

2002

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## Recommended Citation

Nelson, C.D.; Rowland, R.R.; Zeman, D.H.; and Benfield, D.A., "Evaluation of a Macrophage Attenuated Isolate of PRRSV as a Vaccine for Porcine Reproductive and Respiratory Syndrome Virus" (2002). *South Dakota Swine Research Report, 2001*. 20.  
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## Evaluation of a macrophage attenuated isolate of PRRSV as a vaccine for porcine reproductive and respiratory syndrome virus

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**SWINE 2001-19**

PRRS continues to be the most economically important disease of swine. While the acute reproductive disease is still prevalent, chronic or endemic PRRS in nursery and grow/finish pigs is a major problem confronting most swine producers. Post-weaning problems in these herds include a 50-85% reduction in growth rates; a 10-30% increase in unmarketable pigs; and a 10-25% increase in post-weaning mortality. Popular protocols to manage PRRSV infections include breeding herd stabilization; elimination of seronegative sub-populations of susceptible gilts; nursery depopulation; and more recently mass vaccination/unidirectional pig flow in the grow/finish unit. Most of these control programs also use the commercial modified-live vaccines, RespPRRS<sup>®</sup> or PrimePac PRRS<sup>®</sup> as part of the management protocol.

Although modified-live PRRSV vaccines are useful management tools, producers and veterinarians are mindful of their undesirable traits and disadvantages, which include: induction of viremia; infection of fetuses in vaccinated pregnant animals; transmission of vaccine virus to naive pigs; persistence of vaccine virus in pigs; shedding of vaccine virus in semen; and the potential for vaccine virus to revert to virulence. These problems explain the recent popularity of using autogenous and commercial (PRRomiSe<sup>™</sup>) killed vaccines, which are safer than modified-live vaccines. However, many of the problems inherent to modified-live PRRSV vaccines are related to the ability of vaccine viruses to imitate virulent field viruses and replicate in macrophages, which results in: 1) dissemination of the virus in the pig; 2) shedding through bodily secretions; 3) transplacental transmission; and 4) persistence in lymphoid tissue. Both RespPRRS<sup>®</sup> and PrimePac PRRS<sup>®</sup> replicate in pig alveolar macrophages and this may partially explain why vaccinated pigs develop viremia and shed virus to contacts. It may also explain why the virus is able to reach the fetus in

pregnant animals. If replication in macrophages accounts for the undesirable traits of modified-live PRRSV vaccines, then a vaccine virus that is "macrophage-attenuated" (reduced or no replication in macrophages) would be safer. This vaccine can be produced in the conventional fashion; would be more economical to produce than molecular or subunit vaccines, and would avoid the loss of structural antigens (antigenicity), which is a problem with subunit and killed vaccines. The purpose of this study was to test two PRRSV isolates that replicate poorly in porcine alveolar macrophages, for safety and efficacy in young pigs and pregnant animals.

The goal of this project is to determine if an isolate of PRRSV, that has been modified by serial passage in monkey kidney cells (MARC-145) and replicates at very low levels in porcine alveolar macrophages, is avirulent for pigs and pregnant gilts. The original aims were to determine if this macrophage-attenuated isolate replicates in neonatal pigs, induces viremia and/or lesions and results in seroconversion.

### Experimental Procedures

Objective 1: Does the macrophage attenuated isolate of PRRSV 23983 replicate in neonatal pigs, induce viremia and/or lesions and cause seroconversion? The passage-136 (P136) isolate of PRRSV 23983 does not replicate well in alveolar macrophages, but does grow to high titers in the MARC-145 cells to which the virus is adapted. Thus, we compared the virulence of the parental wild-type virus passage-6 (P6) and the macrophage-attenuated P136 to the two commercial modified-live virus vaccines (RespPRRS<sup>®</sup> and PrimePac PRRS<sup>®</sup>). In these experiments, 58, 6-day old gnotobiotic pigs from three litters were inoculated either intranasally or intramuscularly with the P6, P136, commercial vaccines or mock inoculum. Each virus was adjusted to result in a dosage of  $10^4$  tissue culture infectious doses (TCID<sub>50</sub>) per

2 ml of inoculum. Piglets were observed daily for clinical signs and rectal temperatures were recorded for 14 days post inoculation (dpi) with virus. After 2 weeks, pigs were euthanized, examined at post-mortem for gross lesions and various tissues [lung, lymph nodes (trachealbronchial, mandibular, mesenteric, and external inguinal), salivary gland, heart, thymus, spleen, liver and tonsil] were removed for virus isolation and light microscopy examination for microscopic lesions. Serum was also collected at 0, 1, 3, 5, 7, 10 and 14 dpi for serology and virus isolation.

### Results

**Objective 1:** Does the macrophage attenuated isolate of PRRSV 23983 replicate in neonatal pigs, induce viremia and/or lesions and cause seroconversion? Our goal was to determine if the loss of the ability of PRRSV to replicate in alveolar macrophages would result in a virus that is less virulent in pigs than the current modified-live vaccines (RespPRRS<sup>®</sup> and PrimePac PRRS<sup>®</sup>). Sequential passage of the 23983 PRRSV on MARC-145 cells resulted in a reduction in the yield of PRRSV in alveolar macrophages. Fifty-four passages of the PRRSV resulted in only a 10-fold reduction in virus yield compared to the virulent P5. Similarly, there was a 100- and 1000-fold reduction in the yield of PRRSV from passages P94 and P136, respectively (Figure 1).

Comparison of the virulence of the macrophage-attenuated P136 isolate to the virulent P6 isolate of the 23983 PRRSV and the commercial modified-live viral vaccines was done using 58 gnotobiotic pigs randomly assigned to experimental groups indicated in Table 1. Daily clinical scores varied within experimental groups. Surprisingly, the pigs given PrimePac PRRS<sup>®</sup> intranasally had the most severe clinical signs between 4 and 8 dpi, after which the P6 group had the most prominent clinical signs from 8 to 17 dpi. Clinical signs included lethargy, inappetance, diarrhea, eyelid edema and occasional lacrimation. Milder clinical signs were observed in the P136 pigs for the first 5 dpi. Clinical signs of lethargy and lacrimation were also observed in 2/10 mock-infected pigs. In general, the P136 pigs had fewer and milder clinical signs of PRRSV compared to the other virus infected pigs.

There was no significant variation in daily temperatures between the inoculated groups of pigs. Temperatures were highest in the P6 inoculated pigs between 7 to 14 dpi. The rectal temperatures in the P136 pigs tended to be similar to those of pigs given the modified-live vaccine viruses.

Dyspnea (severe, labored breathing) was only observed in pigs receiving the P6 virulent isolate of PRRSV. This condition was principally observed from 10 to 18 dpi in pigs given P6 intranasally and in a one pig at 15 dpi given the P6 intramuscularly.

Pigs given the P6 PRRSV isolate had lesions typical of PRRS induced interstitial pneumonia in 9/10 animals and virus was isolated from all tissues sampled in 10/10 pigs. Less severe lesions were observed in the lungs of 1/10 pigs inoculated with PrimePac PRRS<sup>®</sup> and 5/15 given RespPRRS<sup>®</sup>. Similar to the P6 inoculated pigs, vaccine virus was isolated from all pigs (10/10 and 13/13 pigs, respectively). In contrast, virus was only isolated from 1/13 pigs given the macrophage-attenuated isolate and none of these pigs had lesions. Two mock-inoculated pigs also had early clinical signs of lethargy and lacrimation. No lesions were observed and no virus was isolated from tissues of the mock-inoculated pigs (see Table 1).

Seroconversion was monitored using the commercial enzyme-linked immunosorbent assay (ELISA) at 0 and 14 dpi. The pigs given the P6 inoculum either intranasally or intramuscularly all seroconverted by 14 dpi. In contrast, only 50% and 30% of the pigs given PrimePac PRRS<sup>®</sup> and RespPRRS<sup>®</sup> intramuscularly seroconverted by 14 dpi. A lower number of pigs receiving PrimePac PRRS<sup>®</sup> and RespPRRS<sup>®</sup> intranasally seroconverted at this time, 25% and 20%, respectively. None of the P136 or mock-inoculated pigs was seropositive at 14 dpi.

Significance of results from Objective 1. The above results indicate that the P136 macrophage-attenuated isolate is less virulent in pigs than either the P6 wild type or the commercial modified-live vaccine viruses. This is indicated by the less severe clinical signs, lack of febrile response and absence of lesions observed in pigs inoculated with the P136 PRRSV. The commercial vaccines did

replicate extensively in the gnotobiotic pigs and virus was isolated from most tissues of these pigs regardless of the route of inoculation. However, the P136 virus was recovered from the lung of only one pig inoculated intramuscularly indicating that there is still rare potential for reversion to virulence of the P136 isolate. There is probably less risk of transmission considering the lack of recoverable virus from tissues of the P136 pigs compared to

the other virus isolates. Thus, these results indicate that macrophages do play a significant role in the pathogenesis of PRRSV and that a macrophage-attenuated isolate of PRRSV is less virulent in young pigs. It was disappointing that the P136 isolate did not result in seroconversion of the pigs at 14 dpi indicating that this attenuated virus may be too avirulent to induce an immune response.

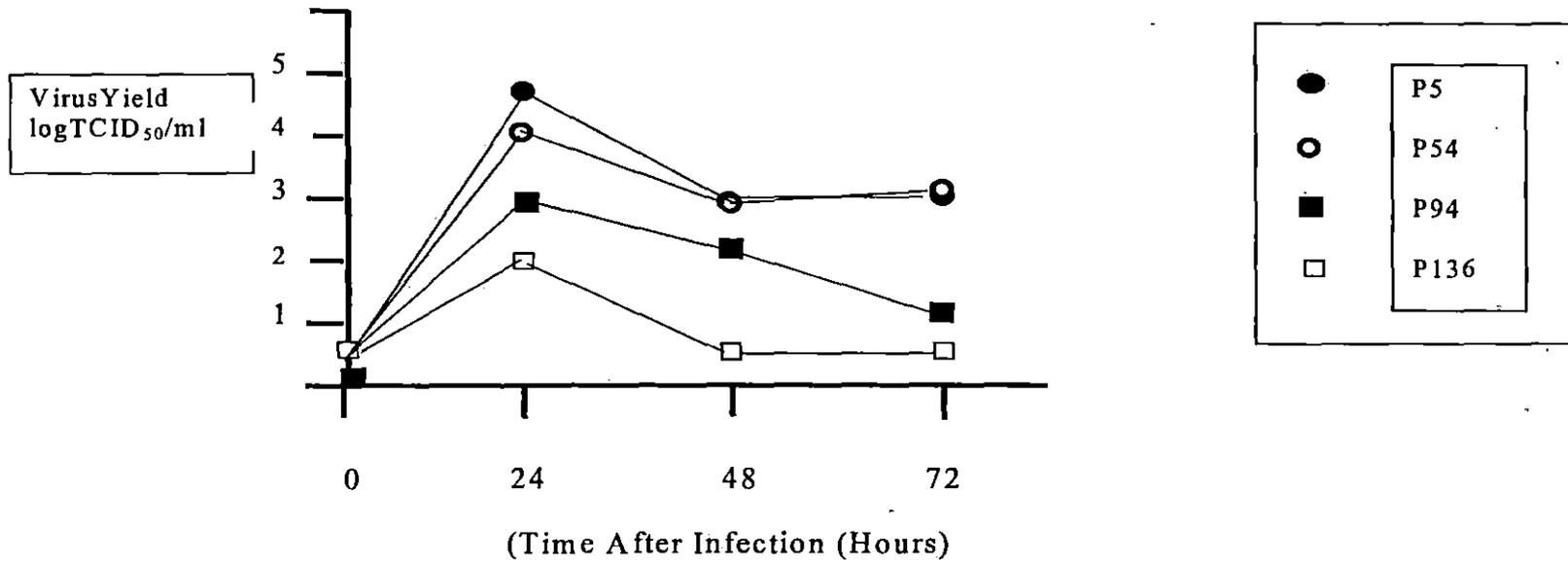
**TABLE 1. SUMMARY OF RESULTS FOR GNOTOBIOTIC PIGS INOCULATED WITH P6, P136, RESPPRRS<sup>®</sup> AND PRIMEPAC PRRS<sup>®</sup> VIRUSES**

RRPS virus	Route of inoculation	Number of pigs in group	Number of pigs with clinical disease	Number of pigs with lung lesions	Number of pigs positive for virus isolation (ELISA)
P6	intranasal	5	5/5	5/5	5/5 (5/5)
	intramuscular	5	5/5	4/5	5/5 (5/5)
P136	intranasal	6	1/6	0/6	0/6 (0/6)
	intramuscular	7	5/7	0/7	1/7 (0/7)
Prime-Pac	Intranasal	4	2/4	0/4	4/4 (1/4)
	intramuscular	6	6/6	1/6	6/6 (ND)
RespPRRS	intranasal	5	5/5	5/5	5/5 (1/5)
	intramuscular	10	6/10	0/10	10/10 (3/10)
Mock	intranasal	4	0/4	0/4	0/4 (0/4)
	intramuscular	6	2/6	1/6	0/6 (0/6)

Numbers in parenthesis in the last column indicate the number of pigs positive for antibodies by ELISA/number of pigs inoculated with virus.

## References

Dee, S. A. 1997. PRRS review. JUST PIGS, March issue, p. 9.



**Figure 1. Replication of various passages of the 23983 PRRS virus on alveolar macrophages. Note that each successive passage of the virus in MARC-145 cells resulted in a reduction or attenuation of the virus yield in porcine alveolar macrophages.**