RESEARCH REPORT
SWINE HEALTH

Title: Impact of pigs entering a region on feasibility of PRRSv eradication - **NPB #10-114** Revised

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Industry Summary:

The goal of this project was to understand the impact of PRRS infected pigs on neighboring herds that are negative. In the original design, we proposed to collaborate with regional PRRS control projects and use data being collected to address the objective. After starting the project, we realized that not enough data was available to address the objective. In 2011, we requested and received approval for a change in protocol. In this new protocol, we proposed a prospective study assigning growing pig sites to vaccinated / not vaccinated. We proposed to collect data on virus dissemination and determine whether vaccination might influence this dissemination. We have aggressively sought out sites to participate.

Unfortunately, there seems to be sufficient sentiment that negative pigs at high risk of becoming infected should be PRRS vaccinated and therefore, we were unable to find enough owners willing to leave pigs unvaccinated. Consequently, in 2012 we proposed a different approach to address this same objective. The proposal built on work recently reported by Dee (2012) where he reported detecting PRRS virus in up to 75% of air samples in the month of March outside selected sow barns. Unfortunately, this request was declined and we were asked to terminate the study. BobM@UMN.Edu

Keywords: PRRS. Risk factors, Vaccination

Scientific Abstract:

We proposed to identify the incidence of infection associated with the introduction of positive or unknown pigs "imported" into a PRRS control region. We expected to measure the risk factors, understand and show their possible interactions and also predict time to regional virus spread depending on the combination of factors. Finally, a model to estimate the economic feasibility of eliminating PRRSv from a region is needed and this study could have contributed to that. Unfortunately, we were over aggressive in proposing more than we could accomplish. The data were not available to address the objectives.

Introduction:

Due to the high antigenic variation of circulating field strains and the mutagenic property of PRRSv, development of effective control measures has been limited. Consequently, pork producers and veterinarians have attempted to control the disease through eliminating the virus from farms and employing strict biosecurity measures to prevent re-infections. Although elimination methods are well understood and economically feasible, re-infections through different routes including fomites and aerosol have been a constant challenge for pork producers.

Frequent re-infection of herds has motivated leaders of the industry to coordinate efforts directed at eliminating the virus from circumscribed regions. At the time this proposal was initiated, there were 9 geographical areas in the United States in different phases of PRRSv regional control. A concern in all these regions is the risk posed by pigs entering the region.

We hypothesized that there are identifiable risk factors for PRRSv spread that are specific to each region. This study was to focus on understanding the role that pigs play in the spread of PRRSv when entering a region. Unfortunately, the study could not be carried out due to insufficient data being available.

Objectives:

1. Measure the impact of introducing PRRSv positive and unknown status pigs on PRRSv transmission into the regions.
2. Characterize and measure risk factors associated with PRRSv infection in regions pursuing virus elimination and compare those risk factors between the regions.
3. Estimate survival time of surrounding PRRSv negative sites after unknown or positive pigs are moved into a region and evaluate associated risk factors.
4. Develop a model that incorporates expected incidence of infection posed by incoming pigs to a region, with estimated cost of regional elimination, and estimated benefit of PRRSv elimination to arrive at the feasibility of regional elimination

Materials & Methods:

To achieve specific aims 1, 2 and 3, we proposed a longitudinal observational study involving existing PRRSv elimination regions. We had agreement to participate from coordinators for all regional elimination projects that were funded by PRRS CAP II (MN, IL, MI). In addition, Dr. Dale Polson at Boehringer Ingelheim is working with other regions and encouraged their participation (see letter of support). Data from each participating project was to be examined to identify all sites with growing pigs entering the site on at least one occasion since the project began. For the purpose of this project, such pigs were to be referred to as "imported pigs". These sites would have been part of the cohort of sites that were to be followed over time.

Based on existing test data, imported pigs were to be characterized as being in one of three states for PRRSv infection: (1) confirmed seronegative, (2) confirmed infected, or (3) unknown.

Unfortunately, this project proposal preceded available data. The regional projects were getting started at that time and did not have enough cases of "imported pigs" to warrant proceeding.

Remaining methods were proposed as follows. All sites located within 3 miles of sites receiving imported pigs will be identified and will represent the remainder of the cohort of sites to be followed. All cohorts will be followed for 6 months after the arrival of imported pigs. A new case of spread within the region will be assumed to have happened if the following criteria are fulfilled:
(a) If there is detection of a "new" infection at any of the sites within 3 miles of the site receiving the imported pigs;

(b) If there is no evidence of transmission by direct introduction of infected pigs from the site receiving the imported pigs;

(c) If the new isolate is at least 99% similar to the isolate present in the imported pigs and at least 2% different from any existing PRRS isolates at the farm.

Having compiled a set of presumed cases of area spread of PRRSv, the following factors will be recorded to each identified farm, and their impact (hazard) will be estimated in regards to risk of regional PRRSv spread: (a) Type of PRRSv strain (defined by local veterinarians as high or low virulent); (b) % homology of the newly detected isolate to live virus PRRSv vaccine; (c) PRRSv vaccination history; (d) Season of year during farm population; (e) Pig density for the neighborhood characterized by # farms/3miles radius; (f) "Imported farm" herd size; (g) Type of farm [GDU, growing-finishing, nursering] and if its commercial farm or genetic multiplier/daughter nucleus farm; (f) Presence of vegetation surrounding the farm with infected imported pigs; (g) Biosecurity level; (h) Type of barn ventilation; (i) Distance to site receiving imported pigs

Logistic regression will be used to estimate the probability of occurrence of the event "PRRSv infection state change" by fitting the collected data to a logistic curve. Our experimental units are the "farms". We will start with the full model, that is, with all factors listed above and their interactions. The final model, as described by McCullagh & Nelder (1989) will include factors, significant at $p<0.20$.

Survival analysis will be performed to compare the time to PRRSv spread within farms of each cohort. Kaplan-Meier Survival analysis will produce graphical data comparing time to PRRSv spread of selected significant models shown to be significant on Logistic regression model. Log Rank test (alpha 0.05) will be used to analyze difference of curve patterns. Proportional Hazards models will be applied to measure the differences using proportional ratios for each model compared in Kaplan-Meier.

The combination of logistic regression, Kaplan-Meier survival analysis and proportional hazards models to analyze impact of risk factors of PRRSv infection was also used by Mortensen et al. 2002.

Methods to answer specific aim 4:

We will estimate time (in months) to return of investment on PRRSv elimination from a single farm and from the studied regions based on:

- Probability of elimination success calculated on logistic regression described above.
- Benefit of PRRSv elimination: we will develop a model applicable to farms and each region using herd sizes and applying the PRRSv infection costs estimated from Neumann et al (2005) study: breeding-farrowing phase $74.16/litter, nursery $6.01/pig and grower-finisher phase $7.67/pig
- Cost of PRRSv elimination: The cost for each farm will depend on type of eradication method utilized. Estimates calculated from Yeske (2010) will be used as follows: depopulation: $9.39/pig, closure $1.48, closure with offsite breeding $1.75/pig, closure offsite GDU $1.39. Regional cost will depend on number of farms requiring PRRSv elimination and proportion of each type of single site eradication method used. Payback times for PRRSv elimination (single farms and regions) will be reported in months with 95% Confidence Interval.

We proposed change in protocol as follows:

We proposed to expand the inclusion criteria to include PRRS vaccinated wean to finish groups. PRRSv vaccinated sites represent an important fraction of wean-to-finish sites particularly in high pig-sites dense areas, but were excluded from the original proposals due to budget constraint to differentiate between vaccine and wild-type virus.

The proposed methods were as follows. A group of 40 wean-to-finish sites that fulfill the eligibility criteria (box 1) will be selected and monitored for PRRSv infection. All sites will have PADRAP assessed and will be enrolled in the new ISU / UMN PRRSv risk factor study, which has a target to enroll 250 wean-to-finish sites. Risk factors associated with PRRSv infection will be investigated using the information of PADRAP assessments.

Box 1. Eligibility criteria to enroll sites:

1. Wean-to-finish site.
2. Populated from breeding sites that qualify as provisional negative (III) or negative (IV), as defined by Holtkamp *et al.,* 2011.
3. Pigs vaccinated with Ingelvac ATP at or before placement.
4. Willingness from producer and veterinarian to participate.

PRRSv surveillance protocol. Oral fluids will be used to monitor pig populations in each finishing site from placement to 24 weeks of age. Procedures described by Prickett *et al*., 2008 will be used to collect 3 oral fluid samples per site at 6 points in time (monthly testing starting on piglet placement). One rope will be positioned at each end of the barn and one in the center. ATP vaccine-specific PCR, recently developed by Dr. Murtaugh's lab (UMN), will be used to differentiate vaccine virus in the PRRSv positive oral fluids from wild-type.

Unfortunately, we were unable to locate sites willing to participate. The owners of sites in high density regions intended to vaccinate and were unwilling to leave sites unvaccinated.

We proposed a change in protocol as follows.

This proposal builds on work recently reported by Dee (2012) where he detected PRRS virus in up to 75% of air samples in the month of March outside selected sow barns. We propose to identify sow herds that fulfill certain inclusion criteria and monitor air outside the sow barn. Furthermore, we will survey all sites within 3 miles of the sow barn for PRRS virus vaccination practice. We will also request herd owners to share PRRS testing results, although this is supplemental and not required to address the objective. With a sample of 20 clusters, we will be able to detect a reduction in frequency of virus detection of at least 50% with alpha .05 and 80% power, should it exist. We believe this study will also yield important knowledge on frequency of PRRS virus dissemination, diversity of sequences detected, effect of weather, density of sites, and type of production in the region.

If approved, we would have had preliminary data in spring of 2013 but would not have enough clusters to complete the study until spring of 2014. This request for change in protocol was not approved.

Results:

Initial protocol: impact of importing pigs:

Coordinators from four PRRS CAP regional elimination programs were contacted at the moment of the grant writing to solicit their willingness to collaborate (this proposal was written in 2009 when there were relatively few regional projects). The study design assumed that PRRSv-negative growing sites located in regional elimination areas would have a PRRSv-surveillance system in place that would be able to diagnose new PRRSv infections. The study needed retrospective and prospective data on PRRSv introduction in growing pig sites. As we started the study, we learned that the diagnostic information was available for a few sites, but was not close to the minimum sample sized needed to provide sufficient statistical power for the comparisons.

Revised protocol: likelihood of PRRSv infection between vaccinated and non-vaccinated sites.

To conduct the experiment, we would get matching funds from Boehringer Ingelheim Vetmedica (it was approved) for diagnostic testing. We received IACUC protocol approved at the University of Minnesota to conduct the study.
The study would fulfill the following objectives:

- To document the time of PRRSv infection in growing pigs placed in all-in / all-out wean-to-finish sites located in high pig density areas
- To compare the duration of PRRSv infection detected by PRRSv PCR in oral-fluids between MLV vaccinated and non-vaccinated growing pigs
- To describe the interaction between age and duration of PRRSv infection in growing pigs
- To evaluate the association between time of PRRSv infection and specific risk factors (we would generate data for Dr Holtkamp's analysis)

To accomplish this, power analysis indicated that we needed 22 vaccinated and 22 non-vaccinated wean-to-finish sites located in high pig density area. Sites needed to be populated from a PRRSv-negative source (at least AASV category IIb). Oral fluids samples would be collected periodically from each site for PRRSv monitoring by PCR. Positive samples would be sequenced (ORF5) to differentiate between vaccine strain vs wild type virus (costs would be covered by B.I.).

To reduce confounding, we proposed to match vaccinated vs non-vaccinated site by source breeding herd and region. We contacted at least 12 production systems and veterinary clinics to solicit participation.

There was a challenge to match vaccinated vs non-vaccinated sites according to genetics, nutrition, veterinary services, and regional density. Two systems were interested but didn't have enough sites that matched the inclusion criteria. One system would not consider using attenuated PRRSv vaccine believing it negatively affected performance. We had several meetings with one system, which at one point considered joining with a few matched sites. However, there was no agreement to use MLV vaccine in their growing pigs, they had few PRRSv-negative sow farms and low number of eligible growing pig sites located in high pig dense region. One system was confirmed to participate but PRRSv infected their major sow farms that would have been the source population and they were no longer eligible to participate. The remaining systems were not willing to leave pigs nonvaccinated or believed they already knew the answer to the question being pursued.

In summary, we failed to find enough wean-to-finish sites that fulfilled the eligibility criteria of the study. Relaxing the eligibility criteria would result in a study with very limited external validity.

Discussion: We were overly aggressive in proposing what we thought we could accomplish. The data were not available to address the objectives.