Pigs immunized with Chinese highly pathogenic PRRS virus modified live vaccine are protected from challenge with North American PRRSV strain NADC-20

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Modified live virus (MLV) vaccines developed to protect against PRRSV circulating in North America (NA) offer limited protection to highly pathogenic (HP) PRRSV strains that are emerging in Asia. MLV vaccines specific to HP-PRRSV strains commercially available in China provide protection to HP-PRRSV; however, the efficacy of these HP-PRRSV vaccines to current circulating NA PRRSV viruses has not been reported. The aim of this study is to investigate whether pigs vaccinated with attenuated Chinese HP-PRRSV vaccine (JXA-1) are protected from infection by NA PRRSV strain NADC-20. We found that pigs vaccinated with JXA1-R were protected from challenges with HV-PRRSV or NADC-20 as shown by fewer days of clinical fever, reduced lung pathology scores, and lower PRRSV virus load in the blood. PRRSV-specific antibodies, as measured by IDEXX ELISA, appeared one week after vaccination and virus neutralizing antibodies were detected four weeks post vaccination. Pigs vaccinated with JXA1-R developed broadly neutralizing antibodies with high titers to NADC-20, JXA1-R, and HV-PRRSV. In addition, we also found that IFN-α and IFN-β occurred at higher levels in the lungs of pigs vaccinated with JXA1-R. Taken together, our studies provide the first evidence that JXA1-R can confer protection in pigs against the heterologous NA PRRSV strain NADC-20.

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1. Introduction

Porcine reproductive and respiratory syndrome (PRRS) is a highly devastating swine viral disease, which causes more than $664 million in losses within the U.S. annually [1]. PRRS is caused by the porcine reproductive and respiratory syndrome virus (PRRSV), which is a member of the genus Arterivirus, family Arteriviridae. PRRSV infection leads to respiratory distress in pigs of all ages and reproductive failure in sows, and pigs infected with PRRSV have enhanced susceptibility to secondary microbial infections [2]. The North American prototypic strain of PRRSV, VR-2332, was isolated in 1987 [2]. Pigs infected with VR-2332 in an experimental setting display few clinical symptoms and only slight changes in lung pathology [3]. Modified live virus (MLV) vaccines based off VR-2332 (Ingelvac PRRS MLV) have been shown to provide complete protection from infection by VR-2332 [4]. However, these vaccines are only able to partially protect pigs from newly emerging heterologous PRRSV strains [5,6]. In 2001, the NADC-20 strain was first isolated in an “atypical PRRSV abortion storm” and, compared with earlier isolates, NADC-20 is more virulent as it can cause dyspnea, mild lethargy, and moderate proliferative interstitial pneumonia in infected pigs [7]. Today, NADC-20 remains one of the most virulent strains of PRRSV circulating in North America, which makes it a suitable challenge strain for PRRSV vaccine efficacy evaluation [8,9].

In 2006, highly pathogenic PRRSV (HP-PRRSV) strains first emerged in China, and infected pigs developed clinical signs including high fever (>41°C), anorexia, listlessness, red discoloration of skin, respiratory distress with very high morbidity and mortality...
rates [10]. Thus far, HP-PRRSV strains have been detected in most countries in East Asia including Cambodia, Laos, Philippines, Bhutan, Myanmar, Thailand, South Korea, and Russia [11]. JXA-1 (GenBank ID: EF112445.1), one of the earliest HP-PRRSV strains, was isolated in 2006 and shares 91% homology with the genome of VR-2332 (GenBank ID: AY150564.1). However, during the prevalence of HP-PRRSV from 2006 to 2009, commercial PRRSV vaccines based off the VR-2332 strain failed to protect pigs from HP-PRRSV infection. This challenge led to the development of the first HP-PRRS MLV (JXA1-R) from the JXA-1 isolate in China [12–14]. Subsequently, two additional commercial HP-PRRS MLV vaccines, TJM-F92 and HuN4-F112, were also introduced into the Chinese swine industry, all providing adequate protection of pigs to HP-PRRSV infection [15,16].

Although HP-PRRSV has not been detected in swine farms in the U.S., the possibility of an outbreak of this high virulent PRRSV in North America cannot be dismissed. This danger has become more relevant in light of recent epidemic of Porcine Epidemic Diarrhea Virus (PEDV) in North America [17–19], another high consequence swine virus that was widespread in Asia. Therefore, it is important to determine whether current commercial vaccines for HP-PRRSV (i.e. JXA1–R) are safe and effective against HP-PRRSV in a U.S. setting in order to prepare U.S. swine farms from a potential attack of HP-PRRSV.

Furthermore, compared with VR-2332, HP-PRRSV can elicit stronger immune responses as evidenced by a striking induction of cytokines associated with both innate and adaptive immunity in pigs infected with HP-PRRSV [16]. Therefore, it is possible that HP-PRRS MLV vaccines may also elicit a stronger immune response in pigs that can result in broader protection against heterologous PRRSV strains circulating in the U.S. To test our hypothesis, pigs vaccinated with JXA1–R were challenged with HV-PRRSV (a highly pathogenic strain of PRRSV from China) or NADC-20. Here we provide the first evidence that JXA1–R not only is safe and efficacious against HP-PRRSV in an experimental setting in the U.S., but also can protect pigs against virulent NA PRRSV strain NADC-20. This protection likely results from the induction of high levels of NADC-20-specific neutralizing antibodies and pulmonary IFN-α and IFN-β in pigs vaccinated with JXA1–R.

2. Materials and methods

2.1. Cells and virus

MARC-145 cells were maintained in modified Eagle’s medium (MEM) as described previously [20]. HV-PRRSV is a Chinese HP-PRRS virus isolated from the lymph node of an infected pig and sequenced in 2012 (GenBank accession no. JX317649) [21]. Construction of the plasmid encoding the infectious clone cDNA has been previously described [22]. This plasmid was transfected using Lipofectamine (Life Technologies) into MARC-145 cells. CPE was evident several days after transfection, and the virus was expanded for two more passages in MARC-145 cells. Virus harvested after passage three was used in the study. NADC-20 PRRSV was a kind gift from Dr. Kelly Lager (National Animal Disease Center, USDA-ARS, Ames, IA). JXA1–R (a HP-PRRS MLV vaccine) was a kind gift from Guangdong Dahanuong Animal Health Product Co., Ltd. VR-2332 and MN184A strains were described previously [3].

2.2. Pigs, vaccination and challenge

Thirty five conventional Large White–Duroc crossbred weaned specific-pathogen free piglets (3 weeks of age) were used for this study. The study protocol was approved by the Institutional Animal Care and Use Committee at Kansas State University. All piglets were confirmed sera-negative for antibodies to PRRSV by ELISA and PRRSV-free in the blood by RT-PCR. Pigs were allowed to acclimate for one week before initiation of the experiments. The 35 pigs were divided into seven groups (5 pigs/group) with three groups (15 pigs) housed within BSL-3Ag conditions at the Biosecurity Research Institute (BRI) and the other four groups (20 pigs) housed within BSL-2 conditions at the Large Animal Research Center (LARC) facility, Kansas State University.

For the 15 pigs housed in the BSL-3Ag facility, on day 0, five pigs in one group were immunized intramuscularly with a single dose of JXA1–R, and the other two groups of pigs were mock vaccinated with PBS as controls. Vaccinated pigs and controls were housed in different rooms. On day 28 post vaccination, 10 pigs (five pigs from the vaccinated group, five pigs from the control group) were challenged with HV-PRRSV (2 × 10^5 TCID50/pig), and these two groups of pigs were housed in separate pens in the same room. The other five control pigs were mock challenged with PBS and housed in a separate room. Half of the challenge virus or mock control was administered intramuscularly and half was administered intranasally. Pigs were monitored for rectal temperature for the first eight days after challenge. Pigs with rectal temperatures above 40 °C were considered to have fever [23]. Body weights were recorded and blood samples were collected every seven days after vaccination and every three days after viral challenge. All pigs that survived challenge were humanely euthanized at 10 days post challenge (DPC).

For the 20 pigs housed in the BSL-2 facility, on day 0, two groups of pigs (10) were immunized intramuscularly with a single dose of JXA1–R vaccine. The other two groups of pigs were mock vaccinated with PBS as controls. Vaccinated pigs and controls were housed in different rooms. On day 28 post vaccination, 10 pigs (one group of vaccinated and one group of control pigs) were commingled and then challenged with NADC-20 (2 × 10^5 TCID50/pig), the other 10 pigs were commingled and mock challenged with PBS and housed in a separate room. Half of the challenge virus or mock control was administered intramuscularly and half was administered intranasally. Pigs were monitored for rectal temperature for the first eight days after challenge. Body weight and blood samples were collected every seven days after vaccination and every three days after viral challenge. All pigs were humanely euthanized at 10 DPC.

Gross lung lesions were evaluated independently by two pathologists. Blood and lung samples were further processed for lung pathology, viral load, cytokine expression, PRRSV-specific antibodies, and PRRSV neutralizing antibody titer, as described below. Sera collected during a previous study [3] and stored at −80 °C were used to determine if pigs immunized with Ingelvac PRRSV MLV developed HP-PRRSV-neutralizing antibodies.

2.3. Serum isolation, ELISAs, virus quantitation

Serum was separated from clotted blood and preserved at −20 °C. Serum samples were used for evaluation of viral titer and PRRSV-specific antibody titers using IDEXX HerdChek ELISA kits as described previously [3]. Pig serum samples at 6 DPC and the supernatants of lung homogenates were used to analyze cytokine expression. Lung homogenates were prepared as described previously [16]. IFN-α and IFN-β ELISA kits were purchased from Abcam (Abcam, Cambridge, MA). The TNF-α ELISA kit was purchased from Invitrogen (Life Technologies, Carlsbad, CA). All ELISA procedures were performed as per the manufacturer’s instructions. The One-step Taq-Man qPCR was performed to calculate PRRSV RNA copy number in the serum sample according to manufacturer’s instructions (EZ-PRRSV™ MIPX4.0 Real Time RT-PCR, Tetracore Inc., Rockville, MD) as we described earlier [5].
2.4. Virus neutralizing titer in serum

Serum samples were heat inactivated (56 °C, 30 min) and serially diluted before the titration. The serial dilutions of serum were mixed with equal volume of PRRSV strains: VR-2332, MN184A, NADC-20, JXA1-R, and HV-PRRSV containing 200 TCID₅₀ of virus. After incubation at 37 °C for 1 h, the mixtures were transferred to MARC-145 monolayers in 96-well plates and incubated for an additional 72 h at 37 °C in a humidified atmosphere containing 5% CO₂. Cells were then examined for cytopathic effects (CPE). End-point titers were calculated as the reciprocal of the first serum dilution to eliminate 90% of the CPE resulting from the inoculation of 200 TCID₅₀ into wells of a 96-well plate. A neutralizing titer of 20 indicates that a 1 to 20 dilution of serum eliminated 90% of CPE in the wells relative to control wells. Each sample was assayed in duplicate. Animals with a titer of greater than eight are considered to be protected from viremia [24].

2.5. Lung pathology analysis

At necropsy, the lungs were macroscopically evaluated as previously described [25]. Briefly, the dorsal and ventral surfaces of each lung lobe were given a score representing the approximate proportion that was consolidated. Individual lobe scores were used to determine an overall lung score representing the percentage of the total lung that was macroscopically pneumatic. Postmortem findings and scoring of macroscopic lung pathology were done in a blinded fashion by two veterinary pathologists at the Kansas State Veterinary Diagnostic Laboratory.

2.6. Statistical analysis

All data were expressed as the mean ± SEM (5 or 10 pigs/group). The differences in the level of body temperature, lung pathology score, humoral response, cytokine production, and viremia among each group were determined by the one-way analysis of variance (ANOVA) followed by post-hoc Tukey's test using SigmaPlot 11 software (Systat Software Inc., San Jose, CA). Differences were considered statistically significant when p < 0.05.

3. Results

3.1. Pigs vaccinated with JXA1-R were protected from challenges with HV-PRRSV or NADC-20

Although JXA1-R is a commercial vaccine that has been widely used in China, its efficacy in the U.S. has not been reported before this study. Consistent with our previous report [26], HV-PRRSV caused significant mortality in young pigs (Fig. 1A). Under the BSL-3Ag setting in the current study, three of five unvaccinated pigs died within 6 DPC with HV-PRRSV. Compared to control and pigs vaccinated with JXA1-R, unvaccinated pigs had more days with high fever (>40.5 °C, Fig. 1B), lost significant body weight (Fig. 1C), and exhibited higher levels of viremia (Fig. 1D) after HV-PRRSV challenge. These results demonstrated that pigs vaccinated with JXA1-R were protected from HV-PRRSV challenge.

NADC-20 has been used for virus challenge to evaluate the efficacy of potential PRRSV vaccines in the U.S. [8]. We vaccinated pigs with JXA1-R on day 0 and challenged them with NADC-20 28 days post vaccination (DPV). In contrast to pigs challenged with HV-PRRSV, all pigs challenged with NADC-20 survived (Fig. 1E). We found that unvaccinated pigs challenged with NADC-20 developed clinical fever (>40°C) at 1, 5, 7, and 8 DPC (Fig. 1F), which is consistent with a previous study [8]. Unvaccinated pigs also had slightly reduced weight gain following challenge relative to vaccinated animals (Fig. 1G). Consistent with previous studies [20], vaccine virus circulating in the blood peaked at 14 DPV and returned to undetectable levels by 28 DPV (Fig. 1H). After HV-PRRSV and NADC-20 challenge, the circulating viral loads in all groups of pigs were at similar levels three days after challenge. However, by 7 DPC, the level of circulating HV-PRRSV (Fig. 1D) and NADC-20 virus (Fig. 1H) in surviving unvaccinated pigs was significantly higher than in pigs that had been immunized.

The body weight gain of pigs was monitored throughout the study. Interestingly, pigs vaccinated with JXA1-R had significantly (p < 0.05) lower body weight gain than that of unvaccinated pigs at three and four weeks post vaccination when they were housed at the BSL-2 facility (Fig. 1G). However, after NADC-20 challenge, the unvaccinated pigs challenged with NADC-20 showed minimal growth, while vaccinated pigs had significantly higher body weight gain during the challenge period. Thus, by the end of this study, no significant differences in body weight gain were observed among the three groups: pigs vaccinated with JXA1-R with or without NADC-20 challenge and unvaccinated pigs challenged with NADC-20. Notably, a significant difference in body weight existed between unvaccinated pigs and the pigs exposed to JXA1-R and/or NADC-20 at the end of this study (Fig. 1G). However, this vaccination-related impact on growth was not seen when pigs were housed at the BSL-3Ag facility (Fig. 1C).

3.2. Pigs vaccinated with JXA1-R had reduced lung pathology following PRRSV challenge

Pigs infected with HV-PRRSV showed more severe and extensive pneumonia than NADC-20 infected pigs, and the mean gross lung lesion scores for pigs challenged with HP-PRRSV and NADC-20 were 51% and 25.6%, respectively (Fig. 2). Lung damage in pigs vaccinated with JAX-1R and then challenged with HV-PRRSV or NADC-20 was significantly less than that in control pigs following PRRSV challenge (Fig. 2).

3.3. Pigs vaccinated with JXA1-R developed higher titers of PRRSV-specific IDEXX ELISA antibodies and NADC-20 strain-specific neutralizing antibodies

To monitor the humoral immune responses in pigs exposed to PRRSV virus, we measured serum PRRSV-specific antibody levels before and after vaccination and challenge. As shown in Fig. 3A, low levels of PRRSV-specific antibodies can be detected at 7 DPV only in pigs vaccinated with JXA1-R, with all vaccinated pigs seroconverting by 14 DPV. After NADC-20 challenge, all serum samples from unvaccinated-NADC-20 challenged pigs became PRRSV-positive with an average s/p value of 0.5 at 6 DPC (34 DPV). Unvaccinated pigs that were co-mingled with pigs that received the live vaccine but were never challenged had PRRSV-specific serum antibodies eight days after co-mingling (Fig. 3A).

To determine whether JXA1-R can induce a broad spectrum of PRRSV neutralizing antibodies, the titers of PRRSV neutralizing antibodies directed against NADC-20, JXA1-R (vaccine strain), and HV-PRRSV (a HP-PRRSV strain similar to JXA-1) were determined at 28 DPV and 10 DPC (Fig. 3B–D). Twenty-eight DPV, neutralizing antibodies to the three strains of PRRSV tested were not detected in serum from unvaccinated pigs. In contrast, serum from pigs vaccinated with JXA1-R contained measurable titers of NADC-20-neutralizing activity (>4, Fig. 3B), JXA1-R (>12, Fig. 3C), and HV-PRRSV (>8, Fig. 3D). Furthermore, neutralizing antibody titers to NADC-20 increased in vaccinated pigs following challenge and resulted in titers above 12 by 37 DPV (Fig. 3B).
Fig. 1. Pigs vaccinated with JXA1-R were protected from challenge with HV-PRRSV and NADC-20. Pigs were immunized with JXA1-R on day 0 and challenged with HP-PRRSV strain HV PRRSV or North American strain NADC-20 on day 28. Shown are survival rates of pigs after challenge with HV-PRRSV (A) or NADC-20 (E). Rectal temperature of all pigs was monitored daily after PRRSV infection with HV-PRRSV (B) or NADC-20 (F). Fold of total body weight gain during the study was calculated by considering the weight of the pig on day 0 as 1 (C and G). The copies of PRRSV RNA in serum samples were determined by qPCR (D and H). Data are shown as mean ± SEM for five pigs per group. * p < 0.05.
strains in Asia [27,28]. Here we present data showing vaccination with JXA1-R protects pigs from challenge with homologous HP-PRRSV and reduces viremia and lung pathology in pigs challenged with NADC-20, a heterologous A North American native PRRSV strain that often is used for vaccine efficacy evaluation in the U.S. [89]. JXA1-R has been shown previously to stimulate effective immunity against its parental virus [29]. Our report is the first to show that JXA1-R is safe and effective at stimulating immunity against commercially relevant North American PRRSV viruses in an experimental setting in the U.S.

Although current attenuated PRRS vaccines in the U.S. are effective against NADC-20, they can only provide partial protection against HP-PRRSV [30]. This phenomenon is consistent with the notion that PRRS vaccines are less effective in inducing cross-protection against viral isolates that are significantly different from the vaccine strains. Thus, we were surprised that JXA1-R, a HP-PRRSV-derived vaccine can provide effective protection against NADC-20, a non-HP-PRRSV strain that is genetically heterologous to JXA-1, the parental strain of JXA-1R.

The ability of JXA1-R to stimulate effective immunity against the heterologous NADC-20 likely results from two aspects of the virus’s biology. JXA1-R differs at 47 amino acids relative to its parental virus, and over half of these difference occur in the non-structural proteins (nsp) [29]. Though the exact role of all these mutations in the pathogenesis of the virus has not yet been determined, the role of the nsp in suppressing the innate immune response is well-established [31–33]. Mutations in genes coding for nsp likely account for our observation of increased IFN-α and IFN-β levels in the lungs of pigs immunized with JXA1-R (Fig. 4). The reduced ability of JXA1-R to disrupt the innate immune response ultimately results in a more effective adaptive response.

A significant portion of this adaptive response is directed against GP5. The GP5 proteins of JXA1-R (GenBank ABLE69002.1) and NADC-20 (GenBank AF988878.1) are both 200 amino acids long share 90.5% identity. Most of the differences between the two proteins occur towards the amino terminus of the protein, with 13 of the 19 differences occurring in the first 60 amino acids. These 60 amino acids constitute a large portion of the ectodomain of GP5 which comprises neutralizing epitopes [34–36], a decoy epitope [37], and multiple glycosylation sites [38–40]. Five variable sites that correspond to cross neutralization have been identified in GP5 of North American field isolates, and three of these sites (aa 32–34, 38–39, and 57–59) lie within the first 60 aa of GP5 [41]. JXA1-R and NADC-20 have aa sequence differences at each of the sites located in the first 60 aa, but the remaining two sites are conserved between the two viruses. Thus the ability of JXA1-R to stimulate heterologous immunity may result from the elicitation of antibodies that recognize these previously defined and conserved targets.

However, this proposed mechanism underlying the heterologous immunity elicited by JXA1-R does not explain why live vaccines based on North American strains of PRRSV, such as Ingelvac PRRS MLV, are unable to stimulate protective levels of neutralizing antibodies specific for HP-PRRSV strains (Fig. 3E) or significantly reduce lung pathology scores following challenge with HP-PRRSV [27]. Three of the 13 amino acid differences in the N-terminal of GP5 result in asparagine (N) residues in JXA1-R that are not present in NADC-20 (for a net increase of two N residues). These residues may create glycosylation sites that prevent this portion of JXA1-R GP5 from being a dominant target in the GP5-specific antibody response. Others have previously shown N-linked glycosylation in this region of GP5 to have a significant impact on the neutralizing antibody response [39,40] and that immunization with inactivated PRRSV bearing hypo-glycosylated GP5 results in enhanced immunity [42]. If the dominant neutralizing epitopes in the JXA1-R GP5 are glycan shielded, the shielding may focus the immune response toward the conserved epitopes elsewhere on the
Fig. 3. Pigs vaccinated with JXA1-R developed higher titers of PRRSV-specific IDEXX ELISA antibodies and NADC-20 strain-specific neutralizing antibodies. (A) PRRSV-specific antibodies in serum samples were measured with IDEXX HerdChek ELISA kits. The threshold for seroconversion was set at a sample-to-positive (s/p) ratio of 0.4 according to manufacturers’ instructions. (B–D) Serum from each pig was titrated in duplicate in MARC-145 cells for the levels of PRRSV neutralizing antibodies on 28 days post vaccination (28 DPV) or 9 DPC (37 DPV). End-point titers were calculated as the reciprocal of the first serum dilution to eliminate 90% of the CPE resulting from the inoculation of 200 TCID50 into wells of a 96-well plate. Each sample was assayed in duplicate. Animals with a titer of greater than eight are considered to be protected from viremia. (E) Pigs were vaccinated with Ingelvac PRRS MLV on day 0 and challenged with North American PRRSV strains VR-2332 or MN184A on day 28. The levels of PRRSV neutralizing antibodies on 28 days post vaccination [MLV (D28)] or 7 DPC [MLV + VR-2332/MN184A (D35)] were determined as above. Data are shown as mean ± SEM for five pigs per group. * p < 0.05.
Fig. 4. Immunization with JXA1-R results in increased pulmonary IFN-α and IFN-β levels but decreased TNF-α levels in pigs following challenge. Cytokine expression profiles in serum samples collected from pigs at 6 DPC and supernatants of lung homogenates at the necropsy (10 DPC) were tested by quantitative ELISA. Shown are the concentrations of IFN-α (A), IFN-β (B), and TNF-α (C). Data are shown as mean ± SEM for five pigs per group. * P < 0.05.

virus. Trujillo and colleagues have reported that glycosylation of immunodominant epitopes in caprine arthritis encephalitis virus can enhance vaccine-induced, cross-reactive neutralizing antibody responses [43]. In that study, the immunodominant epitope was not a neutralizing epitope and glycosylation redirected the immune response to a neutralizing epitope. For PRRSV, additional glycosylation in the immunodominant region of JXA1-R GP5, may redirect the immune response to subdominant, neutralizing epitopes that are shared with NADC-20 GP5. This scenario is a plausible explanation for the ability of JXA1-R to elicit NADC-20-neutralizing antibodies without North American strains eliciting a protective response against strains of HP-PRRSV circulating in China. Additional studies are required to confirm or disprove this hypothesis.

5. Conclusion

Our study provides the first evidence that JXA1-R can confer protection in pigs against NA PRRSV strain NADC-20. The availability of a safe and effective HP-PRRS vaccine such as JXA1-R in North America may not only increase the preparedness for a possible epidemic of HP-PRRSV in this region, but also offer extra tools to protect pigs against virulent PRRSV strains native to North America.

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