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How we use strategic inoculation to control PRRS

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Introduction

"Impatience is a virtue."

~ Abraham Lincoln

There is no more patience when it comes to Porcine Reproductive and Respiratory Syndrome (PRRS). As an industry we have learned a great deal about the virus: tools have been developed that aid in diagnosis; we understand more about virus interaction with other diseases and vaccines, and there is more focus on PRRSV epidemiology. However, we still have many questions, and we strike out too often while pig producers continue to face the financially and emotionally devastating effects of PRRS. In June, the National Pork Board launched a 15 year national PRRS initiative focused on understanding, controlling, and potentially eliminating the pig industry's number one disease which, by their estimates, accounts for $600 million per year in losses to US producers.[1]

While there have been some reported successes with depopulation/repopulation to eliminate PRRSV from individual sites or farms in a system, there remain geographical areas of production at such high risk for re-exposure that the financial investment in depop/repop is an expensive and questionable insurance. In parts of Southern Minnesota, for example, pig density is high in terms of the number pigs and pig sources. There are 98 known sow herds in Martin County, Minnesota.[2] Weaned pigs from numerous other sources enter the county to be finished. It is the reality for many flows to experience several pig sources within one or two miles of sow, nursery, or finisher sites. Pigs that leave the sow farm typically travel to multiple nursery, finish, and wean/finish sites. These often are spread over wide geographical areas which puts them and other sources of pigs at risk for area spread of disease. Pigs, needles, people, trucks and trailers, insects, and other fomites have been incriminated in the spread of PRRS among populations of pigs.[3]

In Fall 2001/Winter 2002 our part of the Midwest experienced the spread among pig farms of a newly sequenced family of PRRSV generally described by its restriction fragment length polymorphism (RFLP) 1-8-4. Its particularly harsh reproductive effects caused from 10 to 35% herd abortion rates and impacted weaned pig numbers by as much as 40% for the initial five month period post-infection. Production losses caused weaned pig costs to jump by $20 for that quarter on infected sow farms. While this is not new information to those of us who have dealt with PRRS over the past 15 years, the financial ability of farms to "work through" disease breaks such as this is no longer assured in our industry.

As swine practitioners we work hard to bring consultation to farms that keeps the pig foremost in the health plan. Financially based decisions for populations of pigs requires balancing risk and reward for individual animals to arrive at the best plan for the owner's farm. Pig flow strategies, depopulation and repopulation, and vaccine/medication plans often fall short in the field at controlling PRRS in herds for various reasons. These include, but are not limited to the following:

- Location
- Facility design
- Gilt source
- Virus genetic variation
- Cost of implementation
- Compliance or lack of commitment
- Secondary diseases
- Virus transmission within the farm
- Continued poor or unpredictable production and financial results

Research about PRRSV has taught us that cell mediated immunity (CMI) and serum neutralizing antibody (SN) appear to have important roles in protecting against subsequent exposure to virus, and that this immune response takes a relatively long time to develop in individuals.[4, 5] There is evidence to indicate that an adult pig population which has been uniformly exposed to a field strain of PRRS will discontinue shedding virus in a relatively short period of time, and in four month old gilts shedding was < 90 days.[6, 7] Because natural movement of a virus in a naïve population of animals can be a prolonged or incomplete event resulting in subpopulations, there is high risk of extended clinical signs and related costs of disease in the sow farm and, therefore, in the wean/finish

herd. Torremorell et al. describe in some detail the concept of producing negative pigs from PRRSV positive animals which first requires "A uniformly sero-positive herd . . .."[8]

Pigs that have been previously infected with and recovered from PRRSV are immune to homologous challenge,[7, 9] and there is evidence for passive transfer of effective PRRSV immunity in pigs.[10] The risk of re-exposing breeding stock to virus is minimal, even though there are some animals that experience transient viremia at a later homologous exposure.[11] Only those animals that do not generate an effective immune response at the first exposure will be at risk to later homologous exposure.

Just as feedback with infectious material in a transmissible gastroenteritis (TGE) break can be used to effectively expose the breeding herd and generate uniform herd immunity, farms have used infectious PRRSV-positive serum to expose the sow population. Fitzsimmons described injection of live virus culture to gilts in 1999.[12] This is not a new phenomenon in the animal husbandry business, as the following excerpt from the *Live Stock and Complete Stock Doctor, A Cyclopedia*[13] indicates regarding contagious pleuro-pneumonia in cattle on page 815: "Some very high authorities in Europe recommend inoculation of those that have been exposed, and even of whole herds and dairies. It is a disease, the occurrence of which in an animal once, confers immunity from subsequent attacks." The recommendation was to inject liquid extracted from the lungs of affected stock into healthy herd mates at the end of the tail. In discussing hog cholera on page 983, the authors stated "If a competent veterinarian be near, apply to him at once; but beware of quacks who go about doctoring hogs with so-called specifics; they are a delusion and a snare for the unwary."

So with competent veterinarians or quacks, there are farms running short on patience that have used old-fashioned population health approaches for a new-fashioned disease. Below I review for you how our practice has approached the use of PRRSV-positive serum from a herd to inoculate that same population of animals. The goal has been to shorten the duration of the PRRS outbreak and more quickly achieve PRRS stability as defined by return to normal production and the weaning of PRRS negative piglets into the wean-finish system. Because the cost of this herd stabilization plan is less than with depopulation/repopulation,[14] it may be an effective option for some farms and a welcome tool as we continue along the path of virus elimination from herds.

The inoculation process

Confirmation of a problem in the sow herd

Naturally the first step is to confirm that clinical PRRS exists on the farm. There are ample and all-too-well-known clinical signs that include off-feed animals, elevated rectal temperature, abortions, increased sow deaths, weak-born piglets, stillborns, mummies, and elevated prewean mortality in the sow herd. In addition there are available to us very useful and timely laboratory diagnostic tests such as polymerase chain reaction (PCR) and virus isolation (VI), immunohistochemistry, histopathology, and antibody tests including enzyme-linked immunosorbent assay (Elisa) and indirect fluorescent antibody (IFA).

Serum harvest

Once we have a demonstrated clinical problem on a farm, serum samples are routinely used as part of the confirmation of PRRSV activity. It is extremely important that samples are kept cool after collection, at or near 35° F (4° C). We process the serum within two days of collection, and it is stored in a designated refrigerator at the Fairmont Veterinary Clinic. Virus will remain stable and infective for approximately five days at refrigerator temperature. These samples are split at our clinic laboratory, and individual or pooled samples are saved that match the PCR/VI submissions. In this manner we have the viremic serum available for immediate use once results are known from the diagnostic laboratory.

Pooled samples are from three to five animals of like production stage (i.e., piglets in the farrowing barn, gestating sows, lactating sows, open gilts, etc.). Our success with VI on serum has been more variable than with PCR. Therefore in many cases we now request only PCR. Recently "quantitative PCR" has become available and may be useful to estimate the number of virons per milliliter of serum harvested. We are currently trying to understand the dynamics of viremia post-exposure/inoculation in order to collect the best samples for both diagnosis and harvest.

Generally piglet sera from the farrowing barn has provided us with the greatest virus concentration, specifically serum collected from weak-born and clinically sick piglets. We have an easier time finding PCR-positive samples in that population than in the adult breeding herd. If we collect 6 mL of blood from an individual piglet, after centrifugation we will submit 0.5 mL of serum and have approximately 2 mL remaining which can be stored in cryotubes and later used for inoculation. It is critical that only serum collected from the affected site is used for inoculating animals on that site.

Serum handling and storage

Because every freeze/thaw cycle kills virus (likely by 10-100 fold), sera for storage is put immediately into cryotubes (VWRbrand Cryogenic Vial, VWR Scientific Products, West Chester PA 19380). Sera is stored either in liquid nitrogen tanks or in an ultra-low freezer. Each cryotube contains 1.0-4.0 mL of serum. Frost-free freez-

ers do not work adequately to store serum for extended periods of time.

It is important to have an accurate identification system for these samples We store each farm's serum in separate containers and each tube is labeled with descriptive information about that case. We track the following information:

- Veterinarian
- Date collected
- Source/farm
- Production stage
- Sequence/RFLP
- Case ID
- Tank or shelf ID
- Canister or box ID

Once we have confirmed PCR or VI results from those split samples, then PRRSV-negative tubes are discarded.

Inoculation techniques and target populations

When we first used serum to inoculate pigs, we identified two primary target populations: the active sow herd and the gilt pool. Our goal has been to stabilize the sow site in order to shorten the duration of clinical PRRS and to achieve PRRSV-negative weaned pigs as soon as possible. This initially led us to exposing the entire breeding herd with a mass injection of the diluted serum.

A designated room in a building separate from the veterinary clinic is used for the preparation of the serum inoculum. The work area is disinfected before and following each use.

The serum is prepared by diluting the saved serum with a phosphate buffered saline. In our practice we use HyQ(r) DPBS/Modified Dulbecco's Phosphate Buffered Saline (PBS) (1x), manufactured by Hyclone(r), Logan, Utah. The frozen serum is thawed and added to the appropriate volume of chilled PBS in order to prepare a 2 mL dose for each animal.

Dose determination has not been exact. Generally, if split serum has tested PCR-positive, the saved aliquot has been infectious. We know this because of the resulting viremia and seroconversion in negative naïve animals and serologic or clinical response in antibody-positive animals. In most cases we have not known an exact concentration of virus in the exposure. In fact, on two occasions where we have been suspicious of PRRSV activity but had PCR-negative submissions, split sera was still used to expose negative naïve gilts in isolation. In both cases the group of animals seroconverted. Twice we have submitted a sample of the diluted inoculum for PCR analysis and they have tested negative. So while it is appropriate and important to add the science to dose determination, the bottom line is that in our experience it takes only a very small amount of virus to accomplish exposure with inoculation by intramuscular injection.

We do not use purified virus from culture for inoculation. There is the possibility of contamination of cells used in the culture, and there are more people handling samples.

The serum inoculum is contained in plastic bottles and kept cold until used for exposure. A record is made to indicate the preparation date, farm source, isolate ID, veterinarian, number of doses prepared, and volume of serum used. The bottles of inoculum are labeled to reflect the preparation date, farm, isolate ID, and veterinarian.

Case examples

Farm 1: 2400 farrow site

Tables 1-4 contain descriptions and data from Farm 1 case study.

Since April 19, 2002 weaned pig groups are PCR-negative and nurseries are staying negative. AIAO finishers have also been staying negative.

Farm 2: 2400 farrow site, startup farm

Tables 5-9 contain descriptions and data from Farm 2 case study.

Farm 3: 280 sow farrow to nursery, partial finish

Tables 10 and **11** contain descriptions and data from Farm 3 case study.

The following Farm 3 piglet observations and diagnostics were made, post injection exposure.

- Pigs inoculated at weaning experienced 8 to 10% mortality during the nursery phase; 90% of those deads in the first 10 days post-wean. After that groups stabilized clinically and experienced < 3% mortality in finishing.
- Piglets after January 22 were not inoculated. In the nursery these groups averaged 3.4% mortality. Pigs still seroconverted by the end of the nursery.
- Weaned pigs started testing PCR-negative January 29, 2003, 36 days after sows were inoculated. Some weaned groups continued to seroconvert in the nursery.
- After nursery depopulation in May 2003, weaned pigs have started better. (No groups had closed as of this writing, June 18, 2003.)

Table 1: Farm 1 case information.

PRRS status	Serologically-positive but stable sow herd; pigs go serologically negative in nursery and move to multiple finish sites
Gilt source	Negative, naive, gilts purchased every 30 days at 5 months of age
Isolation	2 locations off site; 60 days AIAO flow, then enter sow site breed/gestation barn
Date of clinical PRRS break	August 2, 2001 first abortion
Clinical signs	Abortions, stillborns, mummies, repeats, pig mortality pre- and post-wean, sow mortality
Diagnostic confirmation	Piglet tissues, sow serum, piglet serum, RFLP 1-8-4
Intervention	Feedback to entire herd with placenta, piglet tissue and blood from aborted pigs and weakborns, and live seeder piglet exposure; this was started aggressively on August 17, 2001

Table 2: Farm 1 observations after feedback exposure.

Sows	Piglets
38 aborts from August 2 to September 7, initially the virus moved slowly through the population. Peak abortions occurred September 10-21 (about 50% of the total in these 12 days); this peak was 24 days after seeder pig/feedback and 39 days after the initial PRRS abortion. 251 total abortions by December 31, 2001. Continued sporadic abortions on into January 2002.	PCR-positive sera from farrowing piglets, 99.7 to 99.8% homology at Open Reading Frame (ORF) 5

Table 3: Farm 1 observations after serum inoculation. Sow herd inoculated with PCR-positive serum on January 22, 2002.

Sows	Piglets
6 aborts within 7 days; 37 aborts by February 4 and 32 dead sows.	PCR-positive and -negative piglet serum pools in February and March. PCR-negative piglets on/after April 19, 2002; 241 days since 1st abort; 87 days since serum exposure. Weaned pigs have remained negative in the nursery (June 18, 2003).

Table 4: Farm 1 pig costs (# pigs weaned).

Year	1st quarter	2nd quarter	3rd quarter	4th quarter
2001	$36.12 (12,700)	$30.91 (13,806)	$34.60 (11,728)	$51.12 (7,770)
2002	$37.31 (11,671)	$30.68 (13,703)	$29.88 (13,670)	$31.80 (13,850)

Table 5: Farm 2 case information.

PRRS status	Initially negative, naive, then some gilts went positive during breeding project
Gilt source	Negative, naive gilts now purchased every 70 days
Isolation	All on one location off-site; pigs raised from 5 and 10 weeks age to breeding; enter the east barn for 10 weeks, then move down the hall to the west barn until transported to the sow site where they enter breeding/gestation
Date of PRRS break	January 22, 2002 based on Elisa-positives, PCR-positive, 6/6 pools January 28, 2002
Clinical signs	No clinical signs initially in open or bred gilts during the off site breeding project
Diagnostic confirmation	Isolate was RFLP 1-8-3; the sequence was > 0.5% different than mlv vaccine strains at ORF 5
Intervention	Injected serum to gilts (open and bred) immediately on February 6, 2002

Table 6: Farm 2 observations after first serum inoculation.

Sows	Piglets
First gilts farrowed the beginning of March 2002. Following herd serum exposure, genetic sequencing of PCR-positive post wean pigs in April identified a second strain that was RFLP 1-7-2, differing from mlv vaccines by > 0.5% and from the first strain detected by 15.0% at ORF 5. This continued to be the only strain identified in the wean/finish flow. This isolate continued to show up sporadically in weaned pigs during the summer and fall 2002. The sow herd was exposed by inoculation to this virus with serum on February 14, 2003.	Second strain isolated from piglets in farrowing on September 2002.

Table 7: Farm 2 observations after second serum inoculation.

Sows	Piglets
A dozen off-feed sows and 7 abortions occurred in the first 14 days after inoculation; no other clinical signs were noted.	There was increased pre-wean mortality for 8 weeks (to 16% from 11%) and higher mortality in the early post wean period. Six weeks of weaners are > 15% mortality wean-to-date, and not closed out yet. Piglets weaned since April 30, 2003 are staying negative in wean/finish barns and have experienced < 1.5 % mortality so far. (June 18, 2003).

Table 8: Farm 2 pre- and post-inoculation mortality (%).

	4 weeks pre-inoculation	0-4 weeks post-inoculation	5-8 weeks post-inoculation
Stillborns	8	13	9
Mummies	2	5	3
Pre-wean mortality	11	15	16

Table 9: Farm 2 pig costs (# pigs weaned).

Year	1st quarter	2nd quarter	3rd quarter	4th quarter
2002	N/A	$31.56 (10,520p)	$38.25 (10,518p)	$33.08 (13,086p)
2003	$35.86 (11,349p)	N/A	N/A	N/A

Table 10: Farm 3 case information.

PRRS status	Fall 2002 serologically-positive sow farm; some PCR-positive pigs at weaning; pigs seroconvert in nursery; clinical signs consist of conception in mid 70%; 12 abortions between October 1 and December 24, 2002; nursery mortality of 7 to 12%; uneven pigs throughout wean/finish flow
Gilt source	Negative, naive gilts purchased every 70 days
Isolation	On-site in a building separate from the herd; gilts are raised from 10 weeks age to breeding; continuous flow style MOF building that houses all the gilts by age in 4 separate pens
PRRSV strain	PCR-positive serum from piglets in farrowing and in nursery; RFLP 1-?-4, sequence different than mlv vaccines by > 0.5%
Interventions	Sow herd inoculated on 12-24-02; On-site nursery and on-site finishers inoculated on 1-3-03; continued to inoculate piglets at weaning through January 22, 2003; Depopulated nursery on May 21 and stood empty for 15 days; weaned into finisher for those 2 weeks

Table 11: Farm 3 sow observations, post injection exposure.[A]

Breeding Groups	50-1	2-5	6-9	10-13	14-17	18-21
Conception rates (%RTU)	87	85	87	92	83	90
Farrow rates (%)	86	83	N/A	N/A	N/A	N/A

[A]Herd experienced 6 total abortions, all from last trimester of gestation.

System results

There are 37 sow farms in our practice that have been inoculated via serum injection. Nine of those farms have sequenced one definable isolate; eighteen have sequenced two unique isolates, and ten have had three or more unique strains of PRRS identified. There is evidence that a vaccine-like isolate can induce clinical signs and lesions different than the parent strain,[15] so I am defining isolates as different if clinical disease occurs in pigs previously exposed to the original strain, and/or amino acid homology at ORF is < 99.5%. (sequenced at U of MN). See **Table 12**.

The serum used in each farm was collected from pre-wean piglets or breeding females *always* on that same site in that specific herd. That serum was diluted with PBS according to methods discussed above. Exposure strategy has ranged from a common mass inoculation date for every sow, boar, and gilt on the farm to a modified plan that avoids sows in the farrowing barn and those in the very late gestation phase. Those sows not initially inoculated because of their production stage are later inoculated sometime after weaning but before mid gestation. On approximately one-quarter of farms, the first exposure was followed with a second inoculation as insurance that all animals were properly exposed. Repeat exposure has not prolonged the timeline of viremia in piglets.

In most cases of mass inoculation, clinical signs in sows peaked by 10-14 days post-injection, and by ten weeks weaned piglets were PCR-negative and nurseries with AIAO flow and wean/finish barns stayed negative. In some situations piglets remained viremic beyond ten weeks, and in the majority of those samples identified a strain different than the one used to expose the herd. We cannot be certain whether those "new" strains were present in the farm prior to inoculation because we lack sequence information of isolates prior to late 2001.

While the primary goal of serum inoculation has been to create uniform sow herd immunity to the present "active" virus so as to shorten the clinical episode and return to negative weaned pigs more quickly, a few farms have continued to expose their breeding herd as some type of "maintenance" plan during the first half of gestation. The value of this plan has yet to be determined. Batista et al. have shown that at 120 days after an original strain exposure there is a significant CMI response to homologous challenge.[7] The same is not true for heterologous challenge. This research matches other experiments where vaccine virus has failed to protect adequately against field virus[16, 17] and experience from the field where farms have isolated a second or third strain after herd inoculation with serum.

Of those farms weaning PCR-positive pigs, four have new isolates identified in the two weeks prior to this writing (June 18, 2003), and the other five were herds injected June 2003, June 2003, May 2003, April 2003, and March 2003.

Gilt exposure

There are primarily two types of gilt barn flows in the 37 sow farms that have used serum exposure via injection. The first is "all in all out" flow where individual groups of purchased gilts are isolated, acclimated, and reared in a barn that is not attached to other barns. This facility is then completely emptied of animals and cleaned before another group of gilts enters. The second type of gilt barn is "continuous" flow where multiple gilt deliveries are housed in the same barn—newly delivered gilts through those ready to enter the breeding/gestation facility. Those sow farms using continuous flow for gilt development generally target 35 days of break between the last entry of animals into the gilt barn and any movement of mature gilts into the breeding/gestation facility.

Viremic serum is used to inoculate gilts after newly purchased animals have been screened as negative with PRRS Elisa and vaccinated/acclimated to other herd diseases that may include Mycoplasma, Erysipelas, Swine Influenza, and *H. parasuis*. Just as with natural outbreaks to some field strains of PRRS, secondary diseases can create a severe challenge following serum exposure to PRRS. Mortality in gilt isolation/acclimation barns in our system ranges from 1.5% to 5% (50 days old to 230 days old). All farms use the current (most recent) isolate of PRRS from the sow farm to expose the gilt herd. There are a few farms using more than one isolate in tandem, but not simultaneously.

Table 12: System-wide results as of June 18, 2003.

	Farms exposed	Weaning (-) pigs	Weaning (+) pigs	Unknown status
# 1 Isolate Farms	9	9	0	0
# 2 Isolate Farms	18	10	6	2
# 3+ Isolate Farms	10	7	3	0
Totals	37	26	9	2

Maintenance/prevention plans for previously exposed sow herds

1. Biosecurity
2. Purchase PRRS-negative naïve gilts and negative semen
3. Continued gilt exposure with serum in isolation/acclimation barn
4. Strategic routine use of serum to expose gestating sows post-pregnancy check (untested)

"The pessimist complains about the wind; the optimist expects it to change; and the realist adjusts the sails."

~ William Arthur Ward

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