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# New data on homologous protection

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## Overview of homologous immunity

We have taken homologous immunity for granted. Porcine parvovirus (PPV), transmissible gastroenteritis virus (TGE), and pseudorabies virus (PRV) all stimulated immune responses that protected animals against any further challenge, possibly for life. Vaccines that were effective for one were effective for all. Even influenza virus followed this one-immunity-fits-all pattern (H1N1) for many years (until 1998). So initially we presumed (hoped) this would be the case for porcine reproductive respiratory syndrome (PRRS) virus as well. Early on there was no evidence to the contrary. Experiments on the completeness and duration of immunity against reproductive PRRS indicated that the immunity was complete and of long duration—over 2 years (Lager et al., 1997). It was standard research procedure to challenge with the same isolate of virus. Anecdotally, naive herds that became infected with PRRSV where most sows showed clinical signs/seroconverted have been reported to return to excellent levels of production, both reproductive and growing, approximately six months later. This seems to suggest a protective homologous immunity was achieved for the herd. However, there was at least one clinical report that suggested that homologous immunity may not be complete (McCaw, 1996). A very large herd in North Carolina had initially diagnosed PRRS at the beginning of 1993 and was still experiencing late term abortions, weak-born viremic piglets, and elevated preweaning—as well as nursery—mortality rates in May 1994. The herd produced all of its own replacement production gilts and purchased grandparent boar and gilt replacements quarterly from a single source. All production gilts were raised through the PRRS-affected nursery. All sows and developing gilts were being fed a mash produced from aborted and weak-born pigs. Sows were fed the mash during lactation, gilts weekly from 10 to 22 weeks of age in the continuous flow developer. The late-term abortion/weak-born piglet problem ended 2 months following cessation of the feedback program. This certainly was not proof that feeding PRRS virus-laden tissues could overwhelm protective immunity under production conditions, but it was suggestive. More recently, anecdotal comments suggest that injection of pregnant sows in endemically infected herds with viremic serum can cause illness and abortion. Whether these were due to an overwhelming of homologous immunity, infection of a susceptible subpopulation, or injection with an heterologous virus is not certain. The feed-back observation coupled with reports that a significant proportion of animals went seronegative on the PRRS ELISA following multiple vaccinations lead to the following study.

## Current research results

### Experiment one

#### Objectives

An experiment was designed to investigate the PRRSV ELISA antibody responses following multiple homologous virus exposures and challenge in breeding age barrows. The completeness of homologous immunity was evaluated by testing serum of these animals after final challenge using low or high doses of the original virus.

#### Materials and methods

**Viruses**

Wild-type SD23983 strain PRRSV was used for multiple injections and homologous challenge as well as neutralizing antibody assay. Powell strain PRRSV (acute PRRS isolate) was used for heterologous challenge.

**Assays**

Serum samples were tested using both the IDEXX HerdChek (IDEXX, Westbrook, Maine, USA) at Rollins Veterinary Diagnostic Laboratory, Raleigh, North Carolina. Serum neutralization (SN) antibody was measured by fluorescent focus neutralization assay at South Dakota State University Veterinary Diagnostic Laboratory. Nested PRRSV rtPCR for ORF 7 (first 5 samples post-challenge only) was performed in our laboratory using standard procedures.

**Phase I: Repeated exposures**

Sixty-eight (68) PRRS-negative 6-month-old barrows were used for this project. They were injected twice, one month apart, then every other month approximating a 6/60 type schedule for a total of 6 immunizations using $10^{2.5}$ field strain SD 28983 PRRS viruses per dose. They were bled 3 weeks following each immunization and tested for PRRSV ELISA and SN antibody. Four months after the last immunization (12 months after initial exposure) the

animals were divided into challenge treatment groups based upon current PRRSV ELISA levels (<0.4 or >1.2 on original HerdChek assay).

**Phase II: Homologous challenge**

Three treatment groups plus negative and positive controls were used for the homologous challenge phase of the study. Only 11 of 55 pigs were ELISA seronegative (<0.4 S/P) 4 months after the end of the 6/60 infection protocol, therefore only 2 experimental groups were possible for challenge. Challenge tissue was generated by infecting three-week-old MEW piglets (weaned at 5 days of age) with $10^{2.5}$ $TCID_{50}$ 28983 strain PRRSV. These piglets were euthanized by lethal injection 5 days later for collection of lung tissue that was homogenized in infected piglet serum and held at 4˚C. Two treatment groups of five multiple-immunized ELISA seronegative pigs each were challenged with either $10^{2.5}$ $TCID_{50}$ PRRSV intranasally (low dose) or orally with 40 grams of fresh infected MEW piglet lung homogenate plus 2 ml of this serum IM (high dose). A third multiple-immunized group of the 6 highest ELISA S/P ratio positive pigs (seropositive challenge controls) were challenged with high dose 28983 PRRSV. Controls included 1 immunized seronegative non-challenged control, 4 non-immunized pigs that received $10^{2.5}$ $TCID_{50}$ SD 23983 IN, or 3 that received no challenge at all. The pigs were bled at 1, 2, 3, 5, 7, and 14 days post-challenge.

**Results**

**Phase I**

In this study 20% (11 of 55) of the breeding aged animals were seronegative (S/P ratio <0.400) on the HerdChek ELISA 4 months following the last of 6 repeated injections with SD 23983 PRRSV. These animals had initially developed strong HerdChek ELISA antibody responses ( 0.818, average 1.367±0.31 1), but subsequently returned to seronegative status. Soon after exposure, ELISA antibody was detected in all pigs and no or few pigs had serum-neutralizing antibody. Midway through the experiment the average ELISA antibody levels were falling relative to early peak levels while SN antibody titers were still rising with each subsequent exposure to virus. The overall average S/P ratio was 0.759 by HerdChek for the infected pigs after the multiple exposures to 23983 PRRSV just prior to challenge. Geometric mean SN antibody titers continued to rise with each injection, peaking between 1:16 to 1:64, but fell somewhat by the time of challenge 4 months after the sixth (final) injection.

**Phase II: Homologous challenge**

The two ELISA-negative PRRSV exposed groups had only a modest increase in mean S/P ratio (to approximately 0.700) two weeks following challenge. In contrast ELISA-negative challenge control pigs S/P ratios were nearly 1.0 two weeks later. High ELISA-positive exposed group pigs had little change in mean S/P ratio following challenge. Pre-challenge mean PRRSV SN titers for the two HerdChek seronegative groups were 1:16. Titers rose to approximately 1:50 two weeks post-challenge. These pigs were not initially seronegative for SN antibody, and were capable of producing a modest anamnestic response following challenge. The high PRRSV ELISA treatment group SN titer response was similar to the negative groups.

PRRSV viremia was detected after homologous virus challenge by rtPCR in serum of pigs from all treatment groups. PRRSV was detectable in most of these pigs for only a day after challenge. However, some pigs had detectable virus for multiple days following challenge. The proportion of pigs with at least one rtPCR positive serum sample following challenge ranged from 30% to 50% by treatment group. All challenge control pig sera were positive for PRRSV by rtPCR for all sampling days except for one pig on the last 3 days of sampling. All non-challenge control pig sera were negative for PRRSV by the nested rtPCR.

## Experiment two

PRRSV was also detected by rtPCR for at least a day following homologous virus challenge of sows by Dr. Scott Dee (personal communication). This study is still in progress. Details of study design and results will be presented in the meeting but not printed here out of respect for Dr. Dee's publication rights.

## Experiment three

This experiment was designed to evaluate the ability of purified recombinant outer membrane proteins from an acute PRRSV strain to provide protection from homologous challenge.

**Materials and methods**

An acute strain of PRRSV (Powell) was identified in a vaccinated herd in North Carolina. Following virus isolation, primers were designed from consensus sequences, ORFs 2–5 were cloned by rtPCR, and the genes sequenced and then inserted individually into bacculovirus vectors. The proteins were then expressed in a baculovirus:SF9 expression system. Individual proteins were purified, mixed with an alum adjuvant, and used to immunize 4- to 6-week-old pigs. The animals were immunized with 0.5mg of protein, boosted similarly after three weeks, and finally challenged three weeks later with $10^{3.0}$ $TCID_{50}$ Powell strain PRRSV. Virus replication was monitored for 14 days in control and immunized animals testing serum using rtPCR and virus isolation on porcine alveolar macrophages (PAMs).

**Results**

- Control unvaccinated animals displayed high levels of serum viral RNA and infectious virus throughout the challenge period.

- Levels of PRRSV RNA in serum were reduced substantially by vaccination with ORFs 4 and 5. In half of these animals PRRSV RNA was undetectable 14 days after challenge.
- DTH testing revealed strong reactions against ORFs 4 and 5, the immunogens that reduced viral load most substantially.

## Implications under production conditions

The experimental results described above suggest that PRRSV appears in the blood even after homologous challenge of frequently exposed animals. This viremia (as detected by rtPCR) is brief following multiple repeated experimental infections, as short as 10 days following immunization with homologous individual recombinant PRRSV ORF proteins. How significant are these findings to field efforts to control PRRSV? Is the possibility for viremia following homologous virus exposure dose related? Our limited experiment does not support this hypothesis; however, all animals were *injected* with virus, not naturally exposed. *If* the occurrence of viremia following homologous challenge is dose-related, and *if* this viremia is capable of passing the virus transplacentally to the fetus, then practices that increase exposure of breeding stock may have negative effects upon our efforts to prevent vertical transmission (in utero infection) of PRRS. Such practices may include feed-back of aborted or weak and viremic piglets (it is very difficult to find virus in mummified or stillborn fetuses), infectious serum "therapy" or exposure, insufficient "cool-down" time following exposure of gilts in acclimatization, trough watering, pen versus crate gestation, mass vaccination without changing needles, or opportunities for fighting and biting. How long does this high level of homologous immunity persist? We challenged pigs 4 months following the last "immunization." Would rate and duration of viremia be higher and longer if challenge occurred 3 years later? *If* this were the case, then old sows may be the most susceptible subpopulation for reinfection and transplacental transmission of virus in herds practicing isolation and acclimatization to homologous virus of PRRS-free gilts without subsequent reimmunization. The original homologous immunity experiments were over 2 years in duration and showed total protection against in utero infection following challenge of sows kept in low density, ideal conditions. However, producers keep some sows for 4 years or more under arguably more stressful production conditions. Duration of immunity studies are tremendously expensive, but the duration of totally protective immunity may be a critical component in management decisions for herds trying to control PRRSV circulation and vertical transmission.

## Summary

What do we want? Freedom from clinical disease and cessation of virus circulation. PRV has spoiled us. PRV vaccine not only induced nearly complete protection versus disease, but when used in conjunction with pre-immunizing negative replacement gilts and regular herd revaccinations, it actually allowed us to *stop* virus circulation within the herd. Currently we are struggling with trying to control clinical PRRS, particularly in growing pigs. Frustratingly, *control of clinical growing pig PRRS is dependent upon stopping virus circulation within the sow herd* (vertical transmission), truly a very difficult thing for any virus vaccine to achieve. Control of virus circulation within a sow herd results from both decreasing the amount of virus shed by vaccinated animals following infection, as well as increasing their resistance to infection in the first place. Today we know that PRRS has multiple different "strains" or immunotypes which do not induce effective cross-protective immunity and which greatly compounds our ability to control the disease. Additionally, the results of these studies suggests that the resistance to re-infection by homologous PRRSV is significant, but may not be complete. Whether brief viremia following homologous virus challenge can result in clinically important problems (virus shedding and circulation within breeding herds or moving transplacentally resulting in in utero infections) has yet to be determined.

## Sources cited

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