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An interferon inducing porcine reproductive and respiratory syndrome virus vaccine candidate elicits protection against challenge with the heterologous virulent type 2 strain VR-2385 in pigs

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ABSTRACT

Achieving consistent protection by vaccinating pigs against porcine reproductive and respiratory syndrome virus (PRRSV) remains difficult. Recently, an interferon-inducing PRRSV vaccine candidate strain A2MC2 was demonstrated to be attenuated and induced neutralizing antibodies. The objective of this study was to determine the efficacy of passage 90 of A2MC2 (A2P90) to protect pigs against challenge with moderately virulent PRRSV strain VR-2385 (92.3% nucleic acid identity with A2MC2) and highly virulent atypical PRRSV MN184 (84.5% nucleic acid identity with A2MC2). Forty 3-week old pigs were randomly assigned to five groups including a NEG-CONTROL group (non-vaccinated, non-challenged), VAC-VR2385 (vaccinated, challenged with strain VR-2385), VR2385 (challenged with strain VR-2385), VAC-MN184 (vaccinated, challenged with strain MN184) and a MN184 group (challenged with MN184 virus). Vaccination was done at 3 weeks of age followed by challenge at 8 weeks of age. No viremia was detectable in any of the vaccinated pigs; however, by the time of challenge, 15/16 vaccinated pigs had seroconverted based on ELISA and had neutralizing antibodies against a homologous strain with titers ranging from 8 to 128. Infection with VR-2385 resulted in mild-to-moderate clinical disease and lesions. For VR-2385 infected pigs, vaccination significantly lowered PRRSV viremia and nasal shedding by 9 days post challenge (dpc), significantly reduced macroscopic lung lesions, and significantly increased the average daily weight gain compared to the non-vaccinated pigs. Infection with MN184 resulted in moderate-to-severe clinical disease and lesions regardless of vaccination status; however, vaccinated pigs had significantly less nasal shedding by dpc 5 compared to non-vaccinated pigs. Under the study conditions, the A2P90 vaccine strain was attenuated without detectable shedding, improved weight gain, and offered protection to the pigs challenged with VR-2385 by reduction of virus load and macroscopic lung lesions. Further work is needed to investigate different vaccination and challenge protocols, including routes, doses, timing and strains.

1. Introduction

Porcine reproductive and respiratory syndrome (PRRS) is one of the most important pig diseases in the United States and continues to cause significant economic losses for swine producers globally [1]. Its etiologic agent is the PRRS virus (PRRSV), a member of the *Arteriviridae* [2,3]. There are two established PRRSV genotypes that diverge by about 40%: PRRSV type 1 with the Lelystad virus (GenBank accession number M96262) as prototype strain and PRRSV type 2 with VR-2332 (GenBank accession number U87392) as prototype strain [2,4–6]. Within PRRSV type 2, there are at least nine lineages with three main categories including prototype, atypical, and high pathogenicity (HP) PRRSV isolates [7,8]. The PRRSV RNA genome is prone to continuous recombination and mutation events resulting in *quasispecies*, leading to the emergence of new heterologous variants, which can escape the established immune response [9,10].

While vaccines are often effective against homologous strains, heterologous protection is often difficult to achieve [11]. Several

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modified-live virus (MLV) vaccines are currently available [11]. The use of MLV PRRSV vaccines can be problematic as vaccine virus can be shed for up to 2 weeks and has the potential to revert to virulence under field conditions [12,13].

Recently an interferon-inducing PRRSV strain designated A2MC2 was discovered [14]. The A2MC2 genome is 99.8% identical to the Ingelvac PRRS® MLV strain (Boehringer Ingelheim Vetmedica) and to VR-2332, the parent strain of the Ingelvac PRRS® MLV vaccine [14]. The A2MC2 PRRSV strain has great potential as a live vaccine candidate because *in vitro* it has been shown to induce type I interferons (IFNs) in MARC-145 cells and also in primary porcine pulmonary alveolar macrophages [14]. Subsequent *in vivo* studies in pigs have shown that the IFN induction could be associated with production of PRRSV neutralizing antibodies, which were detected earlier and higher in titer compared to the Ingelvac PRRS® MLV strain [15]. The previous work also showed that serum samples obtained from pigs 7 weeks after A2MC2 infection contained neutralization antibodies against VR-2385, a moderately virulent PRRSV type 2 field strain [15]. A2MC2 has been serially passaged to passage 90 (A2P90) and the A2P90 virus retains the IFN induction feature and elicits higher neutralizing antibody levels than the Ingelvac PRRS® MLV strain [16].

The objective of this study was to determine if A2P90, a vaccine candidate, is safe and effective in protecting pigs against challenge with heterologous strains VR-2385 and MN184.

2. Materials and methods

2.1. Ethical statement

The Iowa State University Institutional Animal Care and Use Committee approved this study (Approval number 3-16-8220-S).

2.2. Pigs and experimental design

Forty 3-week-old pigs purchased from a PRRSV free herd were randomly assigned to five rooms with eight pigs in each group (Table 1). Water and feed were provided *ad libidum*. Vaccination or sham-vaccination was done at 3 weeks of age followed by challenge or sham-challenge at 8 weeks of age (Fig. 1). All pigs were euthanized 10 days post challenge (dpc). Blood was collected in serum separator tubes (Fisher Scientific, Pittsburgh, Pennsylvania, USA) weekly until challenge and on dpc 3, 7 and 9. Blood tubes were centrifuged at 3000*g* for 10 min at 4 °C and serum was stored at −80 °C until testing. Nasal swabs were collected on dpc 1, 3, 5, 7 and 9 using polyester swabs and stored in 5 mL plastic tubes containing 1 mL of sterile saline solution at −80 °C until testing.

2.3. Clinical assessment

All pigs were weighed at vaccination (dpc −35), at challenge (dpc 0) and at necropsy (dpc 10) and the average daily weight gain (ADG) was calculated. Rectal temperature and respiratory scores were assessed every other day starting on dpc −1 (Fig. 1) with scores ranging from 0 (normal) to 6 (severe respiratory distress when at rest) [17].

2.4. Vaccination

The vaccine strain A2P90 used in this study was derived after passaging A2MC2 90 times in MARC-145 cells [16]. The nucleic acid sequence identity between A2P90 and A2MC2 is 99.7%. At 3 weeks of age, pigs in the VAC-VR2385 and the VAC-MN184 groups were vaccinated intramuscularly in the right neck with 1 mL of A2P90 at 10^6 median tissue culture infectious dose ($TCID_{50}$).

2.5. PRRSV challenge

Two heterologous PRRSV type 2 strains were selected for challenge at 5 weeks after vaccination. Strain VR-2385 (GenBank accession number JX044140.1) has an overall nucleic acid identity of 92.3% with A2MC2 and the amino acid identity in GP5 is 89%. Strain MN184 (GenBank accession number AY656992) has an overall nucleic acid identity of 84.5% with A2MC2 and the amino acid identity in GP5 is 84%. The pigs in the VAC-VR2385 and the VR2385 groups were challenged intranasally with 1 mL of VR-2385 at a dose of 2×10^5 $TCID_{50}$. Similarly, pigs in the VAC-MN184 and the MN184 groups were challenged with 1 mL MN184 at a dose of 2×10^5 $TCID_{50}$. The intranasal inoculation was conducted by slowly dripping 0.5 mL of the inoculum into each nostril.

2.6. Serology

To assess the antibody response against PRRSV in serum samples, a commercial PRRSV ELISA (IDEXX PRRS X3 Ab Test; IDEXX Inc., Westbrook, MA, USA) was used according to the manufacturer's instructions. A sample-to-positive (S/P) ratio greater than 0.4 was considered positive. A fluorescent focus neutralization (FFN) assay for determination of amount of PRRSV-specific neutralizing antibodies was done on sera collected on the day of challenge (dpc 0) according to protocols routinely performed at the Veterinary Diagnostic Laboratory at Iowa State University. The PRRSV strain used for the FFN test was type 2 PRRSV strain ISU-P. In addition, FFN on dpc 0 serum samples was also done using PRRSV challenge strains VR-2385 and MN184 as described previously [15]. The nucleic acid sequence identities of ISU-P, VR-2385 and MN184 to A2MC2 strain are 97.7%, 92.3% and 84.5%, respectively.

2.7. Detection and quantification of PRRSV specific nucleic acids

Total nucleic acids were extracted from serum samples, nasal swabs and bronchoalveolar lavage (BAL) samples using an

Table 1
Experimental groups, treatments at different days post PRRSV challenge (dpc) and average daily weight gain (ADG).

Group designation	Number of pigs	Vaccination	Challenge	ADG[1]	
		dpc −35	*dpc 0*	*dpc −35 to dpc 0*	*dpc 0 to dpc 10*
NEG CONTROLS	8	Saline	Saline	487.4 ± 33.1	591.9 ± 34.5
VAC-VR2385	8	A2P90	VR-2385	403.0 ± 30.7	554.8 ± 41.4[a,2]
VR2385	8	Saline	VR-2385	451.1 ± 20.9	444.2 ± 30.4[b]
VAC-MN184	8	A2P90	MN-184	440.6 ± 21.4	376.0 ± 32.7[B]
MN184	8	Saline	MN-184	424.3 ± 28.3	298.6 ± 35.7[B]

[1] Data presented as group mean in grams ± SEM.
[2] Data were analyzed for each PRRSV challenge strain. Different superscripts within a column indicate significantly ($p < 0.05$) different group means between vaccinated and non-vaccinated pigs challenged with the same PRRSV strain (lower case is for pigs infected with VR-2385 and upper case is for pigs infected with MN184).

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