Successful PRRS eradication in a genetic nucleus farm using serum inoculation and herd closure after a failed sequential partial depopulation

**Introduction:**

Porcine Reproductive and Respiratory Syndrome (PRRS) is one of the most significant diseases affecting the global swine industry. In spite of substantial efforts directed at biosecurity and control, it continues to devastate parts of the North American industry. Moreover, the ongoing economic impact of PRRS is the incentive behind the PRRS eradication strategy recently announced by the American Association of Swine Veterinarians (AASV).

PRRS virus (PRRSv) is a small, enveloped single stranded RNA virus belonging to the family *Arteriviridae*. The virus survives poorly off the host, and is susceptible to heating, drying, non-neutral pH (<6 pH >7.5), detergents and disinfectants. PRRSv is highly host specific and individual isolates are exceptionally variable based on their genomic analysis and clinical presentation. Yet, two distinct genotypes of PRRSv exist: Type 1 (European/Lelystad) and Type 2 (North American/VR2332-like), that share only partial cross protection which has implications for vaccine efficacy and diagnostics.

Several diagnostic assays can be used to investigate the PRRS status of individual animals or herds. The most widely used antibody detection assay, the IDEXX HerdChek 2XR PRRS enzyme linked immunosorbent assay (ELISA),⁹ is a non-quantitative assay that detects antibody within 1-2 weeks of exposure³. A positive IDEXX HerdChek 2XR ELISA test reflects exposure to PRRSv, and is not correlated with sterilizing immunity, degree of humoral response, or level of viral exposure. By contrast, the immunofluorescent antibody (IFA) assay may be used to quantify IgG or IgM levels, or confirm the presence of PRRS antibody in the event of an
unexplainable positive ELISA result. In contrast to the IFA and ELISA, the virus neutralization
(VN) assay detects antibodies that are believed to play a role in protective immunity. The VN
assay has low sensitivity but detects a functional anti-PRRS antibody within several weeks of
Exposure.³

A commonly used antigen detection assay is immunohistochemistry (IHC)⁴ used on paraffin
embedded fixed tissue. Because culturing virus is difficult and laborious, virus isolation on tissue
or sera is rarely performed and has low sensitivity. For commercial diagnostic testing, standard,
nested and real time quantitative polymerase chain reaction (PCR) are preferred and readily
available.⁵⁻⁹

In a farrow to finish herd (FF), PRRSv usually circulates continually in the feeding herd as a
result of horizontal transmission in the late nursery or grower stages, corresponding with waning
passive immunity. In some instances, PRRSv transmission can occur among infected and
susceptible breeding animals. All ages of naïve animals are susceptible to infection, but many
viral strains may only cross the placenta in late gestation.¹⁰ Virus can be shed via milk, saliva,
nasal secretions, urine, semen and feces.¹ However, some animals are persistently infected with
live virus for up to 157 days post infection.¹¹ The most common reproductive clinical signs
include premature farrowings, abortions, and the birth of stillbirth and mummified piglets. If live
born, viremic piglets are generally weak and fail to thrive, and if they survive the early post-natal
period, may develop diarrhea, grow poorly and succumb to other diseases contributing to
significant elevations in pre-weaning mortality. In post-weaned pigs, PRRSv primarily targets
the respiratory and lymphoid systems causing interstitial pneumonia and systemic
lymphadenopathy.¹²
While attenuated and killed vaccines are available and can be useful in some situations, the lack of complete strain cross protection is a major limitation. Attenuated vaccines can reduce the incidence and magnitude of viremia\textsuperscript{13}, and significantly limit lung lesions even against heterologous strains. But they may adversely impact reproductive performance if used in late gestation.\textsuperscript{14} The lack of complete cross protection has lead many to attempt serum inoculation using crude preparations containing PRRSv isolates collected from viremic pigs on the infected farm where the inoculum is subsequently used. While the use of serum inoculation is associated with animal welfare and professional liability issues, it has been used successfully in many herds to control and/or eradicate the virus.\textsuperscript{15,16} Other PRRS control/elimination strategies include total depopulation, partial depopulation, segregated early weaning (SEW), test and removal, and herd closure. While several of these strategies were used in the clinical report that follows, serum inoculation, herd closure and SEW were instrumental in the successful eradication program, while vaccination and sequential partial depopulation as performed under the conditions of this herd were not.

**Clinical Report:**

The PRRS eradication was conducted in a 600-sow FF nucleus farm (Farm A) located in an isolated location in Western Canada. The herd originally broke with PRRSv in the mid-1990’s, had initiated a PRRS vaccination program of lactating sows and replacement gilts\textsuperscript{b} and remained free of clinical reproductive and respiratory disease in spite of active viral circulation in 8 to 15 week old pigs. The herd was serologically free of most major swine respiratory pathogens including *Mycoplasma hyopneumoniae* (APP), *Actinobacillus pleuropneumoniae* (App), swine influenza virus (SIV), porcine respiratory corona virus (PRCV), but not porcine circovirus type 2 (PCV2). The breeding herd was vaccinated for erysipelas and parvovirus\textsuperscript{c}, and was clinically free
of leptospirosis. The farm was designed to accommodate the genetic testing and sale of maternal
and paternal replacement stock to downstream daughter nucleus and commercial operations. As
such, the 15-year-old nucleus facility was subdivided into the following areas (ages of pigs
reared in parenthesis)(Figure 1): breeding-gestation including gilt development, farrowing (0-3
wks), nursery (4-8 wks), pregrower (9-12 wks), test (13-21 wks), and pre-sale holding (22-26
wks). A 1,500 head off-site finisher (Farm B) located approximately 20 kilometers northeast
received surplus pigs on a weekly basis. Individual rooms in the farrowing, nursery and test areas
were run all-in-all-out (ie. contained only one week’s production) and were emptied, high
pressure washed and disinfected at the end of each batch. The remaining areas were operated on
a continuous flow basis, but individual pens within each area were sanitized between batches of
pigs. The level of sanitation, husbandry and farm management was very good to excellent.

In October 2002, after carefully considering the relative costs and logistics of complete
depopulation, senior management opted for a PRRSv eradication strategy using sequential partial
depopulation, PRRS vaccination and unidirectional pigflow. PRRS eradication also presented an
opportunity to introduce new breeds and bloodlines into the nucleus facility which more closely
reflected present customer needs and future opportunities. During the program, significant
maintenance was performed.

In November 2002 in anticipation of the eradication program, PRRSv isolates obtained from
two 9-week old pregrower pigs in Farm A were sequenced, and found to be 96.0% and 96.4%
homologous to the PRRS MLV vaccine strain, based on ORF5 sequencing. ORF5 sequencing
had been performed annually in Farm A since 2000, during which time the annual rate of
nucleotide substitutions had been consistent at approximately 0.5% per year (data not shown).
Cluster alignment (Figure 2) confirmed the presence of genetic drift during the period, rather than the introduction of new field strains into Farm A.

The eradication protocols and timeline (Table 1) were developed which included a sequential partial depopulation and cleanout of all production areas from breeding-gestation through to pre-sale holding, the creation of an 8 week empty period, followed by restocking with PRRS naïve animals from another nucleus unit (Farm C) located about 85 kilometers northwest of Farm A. The cleanout procedures included a thorough wash and disinfection of the facilities and equipment; manure pit and water system chlorination; elimination of rodents; maintenance; partitioning; and a minimum 30-day area closure during which time no personnel entry into the cleaned area was permitted. The breeding herd depopulation began in March 2003 when all females were sequentially culled from Farm A at weaning. Shortly thereafter, an off-site gilt breeding project was initiated on April 20th at the PRRS naïve nucleus (Farm C). Farm C was highly biosecure, located at least 8 km from the nearest known pig farm, and was tested monthly or more frequently as required to document its PRRS naïve status (data not shown).

A recognized limitation of the partial depopulation was that animals would still be present in pregrower, test and holding areas when the new PRRS naïve breeding herd was scheduled to enter the breeding barn in July 2003. To mitigate this risk, PRRS MLV vaccination of all suckling and nursery piglets was initiated in April 2003 just prior to the farrowing and nursery depopulation in an attempt to expose and eliminate PRRSV shedding in the PRRS positive population as early in the program as possible, thereby reducing the risk of PRRSV transmission to the naïve breeding herd. In spite of the 3-4% nucleotide difference between the PRRS MLV vaccine and the strain present in the barn, vaccination, unidirectional pigflow, partitioning of “dirty” and “clean” areas in the unit, and enhanced insect control were determined to be
satisfactory safeguards. Furthermore, the majority of the cleanout was scheduled during the summer, when ambient temperatures facilitated viral desiccation. Serum inoculation was proposed, but rejected on the basis of high risk and liability.

The repopulation of the breeding herd began on July 11, 2003 following the complete depopulation and cleanout of the breeding, gestation and farrowing areas. About 2/3 of the bred gilts, as well as all open gilts were transferred to the cleaned breeding barn of Farm A in anticipation of the first farrowings on August 8th. The remaining gilts were transferred to Farm A on August 22nd. All semen used in Farms A and C was confirmed negative for PRRSV prior to use by nested PCR (nPCR).

During a routine veterinary herd health visit to Farm A on August 25th, the breeding staff reported a drop in feed consumption in a number of sows during the preceding week. The problem appeared to coincide with the delivery of feed, but it was recommended that a rectal thermometer be purchased to determine if affected animals were febrile. Since arrival on July 11th, 2 gilts had aborted at gestation days 59 and 109 respectively, which was considered within expectations given the transport and relocation stress placed on the pregnant gilts. Moreover, one of the gilts was delivered 2 days prior to aborting and was pale and anorexic at arrival. It was recommended that in the event of future abortions, some aborted fetuses be frozen at -20°C pending diagnostic work up if warranted.

By August 25th, about 40 gilts had farrowed and except for the sporadic starveout (unthrift) piglet, all litters and dams were in excellent health and condition. To assist the starveout piglets, it was recommended they be cross-fostered onto nurse (extra suckle) sows as the need arose, and fed supplemental fresh or frozen colostrum collected from healthy farrowing sows in the unit. Unfortunately, heat lamps were in short supply and the absence of supplemental heat in some
farrowing pens enhanced piglet chilling and increased the risk of starvation. During the herd health visit, blood was collected from 20 suckling piglets from gilt litters ranging in age from 4 to 14 days, and submitted for PRRS ELISA. Nineteen of 20 samples were clearly negative (S/P ratio <0.1); one sample was positive on ELISA, but confirmed negative on nPCR and was deemed at the time to be a false positive ELISA result (Table 2). The animal was tagged and scheduled to be rebled in 30 days. These PRRS negative serologic results were very encouraging.

Two weeks later on September 11th the farm experienced a sudden increase in pre-weaning mortality from 13% to about 19% associated with weak born piglets and neonatal scour. In addition, 4 gilts had farrowed 3-4 days prematurely with variable quality litters, some including high numbers of stillborn, large and/or small mummified piglets. Fearing a PRRSv outbreak, live, stillborn and mummified piglets were submitted for PRRS diagnostics along with sera harvested from 20 suckling piglets. Seven of 20 sera tested were PRRS ELISA positive and 12/20 sera were PRRS nPCR positive (Table 3). PRRSv was identified by immunohistochemistry (IHC) in 3 of 4 liveborn piglets submitted (Table 4). PCV2 and porcine parvovirus (PPV) results were negative. Four PRRSv isolates were sequenced and found to have 100% nucleotide homology to each other, and 98.8% to 99.3% homology to the isolates obtained from Farm A in November 2002. The cluster alignment of PRRSv isolates collected from the farm between 2000 and 2003 strongly suggested that the source of the PRRSv was the grower and finisher pigs residing on the “dirty” side, rather than the introduction of a novel field strain (Figure 2). Moreover, at this time and subsequently, Farm C remained serologically free of PRRSv, thus ruling out the new gilts as a potential source.

In spite of the setback, the owners were committed to the PRRSv eradication program. Several options were considered but to salvage the project herd closure and mass exposure of the
breeding herd via serum inoculation was approved. When this decision was made, no piglets had been weaned and the Farm A nursery remained uncontaminated. The off-site finisher (Farm B) was quickly retrofitted to receive weaned Farm A (PRRSv positive) piglets which enabled the on-site (Farm A) nursery and pre-grower areas to remain closed until such time that they would receive PRRS negative piglets. Depopulation and cleanout of the Farm A test and holding areas proceeded on schedule. The revised target was to produce PRRS negative breeding stock by the summer of 2004.

On September 22nd, approximately 8-12 mLs of blood was aseptically collected via the jugular vein from 12 suckling pigs demonstrating clinical signs consistent with PRRSv infection (unthriftiness, dyspnea). Piglets demonstrating signs of bacterial infection, septicemia, and piglets with rectal temperatures in excess of 40.3°C were not bled. The serum from the 12 pigs was centrifuged at 2000 rpm for 15 minutes, aliquoted into multiple 1.0 mL cryovials and all except one aliquot per pig were immediately placed into liquid nitrogen. The remaining unfrozen aliquot from each pig was submitted for PRRS nPCR. Ten of 12 samples were nPCR positive, and 6 of these 10 were submitted for quantification of PRRSv RNA to identify the most concentrated samples (Table 5). On October 14th, 12 lactating sows and 12 gilts in late gestation were inoculated IM (neck) with an estimated 1.50 x 10⁶ genomic copies RNA/mL diluted in 2 mL sterile phosphate buffered saline (PBS). The inoculum was derived from 2 piglets (Table 5). The primary purpose of this test dose was to ensure inoculum safety, thus all inoculated animals were monitored closely for 14 days. During this period, there was a notable drop in the feed consumption of gilts inoculated in late gestation as well as higher numbers of unthrifty piglets when these gilts farrowed, but no adverse effects were noted in the lactating sows.
On Oct 24th, a 5-month supply of naïve replacement gilts were transferred into Farm A from Farm C. Thirty of these gilts were selected as sentinels to ensure 100% seroconversion following inoculation. Immediately prior to entry, a 7 mL blood sample was collected from the jugular vein, and the serum frozen at -20°C. With no significant adverse effects evident following the trial dose, inoculation of the entire breeding herd was undertaken on October 27th with an IM inoculum dose of 3.09 x 10^6 diluted in 2 mL PBS. A second IM inoculation was performed on Nov 10th but the inoculum concentration was unknown because the viral RNA was not quantified in some of the aliquots used. On each day of inoculation, the frozen aliquots were removed from liquid nitrogen, immediately transported to the farm on ice, thawed at room temperature, diluted 1:100 in sterile PBS, and injected immediately. After inoculation, records were maintained detailing all clinical signs of disease such as off-feed sows, abortions, mortality, stillbirths etc.

On Nov 24th, 28 days post first inoculation (DPI), sera were collected from 34 randomly selected animals plus the 30 sentinel (Farm C) gilts that had entered about 1 month prior. PRRS ELISA results were positive (S/P>0.4) in 61/64, suspicious (S/P = 0.338, 0.347) in 2/64, and negative (S/P=0.071) in 1/64 samples. This negative sample was retested on ELISA with similar results, was PRRS nPCR negative but IgG IFA positive (1:64). These PRRS ELISA results indicated a very high level of PRRS exposure in animals in the herd.

By mid November clinical signs had stabilized in the farrowing rooms and pre-weaning mortality had dropped to about 12% (Table 6). Beginning December 1st (35 DPI), the staff aggressively implemented a PRRSv reduction program, in contrast to the preceding 6 weeks when PRRSv transmission was encouraged through minimal sanitation. The PRRSv reduction program included the rigorous implementation of a McRebel program, thorough sanitation (wash, disinfect, dry) of all areas of the barn, the placement of boot washes at the entry to the
farrowing area, improved fly control and manure pit management. On Dec 22nd (56 DPI) blood
collected from 10, 3-week old suckling piglets from different litters (pooled 3:1) and lung tissue
from 2 unthrifty 3-week old suckling piglets tested negative on nPCR. These results indicated
the possible cessation of PRRS viremia in this batch of piglets (15 weeks post initial clinical
signs).

On a weekly basis from January 26 to May 24, 2004 (17 weeks; one gestational cycle) one
piglet from each litter scheduled for weaning later in the week (mean 20.3 litters/week; range 9-
25) was aseptically bled via the jugular vein. Whenever possible, unthrifty piglets were selected
as they were considered to be the most likely piglets infected with PRRSv. The serum was
pooled 3:1 for most submissions, but individual piglet vials were tested on 4 of 17 weeks. All
samples in all 17 weeks were PRRS nPCR negative. On March 10th (135 DPI) approximately
120 PRRS naïve sentinel gilts from Farm C were transferred to various locations in the Farm A
breeding/gestation area and exposed to manure, placentae, stillborn and mummified piglets daily
for 14 days. Thirty of these sentinel gilts were bled 2 days pre-arrival and 19 days post arrival
(March 29th; 154 DPI). All sera were PRRS ELISA negative. Moreover, 4 of 30 samples with
S/P ratios >0.1 (0.116, 0.243, 0.249, 0.279) were confirmed negative by nPCR. On April 8th
(165 DPI) the Farm A nursery was recommissioned and on-site weaning commenced. Twenty-
four of the oldest nursery pigs were bled on May 24th and confirmed negative on PRRS ELISA
(S/P<0.288). The pregrower, test and holding areas were opened in succession. All sera
submitted from June to September 2003 from the pregrower, test, holding areas and sentinel gilts
was confirmed negative on PRRS ELISA. Thereafter, PRRS serology was scaled back and the
eradication program was declared a success.

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Discussion:

This case report describes the initial failure but ultimate success of a PRRS eradication program. The failure involved a sequential depopulation to create an 8-week “hole” in production combined with PRRS MLV vaccination of the existing grow-finisher population. ORF 5 sequencing confirmed that the virus transmitted from the “dirty” to the “clean” populations, resulting in clinical disease in naïve animals. The exact method of transmission from dirty to clean populations was never determined, but insect migration and/or aerosols of exhausted air were suspected\(^{18-21}\). Both are potential sources of transmission over short distances. Insufficient cleaning is an unlikely possibility due to the thorough and diligent cleanout undertaken, and because PRRSV is labile in the environment particularly in the presence of detergents and disinfectants. However, it is possible that the introduction of PRRS naïve breeding females into Farm A coinciding with the PRRS eradication program increased the risk of failure. Had the breeding herd been solidly immune, the absence of susceptible animals in this population may have prevented successful PRRSV transmission from the dirty side even if this population was exposed.

After the outbreak was confirmed in the naïve herd in September 2003, double serum inoculation was undertaken in conjunction with herd closure. The use of serum inoculation is controversial. While there are inherent risks such as inducing unplanned bacterial or viral infections, endotoxemia, or exacerbating PRRS-related mortality, serum inoculation ensures the development of acquired immunity to homologous field strains. Hence, it has been popular particularly where field strains are genetically distinct from the vaccines, or on farms where vaccines have failed to satisfactorily control the disease. Serum inoculation was instrumental in
this eradication program and double inoculation was a safeguard performed at the request of
senior management.

ORF5 sequence analysis performed in the herd in September 2003 confirmed that the strain
in the farm was 3.81% different from the PRRS MLV^w vaccine strain, which involved 23
separate ORF5 nucleotide substitutions (data not shown). Using the vaccine rather than serum
inoculation may have resulted in only partial protection, resulting in the ongoing shedding of low
levels of PRRSv and the transmission to naive animals. No severe undesirable effects were noted
following inoculation, except that reproductive performance was temporarily impacted.

Moreover, pre-weaning mortality, stillbirth and fetal mummification rates improved within 6-8
weeks of the first serum inoculation. While beneficial in this herd, serum inoculation should be
used as a last resort and follow adequate communication with herd owners and staff, and a
mandatory safety evaluation in a small group of animals.

In this case, quantitative PRRSv PCR was useful in determining the concentration of RNA
used for the serum inoculation. Aliquots from several different animals were blended together to
manage risk. Quantitative PCR provides a relative quantification of viral RNA or DNA. A
potential drawback of qPCR is that quantifying as genomic copies/mL does not necessarily
 correlate with infective virus concentration (ie TCID_{50}/mL). In this case, it was assumed that a
relationship existed and aliquots were blended to provide inoculum doses of 1.50 \times 10^6 and 3.07
x10^6 for the trial and inoculum #1 respectively. The philosophy behind quantification of the dose
was to help ensure the inoculum was not excessively concentrated and that sow mortality and/or
abortions would be prevented or reduced. While Benson et al.\textsuperscript{22} reported that in an experimental
challenge situation, inoculum doses did not significantly impact the results, it was felt that this
was nevertheless a safer way to proceed. To this end, the technique appears to have been useful.
Unfortunately, the concentration of inoculum #2 was unknown, because some aliquots used in
the inoculum were not tested with qPCR. This in retrospect was an oversight.

The PRRS serologic and PCR testing undertaken in this eradication program were more
intensive than would likely be undertaken in commercial units. While the diagnostic expenses
were very high, they were justified on the basis of the strategic importance of this genetic
nucleus, specifically the need to thoroughly document the eradication’s success in order to
rebuild customer confidence and sales. That being said, it is likely that the same level of
diagnostic testing would not be undertaken if the program was repeated in the future. Diagnostic
rationalization and the faster collection and inoculation would help to speed up the program and
reduce overall costs.

The farrowing rate was unsatisfactory in the herd prior to and during the eradication
program. While this may be partly due to PRRS, it is clearly not the entire explanation. An
analysis of breeding records (data not shown) indicated regular returns accounted for 40-70% of
returns to service in any given month. For this and other reasons beyond the scope of this report,
conception failure was the most likely reason for the ongoing suboptimal farrowing rate.

However, herd closure and the suboptimal farrowing rate contributed to an unacceptable drop in
throughput experienced during and immediately after the eradication program. More effort
should have been directed at solving the reproductive problems faster to help offset the costs of
the eradication program.

Summary:

Successful PRRSv eradication was performed in a 600-sow farrow to finish nucleus
operation located in an isolated location. During the eradication program, serology and PCR
diagnostics were used extensively to document the PRRS negative status, which has since been
maintained for over two years. ORF5 sequencing was useful in determining the source of the
PRRSv and the necessity for serum inoculation over commercial vaccination. Double inoculation
of the breeding herd was undertaken following an uneventful trial dose on a small number of
sows. Farrowing performance improved within about 8 weeks of the first inoculation and severe
side effects were not noted. The suboptimal farrowing rate was largely due to unrelated factors
adversely impacting conception rate. When herd closure is implemented it is imperative that a
superior farrowing rate be maintained. The success of partial depopulation in farrow to finish
herds may be improved by the removal of the entire nursery-grow-finish population.

References:

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   Porcine Reproductive and Respiratory Syndrome Virus (Porcine Arterivirus). In: Straw BE,
   Zimmerman JJ, D'Allaire S, and Taylor DJ, eds. Diseases of Swine. 9th ed. Ames, IA:

2. Nelsen CJ, Murtaugh MP, Faaberg KS. Porcine reproductive and respiratory syndrome virus

   Diagnostic Tools). Toronto, Canada. March 5, 2005;7-16.

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   in paraffin-embedded tissues: comparison of immunohistochemistry and in situ

5. Gilbert SA, Larochelle R, Magar R, Cho HJ, Deregt D. Typing of porcine reproductive and
   respiratory syndrome viruses by a multiplex PCR assay. Journal of Clinical Microbiology.


Table 1. Partial depopulation and PRRS eradication timeline

<table>
<thead>
<tr>
<th>Time</th>
<th>Milestone activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>November 2002</td>
<td>Obtain and sequence PRRS virus from pregrower pigs</td>
</tr>
<tr>
<td>February 2003</td>
<td>Discontinue breeding for 8 weeks to create an 8 week “hole” in production flow; begin breeding herd depopulation by culling all sows at weaning</td>
</tr>
<tr>
<td>April-June 2003</td>
<td>Initiate off-site breeding program (12 wks) at second nucleus farm</td>
</tr>
<tr>
<td>April 2003</td>
<td>Discontinue PRRS MLV vaccination of breeding herd</td>
</tr>
<tr>
<td>April-June 2003</td>
<td>Implement PRRS MLV vaccination of suckling and nursery piglets</td>
</tr>
<tr>
<td>May 2003</td>
<td>Breeding barn empty and partitioned</td>
</tr>
<tr>
<td>June 2003</td>
<td>Gestation barn empty and partitioned</td>
</tr>
<tr>
<td>July 2003</td>
<td>Farrowing barn empty and partitioned</td>
</tr>
<tr>
<td>August 2003</td>
<td>Nursery barn empty and partitioned</td>
</tr>
<tr>
<td>September 2003</td>
<td>Pregrower barn empty and partitioned</td>
</tr>
<tr>
<td>October 2003</td>
<td>Test barns (east &amp; west) empty and partitioned</td>
</tr>
<tr>
<td>November 2003</td>
<td>West holding barn empty and partitioned</td>
</tr>
<tr>
<td>December 2003</td>
<td>East holding barn, loadout and office empty and partitioned</td>
</tr>
<tr>
<td>February 2004</td>
<td>First sales of PRRS naïve animals</td>
</tr>
</tbody>
</table>
Table 2. PRRS ELISA results of 4 to 14 day old piglets bled on 25-August-03

<table>
<thead>
<tr>
<th>ID</th>
<th>S/P ratio</th>
<th>Interpretation</th>
<th>ID</th>
<th>S/P ratio</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.036</td>
<td>negative</td>
<td>11</td>
<td>0.033</td>
<td>negative</td>
</tr>
<tr>
<td>2</td>
<td>0.029</td>
<td>negative</td>
<td>12</td>
<td>0.047</td>
<td>negative</td>
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<tr>
<td>3</td>
<td>0.053</td>
<td>negative</td>
<td>13</td>
<td>-0.042</td>
<td>negative</td>
</tr>
<tr>
<td>4</td>
<td>-0.058</td>
<td>negative</td>
<td>14</td>
<td>-0.042</td>
<td>negative</td>
</tr>
<tr>
<td>5</td>
<td>-0.044</td>
<td>negative</td>
<td>15</td>
<td>-0.031</td>
<td>negative</td>
</tr>
<tr>
<td>6</td>
<td>-0.053</td>
<td>negative</td>
<td>16</td>
<td>-0.011</td>
<td>negative</td>
</tr>
<tr>
<td>7</td>
<td>-0.036</td>
<td>negative</td>
<td>17*</td>
<td>1.99</td>
<td>POSITIVE; nPCR negative</td>
</tr>
<tr>
<td>8</td>
<td>0.027</td>
<td>negative</td>
<td>18</td>
<td>-0.002</td>
<td>negative</td>
</tr>
<tr>
<td>9</td>
<td>-0.009</td>
<td>negative</td>
<td>19</td>
<td>-0.022</td>
<td>negative</td>
</tr>
<tr>
<td>10</td>
<td>-0.031</td>
<td>negative</td>
<td>20</td>
<td>-0.065</td>
<td>negative</td>
</tr>
</tbody>
</table>

S/P > 0.4 = positive

* vial 17 was deemed at the time to be a false positive based on PRRS nPCR result. As the dam of this piglet was sourced from a PRRSv naïve farm, it was very that the positive ELISA result was associated with maternal antibody. The positive piglet was tagged and scheduled to be rebled in 30 days, but wasn’t because the herd broke clinically within 30 days.
Table 3. PRRS ELISA and nPCR results from 20 suckling piglets bled 11-Sept-03 after the onset of clinical disease*

<table>
<thead>
<tr>
<th>ID</th>
<th>S/P ratio</th>
<th>nPCR result</th>
<th>ID</th>
<th>S/P ratio</th>
<th>nPPCR result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-0.033</td>
<td>P</td>
<td>11</td>
<td>-0.008</td>
<td>P</td>
</tr>
<tr>
<td>2</td>
<td>-0.018</td>
<td>N</td>
<td>12</td>
<td><strong>0.826</strong></td>
<td>P</td>
</tr>
<tr>
<td>3</td>
<td>0.006</td>
<td>P</td>
<td>13</td>
<td><strong>0.431</strong></td>
<td>P</td>
</tr>
<tr>
<td>4</td>
<td><strong>0.489</strong></td>
<td>N</td>
<td>14</td>
<td>0.344</td>
<td>P</td>
</tr>
<tr>
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<td>0.025</td>
<td>N</td>
<td>15</td>
<td>0.008</td>
<td>N</td>
</tr>
<tr>
<td>6</td>
<td>0.270</td>
<td>P</td>
<td>16</td>
<td><strong>0.847</strong></td>
<td>N</td>
</tr>
<tr>
<td>7</td>
<td><strong>0.937</strong></td>
<td>P</td>
<td>17</td>
<td>0.270</td>
<td>N</td>
</tr>
<tr>
<td>8</td>
<td>0.301</td>
<td>P</td>
<td>18</td>
<td><strong>1.204</strong></td>
<td>P</td>
</tr>
<tr>
<td>9</td>
<td>0.00</td>
<td>N</td>
<td>19</td>
<td>-0.018</td>
<td>P</td>
</tr>
<tr>
<td>10</td>
<td>0.012</td>
<td>N</td>
<td>20</td>
<td><strong>0.620</strong></td>
<td>P</td>
</tr>
</tbody>
</table>

* The dams of these piglets were sourced from a PRRS naïve herd (Farm C) and entered the empty and sanitized Farm A breeding-gestation area.

S/P ≥ 0.4 = positive
P = positive
N = negative
Table 4. Diagnostic results of liveborn, stillborn and mummified piglets submitted on 11-Sept-03 after the onset of clinical disease

<table>
<thead>
<tr>
<th>Case #</th>
<th>Age</th>
<th>Gross lesions</th>
<th>IHC</th>
<th>FAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>405</td>
<td>2 days</td>
<td>Poor body condition</td>
<td>PRRS positive</td>
<td>PCV2 negative</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Non-suppurative meningitis</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Colitis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>406</td>
<td>1 day</td>
<td>Poor body condition</td>
<td>PRRS positive</td>
<td>PCV2 negative</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Posterior subcutaneous &amp; soft tissue edema</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Moderate interstitial pneumonia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>407</td>
<td>2 days</td>
<td>Poor body condition</td>
<td>PRRS negative</td>
<td>Parvo negative</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cyanosis of ear margins &amp; skin</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mild mesocolonic edema</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dilated &amp; emphymematous alveoli, moderately congested</td>
<td></td>
<td></td>
</tr>
<tr>
<td>408</td>
<td>Day of birth</td>
<td>Septicemia (Pasteurella multocida)</td>
<td>PRRS positive</td>
<td>PCV2 negative</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Posterior subcutaneous &amp; soft tissue edema</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fibrinous exudates in abdomen</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Diffuse congestion most tissues</td>
<td></td>
<td></td>
</tr>
<tr>
<td>409</td>
<td>Stillborn</td>
<td>No abnormal findings</td>
<td>PRRS negative</td>
<td>Parvo negative</td>
</tr>
<tr>
<td>410</td>
<td>Stillborn</td>
<td>Moderate suppurative hepatitis</td>
<td>PRRS negative</td>
<td>PCV2 negative</td>
</tr>
<tr>
<td>411</td>
<td>Mummy</td>
<td>Large fetus</td>
<td>PRRS negative</td>
<td>Parvo negative</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Severe autolysis</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

PCV2 = porcine circovirus type 2
Parvo = porcine parvovirus
Table 5. PRRS nPCR and quantitative PCR results for serum aliquots collected from suckling piglets

<table>
<thead>
<tr>
<th>ID</th>
<th># mLs frozen</th>
<th>PCR results</th>
<th>RNA quantification (genomic copies/mL)</th>
<th>Dates vials were used as serum inoculum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Gilbert\textsuperscript{5} primers</td>
<td>Mardassi\textsuperscript{23} primers</td>
<td></td>
</tr>
<tr>
<td>7968</td>
<td>5</td>
<td>N</td>
<td>P</td>
<td>2.9 x 10\textsuperscript{6}</td>
</tr>
<tr>
<td>7507</td>
<td>2</td>
<td>P</td>
<td>P</td>
<td>4.9 x 10\textsuperscript{6}</td>
</tr>
<tr>
<td>7803</td>
<td>2</td>
<td>P</td>
<td>P</td>
<td>1.5 x 10\textsuperscript{6}</td>
</tr>
<tr>
<td>7964</td>
<td>4</td>
<td>S</td>
<td>P</td>
<td>1.7 x 10\textsuperscript{6}</td>
</tr>
<tr>
<td>7998</td>
<td>3</td>
<td>P</td>
<td>P</td>
<td>1.2 x 10\textsuperscript{6}</td>
</tr>
<tr>
<td>7800</td>
<td>1</td>
<td>P</td>
<td>P</td>
<td>2.2 x 10\textsuperscript{6}</td>
</tr>
</tbody>
</table>
Table 6. Impact of PRRS and the subsequent eradication program on reproductive performance

<table>
<thead>
<tr>
<th>Week ending date</th>
<th>TPB (#/L)</th>
<th>BA (#/L)</th>
<th>BD (#/L)</th>
<th>MUM (%)</th>
<th>PWMT (%)</th>
<th>FR (%)</th>
<th>RR (%)</th>
<th>Observations &amp; events</th>
</tr>
</thead>
<tbody>
<tr>
<td>17-Aug-03</td>
<td>9.1</td>
<td>7.9</td>
<td>1.2</td>
<td>0.1</td>
<td>13.4</td>
<td>23.1</td>
<td></td>
<td>Sows reported off feed</td>
</tr>
<tr>
<td>24-Aug-03</td>
<td>9.2</td>
<td>7.9</td>
<td>1.2</td>
<td>0.1</td>
<td>14.1</td>
<td>72.4</td>
<td>30.8</td>
<td></td>
</tr>
<tr>
<td>31-Aug-03</td>
<td>10.8</td>
<td>10.6</td>
<td>0.2</td>
<td>0.2</td>
<td>12.6</td>
<td>64.6</td>
<td>8.0</td>
<td></td>
</tr>
<tr>
<td>7-Sep-03</td>
<td>9.5</td>
<td>8.9</td>
<td>0.6</td>
<td>0.4</td>
<td>18.8</td>
<td>63.6</td>
<td>23.8</td>
<td></td>
</tr>
<tr>
<td>14-Sep-03</td>
<td>11.8</td>
<td>9.0</td>
<td>0.6</td>
<td>0.4</td>
<td>19.1</td>
<td>63.6</td>
<td>13.3</td>
<td>PRRS positive confirmation</td>
</tr>
<tr>
<td>21-Sep-03</td>
<td>9.1</td>
<td>7.7</td>
<td>1.4</td>
<td>0.9</td>
<td>35.1</td>
<td>63.8</td>
<td>21.9</td>
<td>Serum collected for inoculation program</td>
</tr>
<tr>
<td>28-Sep-03</td>
<td>9.2</td>
<td>7.7</td>
<td>1.6</td>
<td>1.2</td>
<td>28.8</td>
<td>74.8</td>
<td>18.2</td>
<td></td>
</tr>
<tr>
<td>5-Oct-03</td>
<td>8.3</td>
<td>6.9</td>
<td>1.4</td>
<td>1.2</td>
<td>56.4</td>
<td>68.9</td>
<td>35.1</td>
<td></td>
</tr>
<tr>
<td>12-Oct-03</td>
<td>10.1</td>
<td>8.9</td>
<td>1.1</td>
<td>0.4</td>
<td>25.0</td>
<td>71.9</td>
<td>34.4</td>
<td></td>
</tr>
<tr>
<td>19-Oct-03</td>
<td>8.0</td>
<td>7.3</td>
<td>0.7</td>
<td>0.9</td>
<td>38.2</td>
<td>76.4</td>
<td>33.3</td>
<td>Trial dose (24 sows)</td>
</tr>
<tr>
<td>26-Oct-03</td>
<td>11.0</td>
<td>10.2</td>
<td>0.8</td>
<td>0.5</td>
<td>27.9</td>
<td>78.1</td>
<td>30.0</td>
<td>Serum inoculation #1</td>
</tr>
<tr>
<td>2-Nov-03</td>
<td>8.5</td>
<td>6.6</td>
<td>1.9</td>
<td>1.9</td>
<td>35.4</td>
<td>75.3</td>
<td>31.0</td>
<td>Serum inoculation #2</td>
</tr>
<tr>
<td>9-Nov-03</td>
<td>9.0</td>
<td>8.4</td>
<td>0.6</td>
<td>1.4</td>
<td>17.4</td>
<td>79.3</td>
<td>57.6</td>
<td></td>
</tr>
<tr>
<td>16-Nov-03</td>
<td>8.3</td>
<td>7.3</td>
<td>1.0</td>
<td>2.6</td>
<td>11.8</td>
<td>72.2</td>
<td>28.6</td>
<td></td>
</tr>
<tr>
<td>23-Nov-03</td>
<td>11.1</td>
<td>9.9</td>
<td>1.2</td>
<td>0.6</td>
<td>12.1</td>
<td>64.6</td>
<td>32.0</td>
<td></td>
</tr>
<tr>
<td>30-Nov-03</td>
<td>8.0</td>
<td>7.1</td>
<td>0.9</td>
<td>1.0</td>
<td>12.7</td>
<td>60.5</td>
<td>21.0</td>
<td>PRRS reduction program initiated</td>
</tr>
<tr>
<td>7-Dec-03</td>
<td>9.7</td>
<td>8.0</td>
<td>1.7</td>
<td>0.0</td>
<td>12.5</td>
<td>55.4</td>
<td>17.0</td>
<td></td>
</tr>
<tr>
<td>14-Dec-03</td>
<td>7.4</td>
<td>7.0</td>
<td>0.4</td>
<td>1.4</td>
<td>3.6</td>
<td>50.0</td>
<td>25.0</td>
<td></td>
</tr>
<tr>
<td>21-Dec-03</td>
<td>10.0</td>
<td>9.6</td>
<td>0.4</td>
<td>0.9</td>
<td>6.0</td>
<td>53.7</td>
<td>31.3</td>
<td>First suckling piglets confirmed PCR negative</td>
</tr>
<tr>
<td>28-Dec-03</td>
<td>9.7</td>
<td>9.1</td>
<td>0.6</td>
<td>0.2</td>
<td>11.9</td>
<td>58.9</td>
<td>40.0</td>
<td></td>
</tr>
<tr>
<td>4-Jan-04</td>
<td>9.0</td>
<td>8.3</td>
<td>0.8</td>
<td>0.5</td>
<td>6.1</td>
<td>63.8</td>
<td>28.6</td>
<td></td>
</tr>
<tr>
<td>11-Jan-04</td>
<td>9.9</td>
<td>9.4</td>
<td>0.6</td>
<td>0.2</td>
<td>2.7</td>
<td>70.5</td>
<td>26.7</td>
<td></td>
</tr>
<tr>
<td>18-Jan-04</td>
<td>10.5</td>
<td>9.9</td>
<td>0.6</td>
<td>0.5</td>
<td>10.6</td>
<td>67.8</td>
<td>25.8</td>
<td></td>
</tr>
<tr>
<td>25-Jan-04</td>
<td>10.8</td>
<td>10.3</td>
<td>0.6</td>
<td>0.5</td>
<td>13.3</td>
<td>65.9</td>
<td>16.0</td>
<td>Wkly PCR testing of all litters initiated</td>
</tr>
<tr>
<td>1-Feb-04</td>
<td>10.4</td>
<td>9.8</td>
<td>0.6</td>
<td>0.3</td>
<td>14.5</td>
<td>66.7</td>
<td>25.8</td>
<td></td>
</tr>
<tr>
<td>8-Feb-04</td>
<td>10.0</td>
<td>8.9</td>
<td>1.1</td>
<td>0.5</td>
<td>15.2</td>
<td>65.2</td>
<td>4.0</td>
<td>IMPLEMENTED natural service</td>
</tr>
<tr>
<td>15-Feb-04</td>
<td>10.5</td>
<td>9.3</td>
<td>1.2</td>
<td>0.5</td>
<td>9.5</td>
<td>64.8</td>
<td>9.4</td>
<td></td>
</tr>
</tbody>
</table>

COMPUTER RECORDS CHANGED TO NEW SYSTEM - DATA DISCONTINUED
Figure 1. Layout of Farm A and relative locations of Farms B & C

FARM C – PRRS
naïve FF nucleus farm
(not to scale)

~85 km northwest

OFF-SITE FINISHER
FARM B (not to scale)

~20 km northeast

Breeding

Gestation

Farrowing

PreGrower

Nursery

Test

Test

Holding

Holding

FARM A LAYOUT
(not to scale)
PRRS sequences were isolated from 2 9-week-old pregrower pigs in 2002 and from 4 suckling piglets less than 7 days of age in 2003. A high degree of homology is noted in all isolates. The annual rate of nucleotide substitution from 2000-2003 was 0.5%.
Endnotes:

1 IDEXX Laboratories Inc., Westbrook, Maine
2 Ingelvac PRRS MLV, Boehringer Ingelheim (Canada) Ltd, Burlington, Ontario, Canada
3 Parvoerycheck, Bioniche Animal Health Canada Inc., Belleville, Ontario, Canada
4 ORF 5; Minnesota State Veterinary Diagnostic Laboratory, St Paul, MN
5 Ingelvac PRRS MLV, Boehringer Ingelheim (Canada) Ltd, Burlington, Ontario, Canada
6 Profilm, Pfizer Canada Inc., Kirkland Quebec, Canada
7 Ingelvac PRRS MLV, Boehringer Ingelheim (Canada) Ltd, Burlington, Ontario, Canada
8 Prairie Diagnostic Services Inc, Saskatoon, SK.
9 IDEXX HerdChek 2XR ELISA submitted to the Prairie Diagnostic Services Inc, Saskatoon SK.
10 5; Minnesota State Veterinary Diagnostic Laboratory, St Paul, MN
11 Prairie Diagnostic Services Inc, Saskatoon, SK.
12 South Dakota State Veterinary Diagnostic Laboratory, Brookings, South Dakota
13 IDEXX HerdChek 2XR ELISA submitted to the Prairie Diagnostic Services Inc, Saskatoon, SK.
14 Prairie Diagnostic Services Inc, Saskatoon, SK.
15 Biovet Inc., St. Hyacinthe, Quebec
16 Prairie Diagnostic Services Inc, Saskatoon, SK.
17 Prairie Diagnostic Services Inc, Saskatoon, SK.
18 IDEXX HerdChek 2XR ELISA submitted to the Prairie Diagnostic Services Inc, Saskatoon, SK.
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21 Biovet Inc., St. Hyacinthe, Quebec
22 Ingelvac PRRS MLV, Boehringer Ingelheim (Canada) Ltd, Burlington, Ontario, Canada