RESEARCH REPORT

SWINE HEALTH

Title: Evaluation of a macrophage attenuated isolate of PRRSV as a vaccine for porcine reproductive and respiratory syndrome virus – **NPB #98-036**

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Date Received: 9/11/2000

I. **Abstract:** Porcine reproductive and respiratory syndrome virus (PRRSV) continues to be a major economic frustration to the swine industry. Despite well-intended management protocols designed to eliminate the virus from herds, many herds either revert to an active disease status or the virus persists in pigs for extended periods of time. Several of these management protocols involve the use of commercial modified-live virus vaccines. These vaccines in combination with certain management protocols are beneficial, when used judiciously by veterinarians and pork producers. However, these vaccines are not without risk and cannot be used in pregnant animals. In an effort to derive a safer vaccine, we produced macrophage-attenuated isolates of PRRSV, that are less virulent than commercial vaccines. In this study we tested two such isolates, P136 in gnotobiotic pigs and pregnant gilts and P83 in conventional pigs. These isolates produce either no disease or mild clinical signs in pigs and pregnant gilts. Pigs inoculated with these two isolates also have fewer lesions than pigs exposed to either virulent PRRSV or commercial modified-live vaccines. However, the P136 isolate may be too attenuated for use as a vaccine candidate as it does not produce an antibody response.

II. Introduction: 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.[1] 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® or PrimePac PRRS® as part of the management protocol.[1]

These research results were submitted in fulfillment of checkoff funded research projects. This report is published directly as submitted by the project's principal investigator. This report has not been peer reviewed

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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™) 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® and PrimePac PRRS® 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.

III. Objectives. The goal 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:

1. replicates in neonatal pigs, induces viremia and/or lesions and results in seroconversion;
2. can be given to pregnant gilts in late gestation and not cause abortions and stillbirths;
   and
3. protects fetuses when pregnant animals are challenged with virulent virus.

IV. 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® and PrimePac PRRS®). 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 ($TCID_{50}$) 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.

Objective 2. Can the macrophage attenuated PRRSV 23983 be administered to pregnant gilts in late gestation without infecting fetuses? Twelve gilts at 85-days of gestation were obtained from a commercial source and housed in separate isolation rooms in farrowing crates. Four gilts were intranasally inoculated at 90-days gestation with the P6 isolate of PRRSV 23983; four gilts received the P136 isolate; and four gilts were given a mock inoculum (tissue culture media). Each gilt was inoculated with 10^4 $TCID_{50}$ per 2 ml of inoculum. Gilts were then examined each day for clinical disease and allowed to farrow naturally. Liveborn piglets were observed for 7 days after birth for clinical signs of PRRS and then euthanized. Only 12 pigs (3 pigs per litter) from the mock-inoculated group of gilts were euthanized. The same tissues identified in Objective 1 were collected at necropsy for virus isolation and light microscopy. Blood was also collected from the gilts at 0, 1, 3, 5, 7, 10, 14 dpi and then at weekly intervals until farrowing. Blood was also collected from the piglets at 0, 1, 3, 5 and 7 days after farrowing. Sera from the gilts and piglets were used for serology and virus isolation.

Objective 3. Does vaccination of gilts pre-breeding protect fetuses from challenge with virulent PRRSV? Objective 3 was modified to a challenge trial in finishing pigs rather than pre-breeding gilts. Our rationale in doing this was to determine if there was any evidence of a protective immune response being induced by the P136 isolate. Pigs born to the mock-inoculated gilts used in Objective 2 were weaned at 19-days of age and maintained on a starter ration on raised platforms in isolation rooms. At 6-weeks of age, the pigs were randomly allotted by weight into four experimental groups of 7 pigs per group. All pigs received an inoculum of 10^4 $TCID_{50}$ per 2 ml of the 23983 virus as follows: Group 1 received the P6 virulent virus; Group 2 the P136 macrophage-attenuated virus; Group 3 the P83 passage of 23983; and Group 4 pigs received 2 ml of medium (mock infection). Pigs were then monitored daily for 10 dpi for temperatures and clinical signs of PRRS. Pigs were also bled at intervals of 0, 1, 3, 5, 7, 10, 14, 21 and 28 dpi. The sera were used for serology and virus isolation. At 28 dpi, all pigs were challenged intranasally with 10^4 $TCID_{50}$ per 2 ml of the virulent P6 23983 virus. Pigs were then monitored for temperatures and clinical signs for 10 days post-challenge (dpc). Pigs were bled at 0, 1, 3, 5, 7, 10, 14, 21 and 28 dpc. All pigs were euthanized at 28 dpc. The same tissues described for the gnotobiotic pigs in Objective 1 were collected at necropsy for virus isolation and light microscopy.

V. 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® and PrimePac PRRS®). 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® 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® and 5/15 given RespPRRS®. 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 intra-muscularly all seroconverted by 14 dpi. In contrast, only 50% and 30% of the pigs given PrimePac PRRS® and RespPRRS® intramuscularly seroconverted by 14 dpi. A lower number of pigs receiving PrimePac PRRS® and RespPRRS® 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.

Major benefit of results to pork producers. These results indicate that attenuation of PRRS viruses for macrophages can result in isolates that produce less clinical disease, no lesions and little replication in tissues of pigs. Thus, attenuation of PRRSV for macrophages provides a promising approach to the derivation of safer, live attenuated PRRS vaccines.

Objective 2. Can the macrophage attenuated PRRSV 23983 be administered to pregnant gilts in late gestation without infecting fetuses. In this experiment, we wanted to determine if the P136 isolate would infect fetuses in gilts intranasally inoculated with this virus at 90-days of gestation. The data is summarized in Table 2. The four gilts given this isolate developed temperatures (103 to 105 F) within the first 3 to 5 dpi and were off-feed for two to three days. The four gilts given the virulent P6 isolate farrowed only 50% of their litters as live-born pigs. Stillbirths and mummified fetuses accounted for 6% and 30% of the litters, respectively. One litter (gilt 94-74) consisted of only mummified pigs. In general, the live-born piglets had rough hair coats and were not as active as pigs born to gilts inoculated with either the P136 or mock-inoculum. Each of the gilts and piglets also had measurable antibody to the PRRSV. The antibodies in the piglets were probably from colostrum of the immune dam. Each of these pigs had lung lesions of interstitial pneumonia at 7 days after birth and virus was isolated from most tissues of these pigs. Thus, the virulent virus was capable of infecting fetuses.

Gilts intranasally given the P136 isolate did not show any clinical signs of fever or inappetance that were observed in the P6 inoculated gilts. All gilts farrowed live-born pigs with no stillbirths or mummified fetuses. Lung lesions were observed in only 1/30 (3%) of the piglets in these litters. Virus was isolated from the lung or lymph nodes of 4/25 (16%) of these piglets, compared to 100% for the piglets from gilts given the P6. Neither the gilts or the piglets had measurable antibodies to the PRRSV as determined by ELISA.

There was one stillborn pig born to one of the four gilts in the mock-inoculated group. These pigs were free of lesions, virus and antibody.

Significance of results from Objective 2. The P136 virus was definitely less virulent than the P6 for pregnant gilts and fetuses. The lack of fever and clinical signs in the P136 gilts and the absence of stillborns and mummified fetuses indicated that P136 had little effect on fetuses. However, P136 did retain the ability to cross the placenta because virus was isolated from 3/6 and 1/5 pigs in two litters and lesions were present in 1/7 pigs from a third litter, despite the absence of virus. Thus, the P136 isolate of PRRSV demonstrates promise as a candidate for a vaccine virus due to the lack of virulence for pregnant gilts

and fetuses. However, this virus may be "over" attenuated, as the gilts inoculated with the P136 isolate did not develop an antibody response measurable by ELISA.

Significance to the swine industry. These results indicate that attenuation of PRRSV for macrophages may result in a virus that is safer for use in pregnant gilts. Currently, neither of the commercial modified-live virus vaccines are safe for use in pregnant gilts at most stages of gestation.

Objective 3. Does vaccination of gilts pre-breeding protect fetuses from challenge with virulent PRRS virus? The lack of a measurable antibody response in the pigs given the P136 isolate of PRRSV in the above experiments was of concern. Therefore, we decided to modify objective three and test an earlier passage level (P83) of the 23983 in 6-week old
pigs rather than pregnant gilts. Our rationale was that the P83 virus that replicates at approximately a 10-fold higher titer than P136 in alveolar macrophages would have greater capacity to induce a measurable immune response.

Clinical signs were observed in 7/7 of the pigs intranasally inoculated with the P6 isolate of PRRSV. The most prominent clinical signs, eyelid edema and erythema of the ventral hindquarters, ears and neck, were observed between 5 and 10 dpi. Dyspnea was also observed in all of the P6 inoculated pigs during this time. Two of the seven pigs inoculated with the P83 PRRSV developed clinical signs similar to those described for the P6 inoculated pigs. No clinical signs were observed in the pigs inoculated with either P136 PRRSV or the mock-inoculum. Temperatures were slightly higher in the P6 pigs but as a group not significantly different.

At 28 dpi, 7/7 and 5/7 pigs inoculated with P6 and P83, respectively had seroconverted. No antibody was detectable by ELISA in any pigs given the P136 or mock-inoculum. Clinical signs were not observed in any of the pigs in the four experimental groups after challenge with virulent P6 PRRSV at 28 dpi. However, 7/7 and 4/7 pigs in the P136 and mock-inoculated groups did seroconvert 21 days after challenge. There was minimal change in the ELISA values of the pigs in the P6 and P83 groups after challenge.

Significance of results from Objective 3. These results indicated that the P83 isolate of PRRSV was less virulent than the P6, but more virulent than P136. Most of the pigs inoculated with the P83 isolate also demonstrated an antibody response on the ELISA. Unfortunately, the challenge results are difficult to interpret due to the lack of clinical signs in pigs in the mock-inoculated group.

Significance to the swine industry. We now have at least two candidate vaccine viruses for future experimental studies, the P136 and the P83 isolates of PRRSV. The reduced virulence of each of these isolates for pigs indicates that macrophages do contribute significantly to the pathogenesis of the disease and that attenuation of PRRSV for macrophage replication reduces the virulence of the virus.

VI. References

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

VII. Addendum to the Project

We are continuing studies on the P83 macrophage-attenuated isolate of PRRSV with
additional funding from the South Dakota Pork Producers Council.

VIII. Presentations and Publications

Gruber, R, M Steffen, DA Benfield and RRR Rowland. 1998. Sequence differences between passage 136 and a parental strain of PRRS virus. Fourth Annual Raymond A. Moore Biostress Poster Day, August 24, South Dakota State University, Brookings, SD.

Rowland, RRR, M Steffen, C Nelson and DA Benfield. 1999. Studies on the replication of SDSU-23983 P136, a macrophage attenuated PRRS virus in pigs. Proceedings of the 80th Annual Meeting of the Conference of Research Workers in Animal Diseases, November 7-8, 1999, Chicago, IL. Abstract 174.

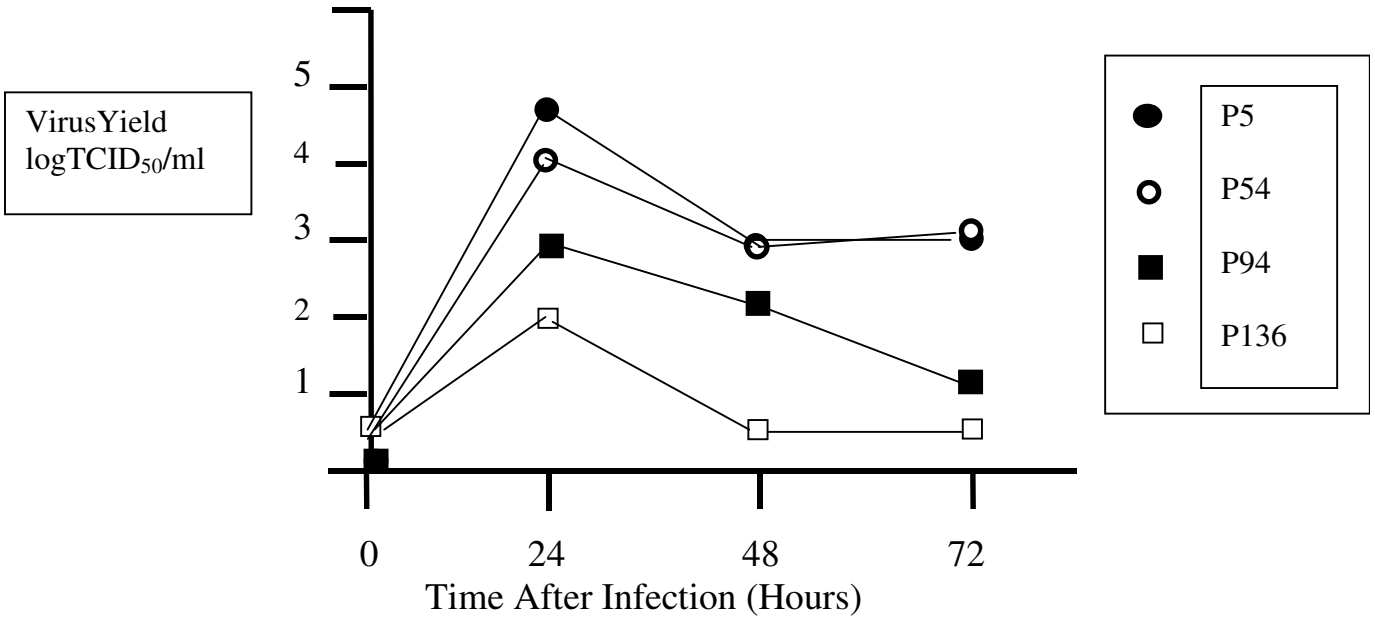

Figure 1. Replication of various passages of the 23983 PRRS virus on alveolar macrophages. Note that each successive passage of the virus in MARC-145 cells resulted in a reduction or attenuation of the virus yield in porcine alveolar macrophages.

Table 1. Summary of results for gnotobiotic pigs inoculated with P6, P136, RespPRRS® and PrimePac PRRS® viruses

PRRS 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.

Table 2. Results of inoculation of 90-day gestation gilts with virulent P-6, macrophage attenuated P-136 and mock inoculum

Gilt No.	Inoculum given intranasally	Number of live-born pigs	Number of stillborn/ mummified pigs	ELISA S/P value for gilt (no. of live-born pigs ELISA positive)	Number of pigs with lesions related to PRRS	Virus isolation from pigs (ELISA)
81-80	P6	4	1/0	0.84 (3)	3/3	3/3 (3/3)
81-81	P6	7	0/0	1.23 (7)	7/7	7/7 (7/7)
94-74	P6	0	0/10	0.51 (no liveborn pigs)	ND due to mummified fetuses	ND due to mummified fetuses
96-80	P6	4	4/0	0.87 (4)	4/4	4/4 (4/4)
	Totals	15 (50%)	5 (6%)/ 10(30%)		14/14	14/14 (14/14)
81-84	P136	10	0/0	0.16 (0)	0/10	3/6 (0/6)
81-88	P136	7	0/0	0.00 (0)	1/7	0/6 (0/6)
96-82	P136	5	0/0	0.00 (0)	0/5	1/5 (0/5)
96-85	P136	8	0/0	0.01 (0)	0/8	0/8 (0/8)
	Totals	30 (100%)	0/0		1/30	4/25 (0/25)
81-87	Mock	11	0/0	0.00 (0)	0/3	0/3 (0/3)
81-89	Mock	11	0/1	0.00 (0)	0/3	0/3 (0/3)
94-75	Mock	9	0/0	0.04 (0)	0/3	0/3 (0/3)
No Tag	Mock	12	0/0	0.19 (0)	0/3	0/3 (0/3)
	Totals	43 (98%)	0/1 (2%)		0/12	0/12 (0/12)

ND = not determined; Numbers in parenthesis in the last column indicate the number of pigs positive for antibodies by ELISA/number of pigs inoculated with virus.