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Efficacy of Type 2 PRRSV vaccine against Chinese and Vietnamese HP-PRRSV challenge in pigs

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ABSTRACT

Porcine reproductive and respiratory syndrome virus (PRRSV) causes significant reproductive losses in the sow herd and respiratory disease in growing pigs. The virus belongs to the family *Arteriviridae* and there are two major genotypes. Type 1 is represented by Lelystad virus, the European prototype virus, and Type 2 is represented by the North American prototype virus, VR-2332. Depending on husbandry, immune status of the herd, and virulence of the isolate, the severity of disease and magnitude of economic loss can be variable. Vaccine use is not always successful indicating a lack of cross-protection between vaccine strains and circulating wild-type viruses. To date, there is no clear method to demonstrate if a vaccine confers protection against a specific isolate except for empirical animal studies. In 2006, a new lineage of Type 2 PRRSV emerged in Chinese swine herds that were suffering dramatic losses resulting in those viruses being described as "Highly Pathogenic PRRSV" (HP-PRRSV). Experimental reproduction of severe disease with HP-PRRSV isolates and virus derived from HP-PRRSV clones demonstrated the causal role of this virus. Recently, partial heterologous protection has been reported for Type 1 and Type 2 attenuated PRRSV vaccines against challenge by different Chinese HP-PRRSV isolates providing some hope for reducing economic loss. This paper reports the efficacy of a commercially available Type 2 attenuated vaccine in young pigs against heterologous challenge with a Chinese and Vietnamese HP-PRRSV isolate. When compared to unvaccinated pigs, vaccination decreased the length of viremia and viral titer, diminished the time of high fever and reduced macroscopic lung scores following homologous and heterologous PRRSV challenge. These results demonstrate the potential use of vaccine as an aid in the control of HP-PRRSV outbreaks.

1. Introduction

Porcine reproductive and respiratory syndrome (PRRS) is a disease of swine that causes reproductive losses in the sow herd and respiratory disease in growing pigs. It is caused by PRRS virus (PRRSV), a virus that was first isolated in 1991 in Western Europe, shortly thereafter in North America, and since then has been detected in almost all pork producing countries [1]. PRRSV is a single-stranded positive-sense RNA virus that is a highly mutable member of the Arteriviridae family. There are two recognized genotypes, Type 1 represented by Lelystad virus, the European prototype virus, and Type 2 represented by the North American prototype virus, VR-2332 [1]. Depending on husbandry, immune status of the herd, and virulence of the isolate, the severity of disease and magnitude of economic loss can be variable. Within a few years of the discovery of the virus, vaccines became available that could reduce economic losses and be used in PRRS control programs [1]. However, vaccine use was not always successful, indicating a lack of cross-protection between vaccine strains and circulating wild-type viruses, a phenomenon confirmed by experimental studies [2,3].

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To date, there is no clear method to demonstrate if a vaccine confers protection against a specific isolate except for empirical animal studies.

In 2006, a new lineage of PRRSV emerged in Chinese swine herds that were suffering dramatic losses resulting in those viruses being described as "Highly Pathogenic PRRSV" (HP-PRRSV) [4–7]. Analysis of the isolates revealed this virus was derived from endemic Type 2 PRRSV, and a common set of mutations (discontinuous deletions in nsp2) was identified that has been used as a molecular marker for HP-PRRSV isolates [4–7]. However, it is not definitive if the common mutations are related to the phenotype [8,9]. Following the initial Chinese reports, PRRSV isolates with similar nsp2 deletions were recovered from high mortality epidemics in contiguous countries indicating probable regional spread of the virus in Southeast Asia [10,11]. Experimental reproduction of severe disease with HP-PRRSV isolates [4–7] and virus derived from HP-PRRSV clones [8,12] demonstrated the causal role of this virus.

The HP-PRRSV isolates HuN4 and TJ have been attenuated by serial passage in cell culture, and when used as an intramuscular live vaccine, both provided protection against homologous challenge with their respective wild-type isolates [13,14]. To date, potential heterologous protection from HP-PRRSV vaccine candidates has not been reported. Recently, partial heterologous protection has been reported for Type 1 and Type 2 attenuated PRRSV vaccines against challenge by different Chinese HP-PRRSV isolates providing some hope for reducing economic loss [15,16]. This paper reports the efficacy of a commercially available Type 2 attenuated vaccine in young pigs against heterologous challenge with a Chinese and Vietnamese HP-PRRSV isolate confirming the potential for vaccine use in HP-PRRSV control.

2. Materials and methods

2.1. Animal study design

Animal studies were conducted under the approval of the Animal Care and Use Committee of the National Animal Disease Center, USDA-ARS, Ames, Iowa. Three-week-old weaned pigs ($n = 77$) from a PRRSV-free herd were used. Upon arrival each pig received an intramuscular injection of ceftiofur crystalline free acid per label dose (Pfizer Animal Health, New York, NY), was randomly identified by ear tag, and 25–26 animals were housed per isolation room for one week of acclimation. At four-weeks-of age pigs were allotted to one of seven equal groups that were housed separately in ABSL 2 isolation rooms. Three groups were vaccinated intramuscularly with an attenuated PRRSV vaccine according to label directions (Inglevac PRRS® MLV, Boehringer Ingelheim, Vetmedica, Inc., St. Joseph, Missouri, USA) and 4 groups were not vaccinated. At ten-weeks-of age (42 days-post vaccination), non-vaccinated non-virus challenge controls received a 2 ml intranasal inoculation with sham inoculum at 0-days-post challenge (dpc). Each remaining non-vaccinated group received a 2 ml intranasal challenge with either VR-2332, rSRV07, or rJXwn06 virus. Similarly, each vaccinated group (Vac) received a virus challenge with one of the three viruses. Prior to challenge, rSRV07 and rJXwn06 challenge groups were transferred to ABSL 3 isolation rooms.

Pigs were weighed on −1, 7, and 14 dpc; bled on −42, −21, 0, 4, 7, 11 and 14 dpc; and rectal body temperature was taken daily from 0 to 13 dpc. At 14 dpc pigs were scheduled for euthanasia and necropsy at which time the lungs were examined for macroscopic lesions and bronchoalveolar lavage fluid (BALF) was collected. Swabs of brain surface, pericardial fluid, and BALF were collected and tested for bacteria. Serum and BALF were tested for infectious PRRSV. Sections of lung were immersed in formalin and processed in a routine fashion for histology. Serum was tested for PRRSV antibody using a commercially available ELISA (IDEXX PRRS X3 Ab Test; IDEXX Laboratories, Maine, USA). A sample was considered positive for PRRSV antibody to if the sample-to-positive (S/P) ratio was equal to or greater than 0.4. All in vitro assays were performed and macroscopic and microscopic lesions were evaluated as previously described [17].

2.2. Challenge virus

Each challenge virus was administered intranasally at a dose of 2×10^6 $TCID_{50}$. Three wild-type challenge viruses were used: passage 6 of the Type 2 North American prototype PRRSV, strain VR-2332 [18] (GenBank U87392); passage 3 of a HP-PRRSV strain rJXwn06 that was rescued from a full-length cDNA of the JXwn06 virus [17]; and passage 3 of a HP-PRRSV strain rSRV07 rescued from a full-length cDNA of a 2007 HP-PRRSV strain detected in the Socialist Republic of Vietnam [10,19]. The viruses were propagated in MARC-145 cells and prepared for challenge inoculums as previously described [17]. A sham inoculum was prepared from virus-free MARC-145 cells.

2.3. Statistical analysis

Quantitative virus numbers in sera were analyzed using a mixed linear model for repeated measures with SAS 9.2 for Windows (SAS Institute, Cary, NC, USA). Linear combinations of the least squares means estimates for virus numbers were used in *a priori* contrasts after testing for a significant ($P < 0.05$) effect of vaccination on each PRRSV challenge strain (VR-2332, rJXwn06, rSRV07 and sham inoculated controls). Comparisons were made between vaccination status groups at each time-point using a 5% level of significance ($P < 0.05$) to assess statistical differences. To determine the effect of vaccination on growth performance, body weights of each pig were converted to a ratio of their starting weight at −1 dpc. The body weight ratios were analyzed using a mixed linear model for repeated measures with SAS 9.3 for Windows (SAS Institute, Cary, NC, USA). Linear combinations of the least squares means estimates for body weight ratios were used in *a priori* contrasts after testing for a significant ($P < 0.05$) effect of vaccination. The GLIMMIX procedure of SAS was used to determine least squares means for macroscopic and microscopic lung lesion scores, and log 10 transformed virus numbers in BALF. The LSMEANS and PDIFF statements provided estimates of least-squares means and differences ($\alpha = 0.05$) between them, respectively. Results were considered significant if $P < 0.05$. Geometric mean back transformations were made for presentation of viral titers as mean (±SEM). Logistical constraints prevented all sera from being tested by ELISA at the same time and statistical analysis was not applied to ELISA values because of plate-to-plate variation. ELISA values reported as mean (±SEM).

3. Results

3.1. Pre-challenge clinical response

All pigs were clinically normal from the beginning of the study (−42 dpc) until time of challenge (0 dpc). At −42 dpc all pigs were negative for PRRSV-specific antibody. All vaccinated pigs developed antibodies by −28 dpc and remained positive until 0 dpc (data not shown). All vaccinated pigs developed a viremia of variable duration post vaccination. At challenge, infectious virus could be isolated from the serum of 2 pigs in each of the 3 vaccinated groups (Table 1).

Table 1
Virus titration in serum and bronchoalveolar lung lavage (BALF).

Vaccination	Challenge[a]	dpc					BALF
		0	4	7	11	14	
No	Sham	0/0[b]	0/0	0/0	0/0	0/0	0/0
No	VR-2332	0/0	**3.6/11**	**3.3/11**	**2.6/8**	2.3/8	**4.1/11**
Yes	VR-2332	1.9/2	2.3/1	1.9/2	0/0	0/0	2.4/4
No	rSRV07	0/0	**4.0/10**	**3.4/11**	**4.6/11**	**3.8/8**	**5.6/11**
Yes	rSRV07	2.1/2	2.9/2	**3.1/6**	**4.6/7**	**2.7/8**	**4.6/9**
No	rJXwn06	0/0	**5.4/11**	**5.4/11**	**5.1(10/10)**[c]	**3.0(6/6)**	**4.9(6/6)**
Yes	rJXwn06	2.2/2	**5.3/11**	**4.3/11**	2.0(3/8)	(0/8)	**3.4(8/8)**

[a] Intranasal challenge at 0 days post challenge (dpc) with sham or virus inoculation. Vaccination was at −42 dpc.
[b] Numerator = mean log 10 virus titer for positive pigs/denominator = number of positive pigs out of treatment group consisting of 11 pigs. Value in Bold font significantly different from control.
[c] In parentheses numerator = number of positive pigs/denominator = number in treatment group following loss of pigs.

3.2. Post-challenge clinical response

Control pigs were clinically normal following sham challenge. Clinical differences between vaccinated and non-vaccinated challenge groups and among challenge virus groups were observed. In both rJXwn06 challenge groups pigs became listless and anorexic 1–2 dpc. Acute respiratory distress, characterized by dyspnea, tachypnea and coughing was common in rJXwn06 pigs beginning 1–3 dpc. Intermittent erythema of the skin was present in most of the pigs beginning 2–3 dpc, and several developed cutaneous petechial hemorrhages and cyanotic extremities (blue ears). Initially, pigs in the vaccinated group developed clinical signs and mortality sooner than the non-vaccinated pigs, but by 5–7 dpc the groups were indistinguishable and from 8 to 14 dpc the Vac/rJXwn06 group began to recover. In contrast, at 14 dpc the remaining rJXwn06 pigs were cachectic with most of them still having overt respiratory disease. Compared to the rJXwn06 challenged groups the magnitude of clinical disease was less in the SRV07-challenged pigs with the non-vaccinated more affected than the vaccinated pigs. Moreover, onset of disease for both groups was delayed several days and no mortality occurred during the experiment although several of the non-vaccinated/rSRV07 challenge pigs were moderately affected at the conclusion of the experiment suggesting that given time they may have succumbed to the infection. There was mild disease in the VR2332 challenge group recognized as increased respiration rates and intermittent anorexia beginning 4–5 dpc and lasting for about 7 days. No clinical disease was recognized in the Vac/VR2332 challenge group.

Clinical differences were mirrored in the rectal temperature data. There were significant differences in mean rectal temperature between non-vaccinated and vaccinated pigs in each challenge group that reflects the magnitude of clinical disease observed, i.e., the lower the rectal temperature the less overall disease (Fig. 1a–c). In the VR-2332 challenge groups, mean rectal temperature for the vaccinated pigs was indistinguishable from controls as was the clinical appearance of the vaccinated pigs. In contrast, severe disease was initially observed in both the rJXwn06 and Vac/rJXwn06 challenge groups during the first 6–7 dpc at which point the mean rectal temperature for Vac/rJXwn06 decreased as the pigs were clinically improving. However, the rJXwn06 challenged pigs continued to maintain high temperatures through 13 dpc and the clinical condition of the surviving pigs was deteriorating at 14 dpc, the scheduled end of the experiment. One rJXwn06 challenged pig was found dead on the morning of 12 dpc and 4 rJXwn06 challenged pigs were euthanized on 10, 11 (2 pigs) and 13 dpc. Three of 11 Vac/rJXwn06 challenge group pigs were euthanized on 7 (2 pigs) and 9 dpc. Although samples were collected from the 7 pigs prior to euthanasia, data from these animals were not included in the 14 dpc analysis. In part, the decline in rectal temperatures at the end of the experiment for the rJXwn06 challenge group is attributed to the severe disease that led to a moribund state. On average, the body temperature decreased 0.79 C in the 24 h preceding death for the five pigs that were euthanized or died 10–13 dpc. In contrast, the pigs that survived until scheduled necropsy had a 0.12 C increase in body temperature in the 24 h preceding euthanasia at 14 dpc. When compared to controls, there were no significant differences in the rate of weight gain among any of the groups at 7 dpc. At 14 dpc there was a significant decrease in weight gain in the rJXwn06 challenge pigs when compared to all other treatment groups including the vaccinated-rJXwn06 challenge group, even though this group did have sick pigs and mortality (data not shown).

Significant differences were found in the presence of macroscopic lung lesions. Similar to body temperature, the more clinically affected groups had more lesions than the less affected groups as well as vaccinated pigs had fewer lesions than non-vaccinated pigs (Fig. 2a). There was also a different character to the lung lesions depending on challenge group. In pigs that either died or were euthanized prior to the scheduled necropsy time at 14 dpc, macroscopic lung lesions consisted of well demarcated consolidated areas of lung that had a purple color with cranial-ventral to multilobular distribution. Cut sections of affected lung often sank in formalin. In addition, pulmonary edema was frequently noted as well as deposition of fibrin in the pleural and pericardial spaces. At 14 dpc lung lesions in the VR-2332 challenge group consisted of diffuse tan-brown discoloration of the lung surface with a predominant cranioventral distribution among multiple lobes. In the rSRV07 and rJXwn06 challenge groups, the lesions observed at 14 dpc were more severe consisting of lung lesions seen in the VR-2332 challenge group in combination with well demarcated consolidated areas of lung similar to pigs examined prior to 14 dpc. Similarly, a spectrum of microscopic pulmonary lesions was observed with the most severe in the rJXwn06 challenge groups and least in the VR-2332 challenge groups with vaccinated pigs having fewer lesions than non-vaccinated pigs (Fig. 2b).

Additional post-mortem findings varied between groups with the VR-2332 challenged pigs less affected than the rSRV07 and rJXwn06 challenged pigs, and with fewer lesions in vaccinated pigs compared to non-vaccinated pigs. The most severe macroscopic lesions were in the rJXwn06 challenged pigs which were similar to what has been previously reported for this isolate in 4- and 10-week-old pigs [17]. Lesions consisted of lymphadenopathy, thymic atrophy, peritoneal and pericardial effusions, renal petechiae, and fibrinous peritonitis with less severe lesions in the Vac/rJXwn06 group, and both rSRV07 challenge groups. Except for lungs and a slight increase in size of lymph nodes, no additional lesions were seen in the VR-2332 challenge groups.

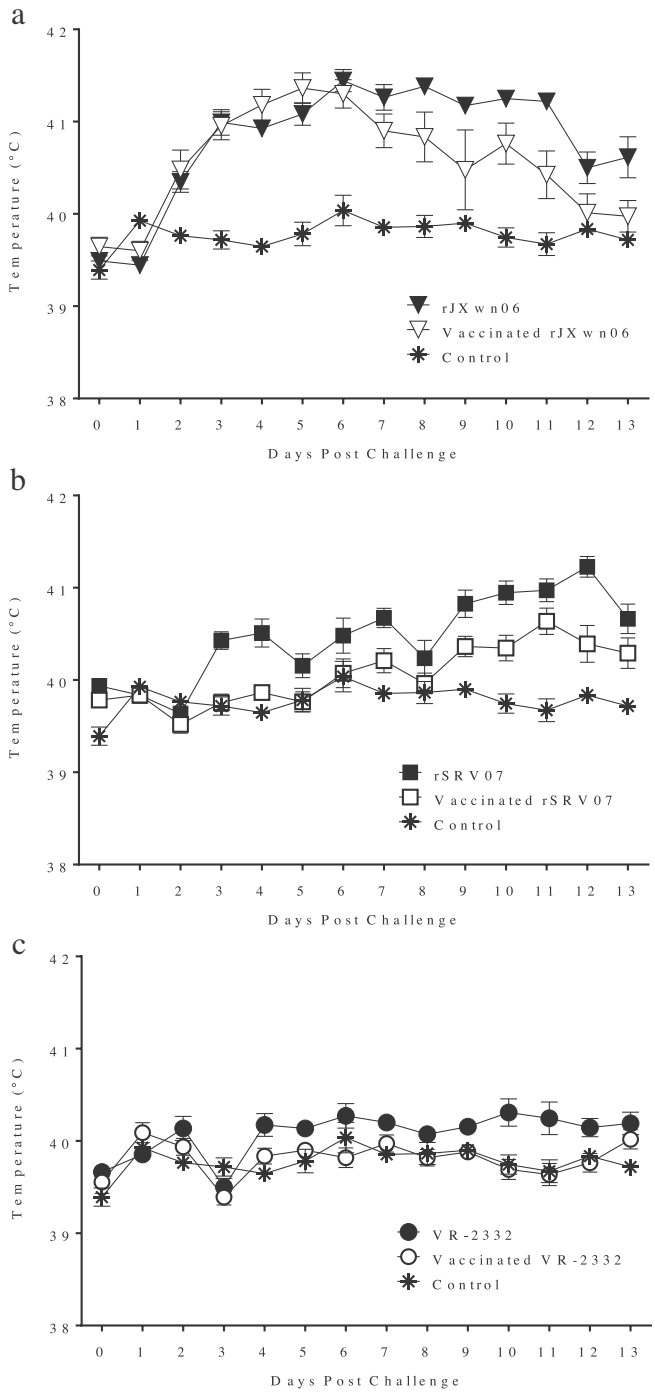

Fig. 1. Comparison of mean rectal temperature (±SEM) among vaccinated, non-vaccinated and control groups: (a) rJXwn06 challenge groups; (b) rSRV07 challenge groups; and (c) VR-2332 challenge groups.

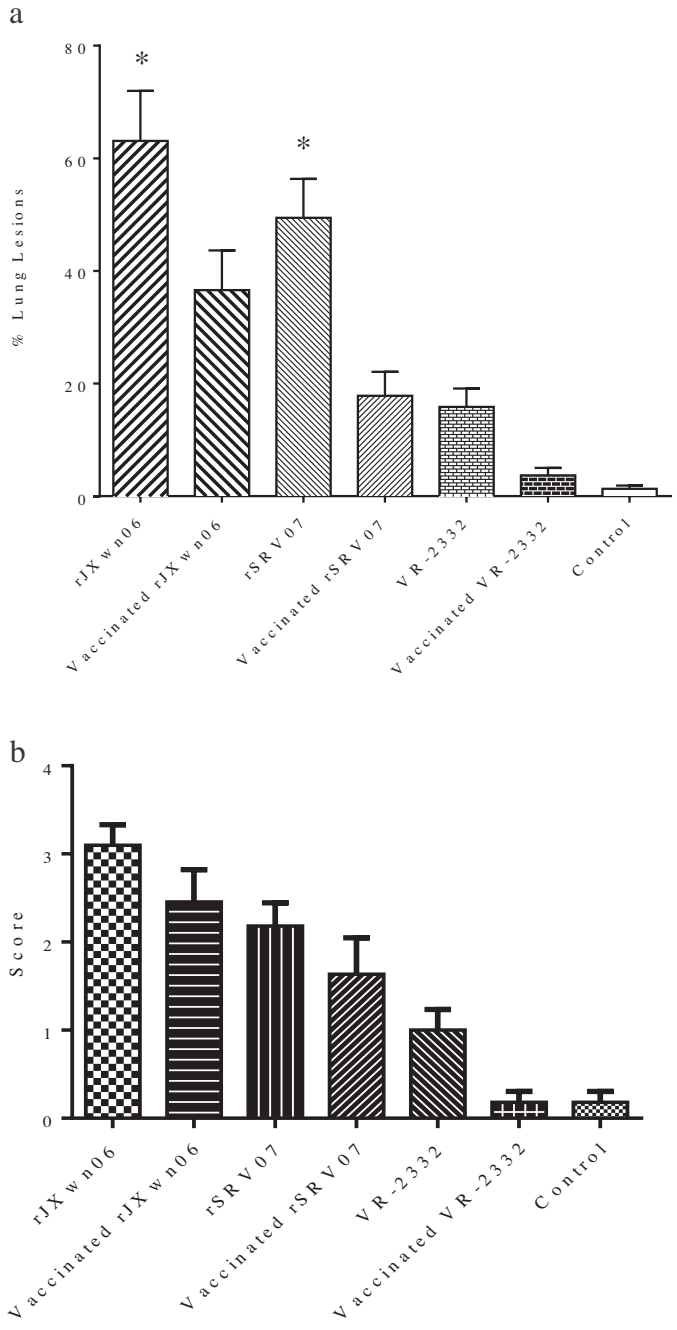

Fig. 2. (a) Macroscopic lung scores: significant differences ($P < 0.05$) between vaccinated and nonvaccinated challenge groups noted with *. (b) Microscopic lesion scores: no significant differences were found between vaccinated and non-vaccinated groups.

3.3. Humoral immune response detected by ELISA

The 0 dpc mean *S*/*P* ratio for each non-vaccinated group was 0.0; and by 14 dpc all pigs had developed antibody with the mean *S*/*P* ratio for the rJXwn06, rSRV07, and VR-2332 groups 1.58 ± 0.13, 0.96 ± 0.10, and 1.02 ± 0.14, respectively. The mean *S*/*P* ratio prior to vaccination at −42 dpc was 0.0 for each of the 3 groups and all pigs were positive by −21 dpc (data not shown). At 0 dpc the mean *S*/*P* ratio was 0.83 ± 0.14, 1.23 ± 0.10, and 1.20 ± 0.18 for the Vac/rJXwn06, Vac/rSRV07, and Vac/VR-2332 groups, and by 14 dpc had increased to 1.07 ± 0.13, 1.94 ± 0.17, and 1.62 ± 0.18, respectively.

3.4. Virus isolation from serum and BALF

No virus was isolated from the control group throughout the experiment. Virus was isolated multiple times from each non-vaccinated virus-challenged pig, and there were significant differences among challenge groups in the quantity of virus detected (Table 1). In vaccinated challenge groups virus was isolated less frequently from serum and significant differences in virus titer were found (Table 1). Isolation of virus from BALF was more

sensitive at 14 dpc than testing serum and vaccinated-challenged pigs had fewer positive pigs and lower virus titer when compared to respective non-vaccinated challenged pigs (Table 1). As for other clinical parameters measured, duration and magnitude of virus replication in serum and BALF were directly related to severity of disease.

3.5. Bacterial isolation from BALF

Haemophilus parasuis was isolated from the BALF of 2 pigs in the sham-inoculated control group. There was an increase in the number of bacterial isolations from the BALF of pigs infected with the Asian strains of HP-PRRSV. Among the non-vaccinated challenge groups, bacteria were isolated from 10/11 pigs challenged with rJXwn06 and 8/11 pigs challenged with rSRV07, but none of the pigs challenged with VR-2332. *H. parasuis* and *Streptococcus suis* were the bacteria most frequently isolated from pigs challenged with rJXwn06 or rSRV07. In addition, *Actinobacillus suis* and *Arcanobacterium pyogenes* were isolated from the BALF of 1 pig each challenged with rJXwn06. Vaccination did not change the isolation rate of bacteria from the BALF of pigs challenged with rJXwn06, as *H. parasuis*, *S. suis*, and/or *Pasteurella multocida* were isolated from 10 of these pigs. However, vaccination did appear to decrease the rate of secondary infection in the rSRV07 challenged pigs, as *H. parasuis* was isolated from the BALF of only 2 pigs in this group, a rate similar to the sham inoculated controls. *H. parasuis* was also isolated from the BALF of 2 of the vaccinated pigs challenged with VR-2332.

4. Discussion

The use of PRRSV vaccine reduced the clinical disease and virus load for each challenge virus in this study. As would be expected, the greatest protection was found in the VR-2332 challenge group which can be considered a homologous virus challenge since the vaccine used was derived from the VR-2332 isolate. In contrast, less protection was found in the heterologous challenge groups, the rJXwn06 and rSRV07 groups. Variable cross protection following heterologous challenge has been a common finding with PRRSV vaccines that has been attributed to antigenic differences between vaccine and challenge viruses [3,20,21]. This has also been recently reported with two HP-PRRSV isolates [15,16]. It is not clear what influence the pathogenic potential of an isolate may have on vaccine protection. Vaccines made from HP-PRRSV isolates attenuated through repetitive cell culture passage [13,14] induced homologous protection like previous attenuated vaccines made from less pathogenic viruses [20,22,23]. This suggests the pathogenic potential does not affect the capacity of the virus to induce homologous protection. To date, there are no reports of these HP-PRRSV vaccines being used in heterologous challenge studies and if they might confer a broader cross protection than less pathogenic isolates.

The clinical disease in the 10-week-old pigs following rJXwn06 challenge in this study was comparable to our previous study using the same virus in age-matched pigs [17]. Our results agree with the original JXwn06 report [8], the parental virus of the recombinant virus used in the present study. Severe disease leading to mortality has been reported with a number of wild-type HP-PRRSV isolates [4–7] as well as infectious clones [8,12] that induced a multi-organ disease with high fever that frequently led to death or the requirement of humane euthanasia. The course of the clinical disease in this study from rJXwn06 infection was compatible with what has been attributed to experimental and field HP-PRRSV infections. The seven pigs that were euthanized prior to the end of the study were not included in the 14 dpc analysis. Although they were more severely affected by the rJXwn06 challenge, and thus euthanized early (from 7 to 13 dpc), all of the pigs had similar virus load, weight loss, and lesion scores despite being vaccinated, or non-vaccinated prior to challenge.

The rSRV07 virus used in this study was rescued from RNA prepared from a 2007 Vietnamese isolate [19]. Clinical disease did occur in both rSRV07 challenge groups, but there was no mortality within the timeframe of the experiment. However, the clinical condition of the non-vaccinated rSRV07 group was deteriorating at the end of the experiment giving the impression that some pigs would have succumbed to the infection given more time. In comparison, pigs in the vaccinated rSRV07 group and the surviving pigs in Vac/rJXwn06 group were beginning to recuperate by 14 dpc. However, the rJXwn06 continued to maintain high temperatures through 13 dpc and their clinical condition was deteriorating at 14 dpc. In the original SRV07 report [10], experimental infection with filtered virus did not induce mortality during the course of the study, however, pigs became quite sick and developed lesions compatible with secondary bacterial disease. Based on our initial infection of 4-week-old pigs with the rSRV07 virus [19], and the current study in 10-week-old pigs, the rSRV07 isolate appears less virulent than the rJXwn06 virus. Additional studies are necessary to determine if these differences are related to actual virus isolate differences, or if production of the rSRV07 virus introduced attenuating mutations.

Since its discovery, PRRSV infections have been associated with an increased incidence of bacterial disease that is typically an exacerbation of endemic disease within the herd. This phenomenon also has been reported in HP-PRRSV field infections. Based on our experience with experimental PRRSV infections over the years, the rJXwn06 virus has a strong capacity to negatively affect the pig's homeostasis allowing subclinical bacterial infections to manifest as acute clinical disease. This was evident in the current study as well as in previous experiments with the same virus [17,19]. Under conditions of this study, the rSRV07 virus was less pathogenic which may be related to a reduced putative immunomodulating capacity. Both the rJXwn06 and rSRV07 viruses are more virulent than the VR-2332 virus which was first isolated in 1991. Based on clinical affect, the VR-2332 and JXwn06 viruses may represent opposite ends of the spectrum, and reflect increasing pathogenicity of the virus as it evolves in swine. The variable host response to a PRRSV challenge was demonstrated in this study with 8 of the 22 rJXwn06 challenged pigs euthanized or dying before 14 dpc as well as 8 of remaining vaccinated pigs appearing to begin recovery the end of the experiment.

Successful control and prevention of PRRS requires a combination of actions that has always involved changes in animal husbandry practices and the utilization of quarantine procedures. Frequently, attenuated vaccines have been used in successful control programs and based on the results of this and previous studies [15,16], it appears control strategies that have worked in the past may work against this lineage of virus. The collective efforts from this study and the work of many others demonstrates the complex PRRSV/host relationship, and reinforces the need for a better understanding of the pathogenesis and biology of this virus if there will be any improvements in PRRSV vaccines.

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