

# Diagnostic investigation into a reproductive failure associated with porcine parvovirus and porcine circovirus coinfection

Emily J. McDowell<sup>1</sup>; Amy L. Woods<sup>2</sup>, DVM; Derald Holtkamp<sup>1</sup>, DVM, MS  
Thomas G. Gillespie<sup>2</sup>, DVM, Dipl ABVP

<sup>1</sup>Iowa State University, Ames, Iowa; <sup>2</sup>Rensselaer Swine Services, Rensselaer, Indiana

## Introduction

Reproductive failure in swine refers to irregular returns due to failure of implantation, failure of pregnancy progressing to term resulting in abortions, or farrowing increased numbers of nonviable piglets resulting in decreased litter sizes.<sup>1</sup> The most common viral agents that can cause reproductive failure include porcine reproductive and respiratory syndrome virus (PRRSV), porcine parvovirus (PPV), pseudorabies (PRV), and porcine circovirus type 2 (PCV2).<sup>1</sup>

PPV is ubiquitous in swine herds across the world.<sup>1</sup> Because the virus is extremely stable in the environment, it is likely that pigs in infected herds are repeatedly exposed to the virus.<sup>1</sup> Clinical signs associated with PPV infection are limited primarily to maternal reproductive failure predominantly in gilts because second and subsequent parity females are more likely to have already been exposed and are actively immune.<sup>1,2</sup> Thus, the goal of managing PPV on farms is to create protective immunity through natural exposure and/or vaccination before breeding. Challenges to controlling PPV in a population are the wide variability in levels of passive antibodies and the long duration of passive antibody decay which may not drop below protective levels until three to six months of age. This may interfere with development of long-term, active immunity. Thus, some gilts are not effectively immunized against PPV until shortly before or even after breeding.<sup>1,2</sup>

Clinical signs of PPV infection in the breeding herd are decreased abdominal girth caused by absorption of the embryos as well as embryonic and fetal fluids, increased incidence of mummified fetuses, and increased number of irregular returns to estrus.<sup>1,2</sup> Macroscopic changes in fetuses under 70 days of gestation include: stunted growth, congestion and leakage of blood into tissues, increased vascularization over the surface of the fetus, accumulation of fluids in body cavities, hemorrhagic discoloration of tissues, and death with subsequent

dehydration (mummification).<sup>1,2</sup> Meningoencephalitis with perivascular cuffs of lymphocytes, plasma cells, and histiocytes are microscopic lesions that can be present with fetal PPV infections.<sup>1</sup>

Typically, infection of a naïve breeding age female will result in viremia and shedding of the virus for approximately two weeks after exposure. The virus can cross the placenta and infect the conceptus 10-14 days after maternal exposure.<sup>1,2</sup> The virus further infects other conceptuses in the litter via intrauterine spread. Thus, death of fetuses can occur at various stages of development within an infected litter. If a dam is exposed to PPV on or before day 56 of gestation, there are two possible scenarios. First, the virus may cross the placenta and infect an embryo (day 10 to 30 of gestation) causing death and resorption of the embryo and associated fluids. Second, the virus may cross the placenta and infect a fetus causing death and dehydration resulting in mummification. If the virus infects the dam after day 56 of gestation, the virus will likely infect the fetus around day 70 to term when the fetus is sufficiently immunocompetent to mount a protective immune response and will survive in utero.<sup>1</sup>

One of the possible clinical manifestations of PCV2 in a mature animal is reproductive failure. Reproductive failure associated with vertical transmission of PCV2 occurs mostly in gilts and is characterized by the virus crossing the placenta, infecting fetuses, and manifesting in increases in mid- to late-term abortions, mummified fetuses, stillborn pigs, weak and nonviable piglets at birth, and not-in-pig events.<sup>5,8</sup> Occasionally, fetuses will show dilated cardiomyopathy and hepatomegaly with secondary ascites. Microscopic lesions associated with PCV2-induced reproductive failure include: non-suppurative and necrotizing myocarditis with fibrosis and mineralization in the fetuses.<sup>5,8</sup> Reported reproductive failures associated with PCV2 were most commonly preceded by a source or housing change prior

to introduction into the breeding herd.<sup>3-8</sup> Subclinical PCV2 infections may be responsible for decreased vaccine efficacy in growing pigs; however, this has not been documented in adults.

### Herd description

The herd in this report is a 2400 sow multi-site pork production operation. The nursery and gilt development buildings are located on a site 0.2 miles from the sow farm and share a common driveway with the sow farm. The isolation barn flows all-in-all-out and is located 0.65 miles from the sow farm. Gilts are introduced, bred and gestated in a separate barn from the sows and are all housed in stalls after breeding. The boar stud is on site and is situated in the middle of the sow barns. There are also three finishing barns located at the sow farm site. The remaining finishing barns for the production system are located near the sow farm.

This is a PRRSV positive stable flow. The sow farm first used PRRSV live virus inoculation via serum injection to broadly expose and stabilize the sow herd on March 3, 2006 and continue to use live virus inoculation to acclimate gilts one week post-entry into the isolation barn. Gilts enter isolation at six months of age and are vaccinated three and seven weeks post-arrival with

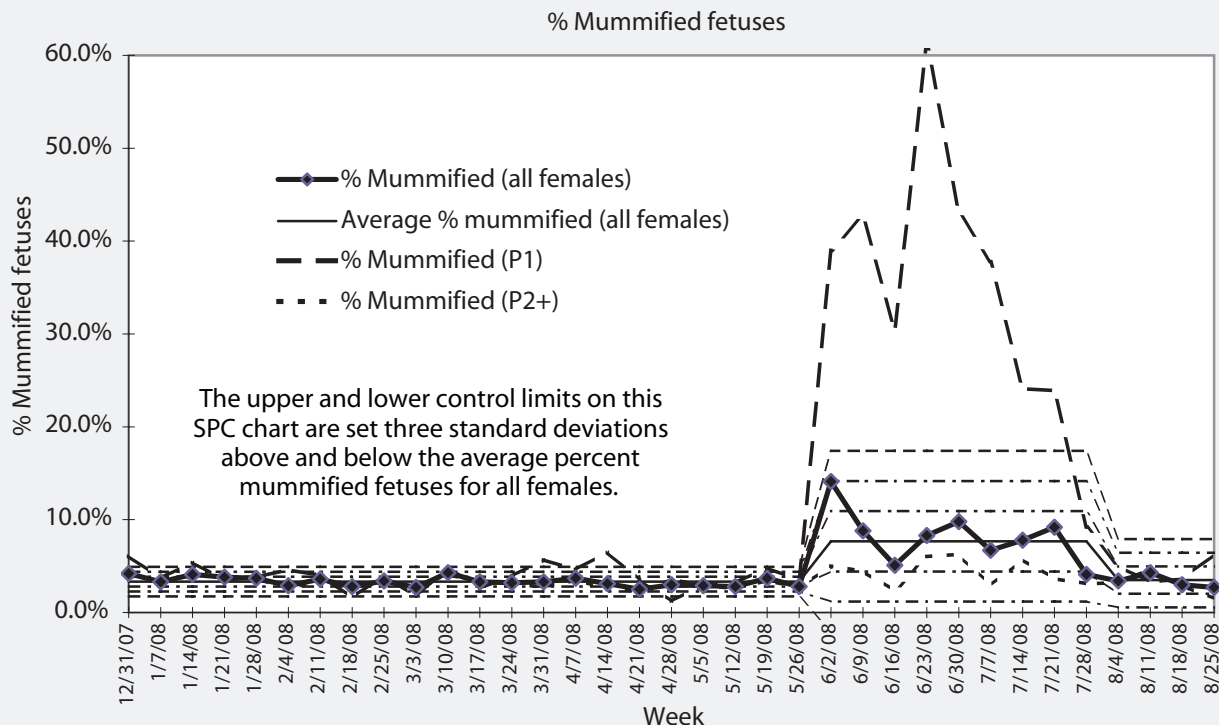
Parvo Shield L5E (Novartis Animal Health US, Inc, Greensboro, North Carolina), a commercially available PPV, *Leptospira*, *Erysipelothrix* (PLE) vaccine. Gilts also receive an Enterisol Ileitis (Boehringer Ingelheim Vet-medica, Inc, St Joseph, Missouri) and an autogenous SIV vaccine (containing H3N2 and H1N2 strains) while in isolation. Sows are vaccinated with Parvo Shield L5E three weeks prior to farrowing.

Farm staff reported changing from FARROWSURE Plus (Pfizer, Inc, Pfizer Animal Health, New York, New York) to Parvo Shield L5E in November 2007. The farm used internally multiplied gilts until the spring of 2008. As noted in figure 5, the farm received their first shipment of purchased gilts on April 15, 2008 and these animals remained in isolation until early June. Following an eight week isolation period, the purchased gilts entered the gilt breeding and gestation barn on the sow farm site.

### Clinical signs, test results, and outcome

Farm staff first reported an increase in late term mummified fetuses and stillborns in April 2008. Five fetuses and a placenta were submitted for diagnostic testing April 11, 2008. The fetuses ranged from 65 days gestation to full-term. Fetal serum IFA tested positive for

**Figure 1:** Statistical process control (SPC) charting that demonstrates the dramatic spike in percent mummified fetuses seen exclusively in gilt litters.



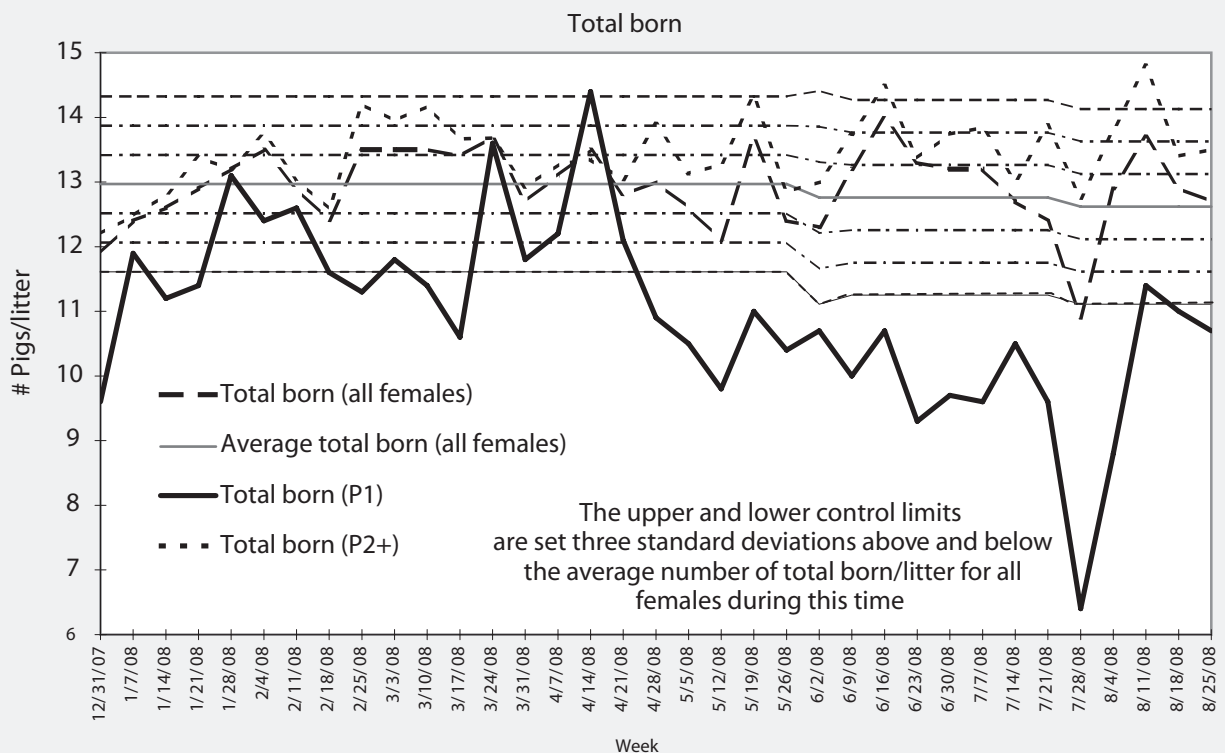
PCV2. PCR testing on fetal lung and spleen as well as a placenta was negative for PCV2 and PRRSV. Antibody tests on fetal serum for PRRSV, PRV, PPV, *Brucella*, and *Leptospirosis* came back negative. No viruses were isolated. Since results from the first submission were inconclusive of the etiology of the stillborn and mummified fetuses, additional fetuses were submitted to the diagnostic laboratory. Eleven fetuses from two different litters and two placentas were submitted on April 25, 2008. The mummified and stillborn fetuses ranged from 55 days gestation to full-term. PCR tests on pooled fetal tissue and thoracic fluids from 11 aborted fetuses were positive for PCV2. The FATS assay run on fetal organs were negative for PCV2 and PPV. Again, no viruses were isolated from fetal tissue or fluids. PCR testing on pooled fetal tissues and body fluids were negative for PRRSV.

Following an acute spike in mummified fetuses the week of May 26 to June 2, more mummified and stillborn fetuses were submitted on June 2 for additional diagnostic testing. PPV antigen was detected via cELISA on serum of a stillborn pool and a mummy on this submission. However, there was no evidence of PCV2 infection on this submission (PCR on fetal tis-

sue pool, IFA on fetal serum, FATS assay on stillborn pool). PRRSV was not detected via ELISA on stillborn or mummified fetal pools or by PCR on fetal lung and liver pools. Another set of fetuses and two live 1-day old piglets submitted June 26 found PCV2 via PCR in piglet lung and kidney and fetal lung, kidney, and thoracic fluid, IFA in piglet and fetal serum and by the FATS assay in a stillborn lung pool. PPV infection was confirmed through virus isolation in stillborn thoracic fluid, fetal organ pool, and piglet organ pools, FATS assay in fetal kidney and lung pools, and via cELISA in fetal and piglet serum. PRRSV was not found on virus isolation of fetal organ and lung pools or via PCR on fetal lung, kidney and thoracic fluid. Gross lesions observed included subcutaneous edema and red fluid in the pleural and peritoneal cavities, hepatic congestion, and interlobular edema in the lungs. Microscopic examination revealed mild multifocal meningoencephalitis with lymphocytic and histiocytic perivascular cuffing. Immunohistochemistry was not used to examine the heart for lesions consistent with PCV2. No significant bacterial pathogens were isolated.

PigCHAMP (PigCHAMP, Inc, Ames, Iowa) production data indicated a herd average mummified fetus

**Figure 2:** SPC charting of total born shows the decrease in total born in gilt litters indicating absorption of embryos before 30 days gestation and a marked decrease in litter size.



rate of 3.3% from January 1, 2008 to May 26, 2008. In contrast, May 26 to July 26 indicates an average mummified fetus rate of 7.7%. As shown in Figure 1, the percentage of mummified fetuses in gilts rose acutely from 3.9% to 31.5% for the same time period while mummified fetuses in multiparous sows rose only slightly from 3.2% to 4.2%. The percentage of mummified fetuses peaked the week of June 23 when 61% of pigs from parity one litters were mummified. Many parity one females farrowed entire litters of mummified fetuses. Total born and live born for parity one females decreased from 11.6 and 10.2 pigs-per-litter respectively for the period of January 1 to May 26 to 9.7 and 5.9 pigs per liter respectively from May 26 to July 26. Figure 2 shows the decrease in total born in parity 0 females which may indicate absorption of embryos infected before day 30 in utero. Multiparous females demonstrated much better protection since the production data showed little effect on mummification. PigCHAMP production data indicated 77 mid- to late-term abortions in the time period of January 1, 2008 to August 4, 2008. As seen in Figures 3 and 4, the abortions were characterized by an increased number of mid-late term abortions that occurred around May 26, 2008. These abortions were predominantly in parity 0 females.

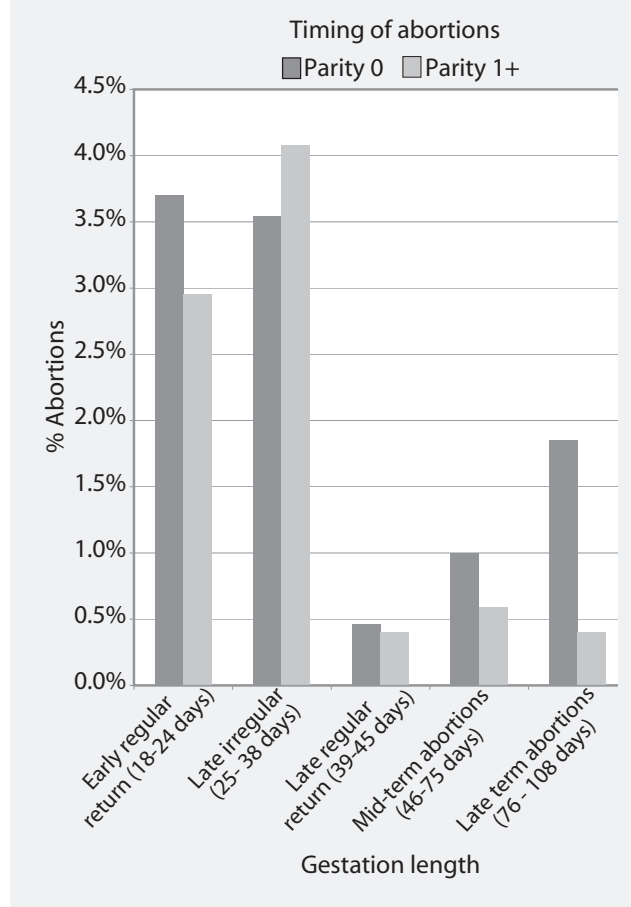
On June 8, 2008, the farm staff mass vaccinated the non-bred and gestating gilts with Ingelvac CircoFLEX (figure 5). At this time, PCV2 was the only pathogenic agent found to be associated with the reproductive failures. On July 2<sup>nd</sup> and 3<sup>rd</sup>, the farm mass-vaccinated the entire breeding herd with FARROWSURE Plus.

## Discussion

Reports of reproductive failure due to PPV have generally been in high health status herds or farms undergoing a PRRSV elimination project.<sup>12</sup> In contrast, previous reports of reproductive failure due to PCV2 have been reported in startup gilt herds, or herds undergoing a source or facilities change.<sup>3-9</sup> However, these scenarios were not present in this case considering the timing and factors involved.

Purchased replacement gilts of unknown serological status for PPV entered the production system on April 15, 2008 into an off-site isolation barn and did not enter the sow farm until early June (figure 5). The PPV HI testing on serum collected June 2 from seven month old purchased gilts, four weeks after a single dose of Parvo Shield L5E, indicated that gilts were positive with very high titers (> 1:512) suggestive of natural infection. Titers typical of a response to vaccination alone

**Figure 3:** Graph of the timing of abortions indicating higher mid- to late-term abortions in parity 0 females between November 1, 2007 and August 4, 2008.



range from 1:64 and 1:128.<sup>13</sup> PPV is transmitted to unexposed animals via the fecal-oral route. Even though PPV is not documented to travel on fomites, given the stability of the virus, it is plausible that it could travel from the isolation barn to the sow farm via manure in the midst of poor biosecurity. However, choring in the isolation barn is predominantly performed by personnel that do not enter the sow unit. In order for the PPV break to have resulted from introduction of purchased gilts, internally multiplied gilts that farrowed around June 2<sup>nd</sup> needed to be exposed before 70 days gestation, or by mid April. If gilts were exposed in mid April, the virus would likely incubate in the sow 10-14 days before crossing the placenta by mid April and resulting in mummification of fetuses. However, purchased gilts did not enter isolation until April 15, 2008. Purchased gilts did not enter the gilt breeding and gestation building on the sow farm site until mid June. Nonetheless, PPV vaccination of internally multiplied gilts at the sow farm should have elicited protection from clinical re-

productive disease. Due to these factors, PPV introduction caused by a source change seems like an unlikely factor in this case.

Reproductive failure in the internally multiplied gilts could have been caused by high maternal antibodies, vaccine failure or a compliance issue. Maternal antibody titers to PPV have been shown to be present for three to six months,<sup>1,2</sup> but their role in this case is unknown. If the problem gilts were still carrying maternal antibody titers at the time of the Parvo Shield L5E vaccination, it is unlikely that the vaccine would be able to override these titers to provide adequate protection. However, no serology was performed to determine the level of maternal antibody prior to the first vaccination. Similarly, no diagnostic serology was done on problem gilts prior to breeding to measure the response to the PPV vaccine. Therefore, we cannot confirm that high maternal antibody levels influenced PPV vaccine effectiveness on the internally multiplied gilts that experienced higher than usual mummified fetuses during the problematic eight week farrowing period. Even though the timing, dosage, and administration of the parvovirus vaccination was said to be performed properly, the acute reproductive failure could be due to non-compliance at the farm. Nonetheless, the change in vaccination product corresponded with the time period of reproductive failure. Despite vaccination for PPV in gilts, stillborn and mummified fetuses tested positive for PCV2 (IFA, PCR, FATS) and PPV (cELISA, FATS, VI).

Current cleaning procedures may lead to poor natural exposure in gilts which would leave them more vulnerable to infections during gestation. Strict biosecurity protocols implemented to control other viruses, multi-site production, and segregation of age groups within a herd may have decreased the probability of natural exposure to PPV. Commercially available disinfectants in the formaldehyde, paraformaldehyde, glutaraldehyde, and hypochlorite classes are expected to be effective at inactivating parvoviruses.<sup>14</sup> Some of these disinfectants are commonly used in production systems today to inactivate other viruses (PRRSV and PCV2).

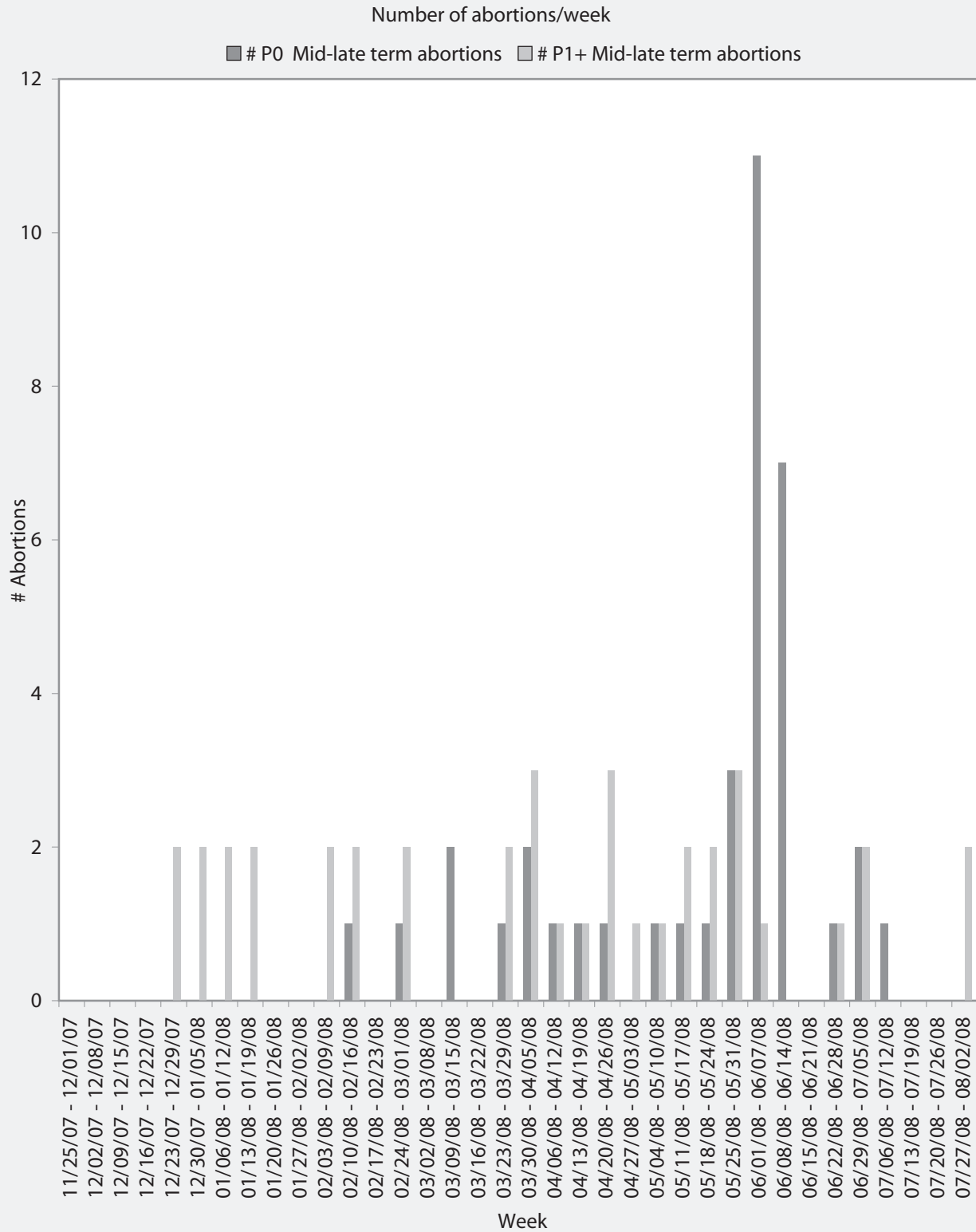
The role of PCV2 in this case is unknown but may be involved by causing subclinical PCV2 infection and thus reducing vaccine efficacy, synergistic effects with PPV to increase the severity of the break, or by causing mid- to late-term abortions not typically seen with PPV infections. Subclinical PCV2 infection or immunosuppression may have worked to decrease the efficacy of the PPV vaccine.<sup>10</sup> PPV and PCV2 have a synergistic effect that may have contributed to the severity of this break. Coinfections of PCV2 and PPV have been

found to experimentally and naturally result in PCVAD in growing pigs.<sup>9,15,16</sup> PPV and PCV2 coinfections in-utero have also been known to enhance the lesions seen in aborted piglets over those infected with PCV2 alone.<sup>17,18</sup> Thus, the synergistic effects of PPV and PCV2 may have contributed to the unusually high percentage of primiparous females farrowing mummified fetuses. Cases of PCV2-associated reproductive failures generally have evidence of diffuse non-suppurative and necrotizing myocarditis with fibrosis and mineralization.<sup>3-9</sup> However, multiple diagnostic reports indicated the absence of heart lesions microscopically in submitted mummified fetuses and stillborns. Finally, there were a high percentage of mid- to late-term abortions in this case which is common in PCV2 infections but not typically found in PPV infections. Nonetheless, PCV2 was consistently found on diagnostic testing in tissues and in serum, indicating exposure.

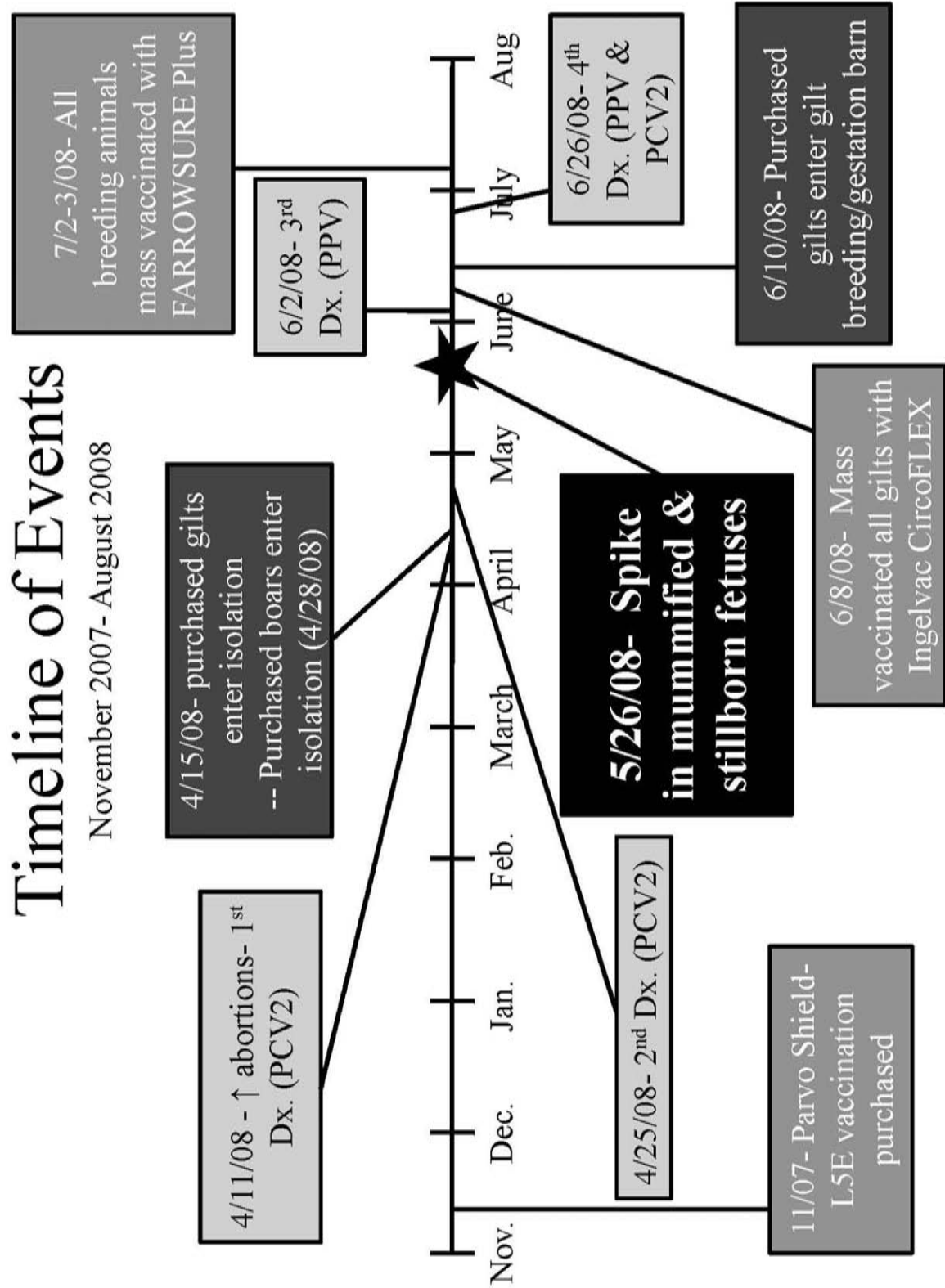
Throughout the investigation, numerous other issues were uncovered and handled accordingly. The ultrasound used for pregnancy detection on this farm was broken and was sent away for repairs during the period of May through August. Also, it was found that the mycotoxin binder had inadvertently been left out of the ration for a time. MTB-100 (Alltech, Lexington, Kentucky) was added to the feed to bind mycotoxins.

Because so little is known about PCV2 and co-factors related to reproductive failure, recommendations were made on the basis of those known to be successful for controlling PPV. The most commonly used management strategy to control PPV is vaccination of gilts or the entire herd to create uniform protection in the herd.<sup>1</sup> Arranged contact between seronegative gilts and seropositive sows and fecal feedback are other management strategies to control PPV.<sup>1,2</sup> The whole herd was mass vaccinated for PPV on July 2<sup>nd</sup> and 3<sup>rd</sup> with FARROW-SURE Plus. Furthermore, the producer was encouraged to feedback mummified fetuses, placenta and feces from animals in farrowing to all gilts in isolation as well as non-bred gilts at the sow farm. Finally, the timing of administering the PLE vaccination in isolation was moved to a slightly older age to avoid the long-lasting maternal antibodies typical of PPV.<sup>1,2</sup> Gilts now receive the first PLE vaccination at six and a half months of age with the booster administered three to four weeks later at entry into the sow farm. Gilts are now vaccinated with Ingelvac CircoFLEX in isolation as well.

**Figure 4:** The number of abortions during the period of November 1, 2007 to August 4, 2008 rose around May 25, 2008 and followed a trend similar to the mummified fetuses.



**Figure 5:** Timeline of events summarizing the events related to this investigation from November 2007 to August 2008.



## Take home messages

- PPV is a ubiquitous organism and still remains a significant cause of reproductive failure and thus, vaccination protocols still need to include PPV.
- Testing for PPV is not routinely done (not done in this case either); however, economically feasible testing for PPV prior to entry into the sow farm may have identified poor PPV exposure prior to seeing clinical problems.
- As in this case, diagnostics for reproductive failures can be challenging; practitioners need to send in multiple fetuses per litter as infection of fetuses may be as low as 10-50% of in-utero fetuses.
- The role of PCV2 as a co-factor in reproductive failure is unknown, but deserves further investigation and attention.

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