1999 NPPC RESEARCH GRANT REPORT

I. In Vivo PRRSV Recombination Studies, NPPC #99-071

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II. Abstract:

The research focus of this grant was to explore whether PRRSV recombination would occur in freshly isolated porcine alveolar macrophages, the host cell, and in swine. Two vaccines, RespPRRS® and Prime Pac® PRRS were used to test this objective, as previous work done in this laboratory had shown that recombination between these two PRRSV strains occurred readily in immortalized MA-104 cells (5). In the course of this study, we found evidence that the two vaccine strains underwent recombination in host macrophages, but that because one vaccine grew more efficiently on macrophages and quickly overtook the dual-infected cells, the recombination events were less frequent using this cell culture method and the rate of recombination could not be estimated with any confidence. The results imply that viral recombination between two vaccines is an infrequent event in host macrophages. When both vaccines were coadministered (i.e., in the same syringe) into young swine, little nascent virus could be directly detected circulating in the hosts and therefore precluded any assessment of viral recombination. The outcome suggests that primary swine hosts of two different PRRSV vaccines do not immediately show evidence of viral recombination.

Nevertheless, evidence of viral recombination in the field has been documented (5). Therefore, it is imperative that the results obtained in this proposal be explained. Conceivably, later than 2 weeks (the sampling timeline for this proposal) viral recombinants may be detected, as persistence is a hallmark of PRRSV infection. Secondary hosts, infected by dual-vaccine shedding, may also provide a demonstration of PRRSV recombination. One might also surmise that highly virulent field strains, capable of replicating to a greater extent in swine, may have the capacity to undergo viral recombination at a pronouced rate.

III. Introduction

Porcine reproductive and respiratory disease virus (PRRSV) is still a leading cause of respiratory problems in infected piglets and abortions in late-term infected sows in spite of efficacious vaccines (1). Recently, more virulent strains of PRRSV have emerged and there are reports of virus spread and persistence of disease symptoms in vaccinated herds (3, 4). In response to the inability to rid some herds from PRRSV,

investigators have proposed a dual vaccination strategy of swine (Barry Wiseman, personal communication). In 1996, Kapur et al. suggested that PRRSV was evolving by processes other than simple accumulation of mutations and proposed that viral recombination may play a role in PRRSV evolution, although no reports of field recombination had been described (2). Our recent systematic study of dual infection and recombination, funded by the Minnesota Pork Producers Association and PIC USA, has revealed that recombination between two PRRSV vaccines occurs readily in a non-host continuous cell line (5). It is necessary to determine whether dual vaccination results in recombination events between the vaccine strains in the swine host, leading to potentially virulent virus progeny. This proposal extends the initial recombinant studies done in this laboratory to possible PRRSV recombination events in host alveolar macrophages and in swine. The results of this proposed research will aid in the refinement of vaccine strategies and may reveal a mechanism for the continuous emergence of new PRRSV strains. The proposed research will provide a firm knowledge base for understanding field experiences with PRRSV and vaccine use. The information gained from the outlined studies will help determine the value of dual vaccination and enable rational decision-making pertaining to herd management. These studies will aid in defining possible outcomes of PRRSV dual inoculation due to possible recombination events between RespPRRS® and Prime Pac® PRRS.

IV. Project Objectives

1) To assess if viral recombinants result from dual infections of freshly isolated porcine alveolar macrophages with PRRSV vaccine strains RespPRRS® and Prime Pac® PRRS.
2) To test if dual infection of 4-6 wk old pigs with vaccine strains RespPRRS® and Prime Pac® PRRS will result in detectable virus recombinants, and if recombination is evident in vivo.
3) establish potential altered viral growth properties on alveolar macrophages of mixed progeny virions.

V. Procedures

1) RespPRRS® (R, Boehringer Ingelheim Animal Health, St. Joseph, MO) and Prime Pac® PRRS (S, Schering Plough Animal Health, Kenilworth, NJ) were reconstituted from vials ($4 \times 10^{5.5}$ plaque forming units (pfu)/ml) and inoculated at 0.1 multiplicity of infection into freshly isolated porcine alveolar macrophages. Samples included strain R alone, strain S alone, strains R and S together and mock-infected. Infected cells were incubated at 37 °C until the cytopathic effect (CPE) was greater than 80%, at which time supernatants were harvested. Viral RNA was isolated from each infected cell supernatant, and subjected to RT-PCR analysis and agarose gel electrophoresis. Specific RT-PCR conditions available on request.

Table 1: Differential primers (R9/, S9/, /R13, /S13) amplify a 1182 base region from nucleotides 12886-14067, encoding part of ORF 3 through ORF 5, by RT-

PCR.

Primer	Sequence
S9/	CAGCGCTACGAACCTGGCAAGGT
R9/	GAGATCTACGAACCCGGTAGGTC
/**S13**	AACCAGACCAACTGTGTCAAGGAAG
/**R13**	GACTAAAGCGACTGTGTCAAGGAAA

2) The animal work described in this report was completed under the direction of Eric Nelson, Ph.D. at South Dakota State University. Pigs were inoculated with standard doses of vaccine strain S, vaccine strain R or co-infected with strains S and R mixed in the same inoculum and delivered by IM and IP injection. A prevaccination serum sample was collected, and then at 1, 2, 4, 6, 9, 13 and 17 days post-vaccination, 4 ml of serum was collected from each pig and stored at 4°C until analyzed by RT-PCR. At day 17, the pigs were euthanized and lung, tonsil and lymph nodes were obtained. Pigs #1-4 were inoculated with both vaccines, pigs #5-6 were inoculated with vaccine strain R alone, and pigs #7-8 were inoculated with strain S alone. RT-PCR was completed as described in aim 1. Because virtually no virus was detected in the serum, pigs #1-4 serum samples from day 2 p.i. were sent to UM VDL for possible viral detection by Taqman RT-PCR. Day 17 serum samples were tested for antibody titer by IDEXX Elisa at SDSU.

3) Because no in vivo viral recombination was detected, we could not complete this specific aim.

VI. Results

AIM 1: **To assess if viral recombinants result from dual infections of freshly isolated porcine alveolar macrophages with PRRSV vaccine strains RespPRRS® and Prime Pac® PRRS.** This objective was more complex than we anticipated. First, it was difficult to obtain lung macrophages that were not compromised by other nonviral infections, so we had to alter the source of pigs to obtain lung macrophages that supported PRRSV growth. In addition, the PRRSV infection conditions which we utilized to elucidate viral recombination in MA-104 cells did not prove successful when repeated on freshly isolated porcine macrophages. Thus, as each ex vivo macrophage preparation had unique PRRSV strain growth properties, several infection conditions were implemented in each experiment in order to uncover recombination between the two vaccine strains. The experimental protocol was repeated several times, yet only positive results are included in this final report.

Both vaccine strains are detected in swine macrophage supernatant. One question to be addressed was whether or not both vaccine strains would be detected in infected macrophage supernatant by RT-PCR. As shown in Figure 1, we could detect both vaccine strains, but the results suggested that we must decrease the amount of strain R inoculated onto the macrophages as compared to strain S, as strain R appears

to have replicated at a faster rate than strain S. Also, the RT-PCR conditions needed to be adjusted to eliminate the nonspecific “strain S band” (Panel R, lane 4) resulting from macrophages infected by strain R alone.

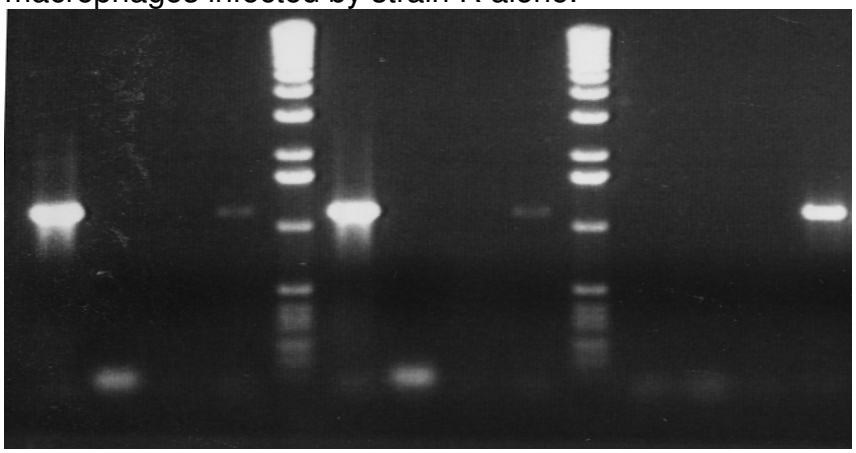

Figure 1. Reverse transcription and polymerase chain reaction (RT-PCR) analysis of first passage infected porcine alveolar macrophage supernatants. Panel R: infection of macrophages with strain R alone (m.o.i. 1); Panel RS: macrophages infection with both strain R and strain S (m.o.i. 1:1); Panel S: infection of macrophages with strain S alone (m.o.i. 1). Lane 1: primer pair R9/R13; lane 2: primer pair R9/S13; lane 3: primer pair S9/R13; lane 4: primer pair S9/S13.

Derivation of vaccine strain co-infection conditions to detect possible viral recombination. Several infection conditions were implemented in order to adjust for overgrowth of RespPRRS in macrophages and to detect recombination. Possible recombination was seen under two infection conditions (Figure 2, lanes 3 and 5). When strain S-infected macrophages (passage 1) were superinfected with strain R at day 4 p.i., possible RS recombinants were detected at day 5 p.i. Alternatively, dual infection of macrophages with strain S:strain R (m.o.i. ratio of 3:1, respectively) yielded possible SR recombinants at day 5 p.i. Control lanes 9 and 10 provide evidence that strain S is still detected after strain R is used to superinfect the same macrophage culture (lane 9) or when only strain S is used to infect the cells.

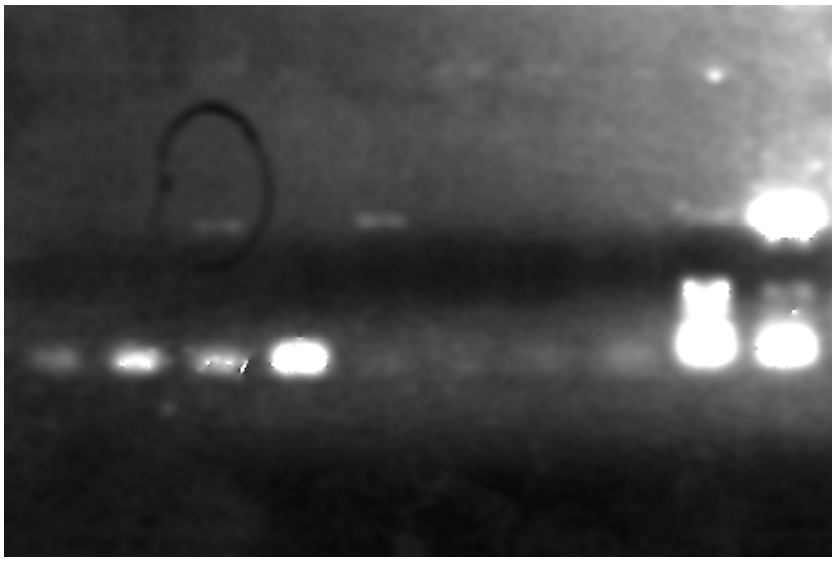

Figure 2. RT-PCR analysis of variable ex vivo macrophage infection conditions. Macrophage culture supernatants were harvested after PRRSV vaccine co-infection and subjected to RT-PCR analysis with primer pairs indicated. Lanes 1, 5: Strain S:strain R infected (m.o.i. ratio of 3:1, respectively) macrophage supernatant at day 5 p.i.; lanes 2, 6: A fresh macrophage preparation (passage 2) was reinfected with strain S-infected macrophage supernatant (passage 1). At day 4 p.i., strain R was used to superinfect the cells; lanes 3, 7, 9: Strain S-infected macrophages (passage 1) were superinfected strain R at day 4 p.i.; lanes 4, 8: S:R (m.o.i. ratio of 1:1) infected macrophage supernatant (passage1) was used to reinfect a fresh macrophage preparation (passage 2). Lane 10: Strain S was used to infect macrophages as a positive control. At day 5 p.i., culture supernatants were harvested and viral RNA purified before RT-PCR analysis.

Detection of viral recombinants on porcine alveolar macrophages. A new preparation of macrophages was infected according to the conditions derived from figure 2. As shown in Figure 3, some probable viral recombinants were detected under infection conditions where strain S infected macrophages were superinfected with strain R at 4 days p.i (Figure 3, Panel S:R (1,1), lane 2). The recombination seen was not easy to produce, which may indicate that recombination frequency is much less in macrophages than in the MA-104 cell culture previously utilized. As we had spent considerable time an effort in order to show that viral recombination could occur in freshly isolated porcine alveolar macrophages, we decided to address aim 2 rather than spend additional time attempting to increase evident ex vivo recombination events.

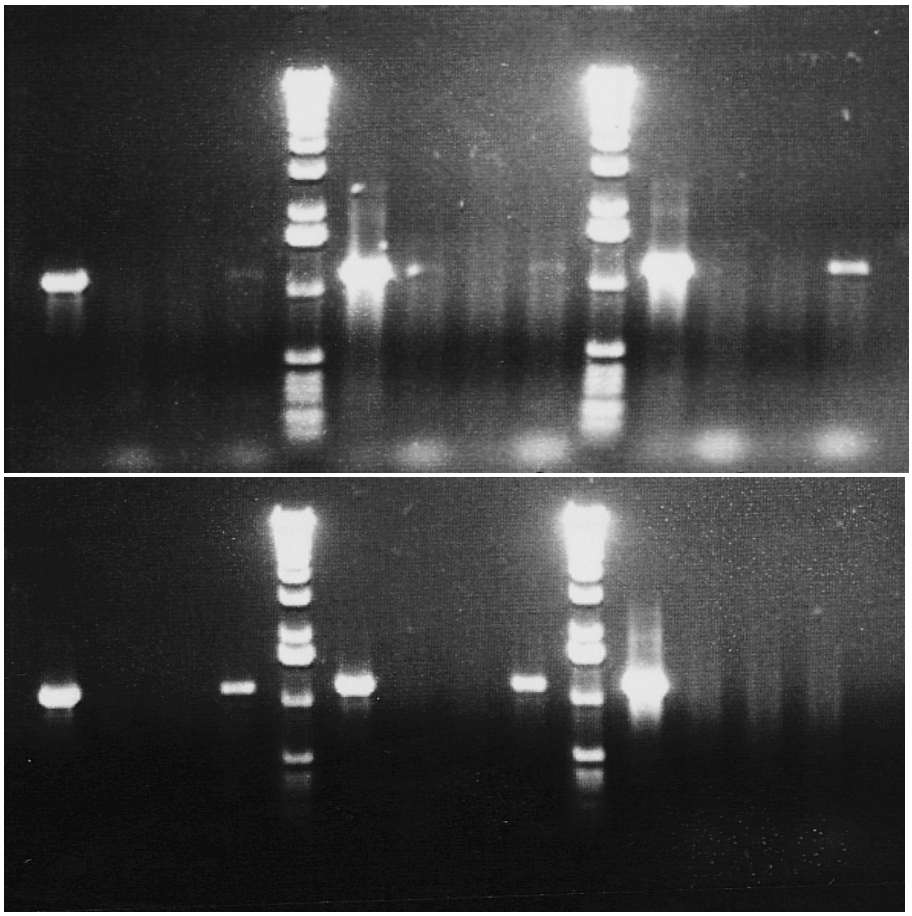

Figure 3. RT-PCR detection of probable viral recombinants of strain S and strain R resulting from co-infection of ex vivo porcine alveolar macrophages. Macrophage passaged vaccine virus strains (S and R, passage 2) were used to infect freshly isolated macrophages at three different conditions. Panel S:R (3:1): m.o.i. ratio of 3:1 (S:R); Panel S:R (1,1): Strain S infected macrophages were superinfected with strain R at 2 hour p.i.; Panel S:R (10:1): m.o.i. ratio of 10:1 (S:R); Panel S+R: Macrophages were infected with each vaccine strain alone, then supernatants were combined prior to RT-PCR analysis; Panel S: Strain S alone; Panel R: Strain R alone. Four days p.i., infected macrophage supernatants were purified and subjected to RT-PCR analysis using strain specific primers: Lane 1: R9/R13; Lane 2: R9/S13; Lane 3: S9/R13; Lane 4: S9/S13. A1 kb ladder was used to determine product length (M). (Note: Panel S, lane 1: Aberrant RespPRRS specific PCR product was probably due to sample contamination prior to RT-PCR. Note that viral recombinants (lanes 2 and 3) are not seen in this panel.)

Aim 2: To test if dual infection of 4-6 wk old pigs with vaccine strains RespPRRS® and Prime Pac® PRRS will result in detectable virus recombinants, and if recombination is evident in vivo. Table 2 documents the samples collected and tested by RT-PCR, Taqman RT-PCR, and IDEXX Elisa.

		Day Post Inoculation						
Sample	Inoculum	1	2	4	6	9	13	17*
Pig #1	SR	RT-PCR (-) TaqMan (-)	RT-PCR (-) TaqMan (-)	RT-PCR (-) TaqMan (-)	RT-PCR (-)	RT-PCR (-)	RT-PCR (-)	S/P 0.16
Pig #2	SR	RT-PCR (+ for strain S only)	RT-PCR (-) TaqMan (-)	RT-PCR (-)	RT-PCR (-)	NT	RT-PCR (-)	S/P 0.71
Pig #3	SR	RT-PCR (-)	RT-PCR (-)	RT-PCR (-)	RT-PCR (-)	RT-PCR (-)	RT-PCR (-)	S/P 0.09
Pig #4	SR	RT-PCR (-)	RT-PCR (-)	RT-PCR (-)	RT-PCR (-)	RT-PCR (-)	RT-PCR (-)	S/P 1.03
Pig #5	R	RT-PCR (-)	RT-PCR (-)	NT	NT	NT	RT-PCR (-)	S/P 1.04
Pig #6	R	RT-PCR (-)	RT-PCR (-)	NT	NT	RT-PCR (-)	NT	S/P 1.04
Pig #7	S	RT-PCR (-)	RT-PCR (-)	NT	NT	RT-PCR (-)	NT	S/P 0.36
Pig #8	S	RT-PCR (+ for strain S only)	RT-PCR (-)	NT	NT	NT	RT-PCR (-)	S/P 0.32

*IDEXX Elisa results obtained by SDSU

The lack of viral detection was not expected, and prevented further analysis of recombinant virus. The results suggest that recombination can not be seen in the primary host immediately after coinfection with both vaccines, which is of value. However, the likelihood of recombinants showing up in secondary hosts is not known and should be explored. In addition, the capacity of more virulent strains of PRRSV to undergo viral recombination should be investigated.

References

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