (1, 0.7, 1.5)	$0/10\ (0.0 \pm 0.0)^{A}$
VAC-IM-PRRSV	10	10	SAVE5	Intramuscular	VR2385	Intranasal	633.6 ± 16.5	14.0 ± 2.5^{A} (13; 8.8, 19.2)	$1.9 \pm 0.2^{B,C}$ (2; 1.5, 2.3)	$16/20\ (1.1 \pm 0.2)^{B,C}$
VAC-IN-PRRSV		10	SAVE5	Intranasal	VR2385	Intranasal	620.3 ± 11.3	6.4 ± 1.4^{A} (2.5; 0.8, 12)	$1.4 \pm 0.4^{B,C}$ (1.5; 0.6, 2.2)	$5/10\ (0.6 \pm 0.2)^{A,B}$
POS-CONTROL	10	9	Saline	Intramuscular	VR2385	Intranasal	654.5 ± 54.8	27.4 ± 5.1^{B} (21; 16.8, 38.1)	2.1 ± 0.2^{C} (2; 1.5, 2.6)	$19/19\ (1.3 \pm 0.1)^{C}$

[a] Group mean ADG in grams ± SEM.
[b] Group mean macroscopic lung lesions ranging from 0 to 100% of the lung surface affected by lesions ± SEM (median; 95% confidence interval).
[c] Microscopic interstitial pneumonia lesions ranging from 0 = normal to 6 = severe and diffuse ± SEM (median; 95% confidence interval).
[d] Prevalence of PRRSV antigen and load ranging from 0 = absent to 3 = abundant as determined by immunohistochemistry.

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