

Ingelvac® CircoFLEX™ PCVAD Symposium Agenda March 2, 2007

2:30 p.m.	Welcome and introduction	Klaas Okkinga BIVI-USA
	Moderator	Dr. Ernest Sanford, BI-Canada
2:40 p.m.	Investigating PCVAD – Results from the MAGIC™ program	Dr. Kent Schwartz, Iowa State University
3:00 p.m.	Sub-clinical PCV-2 infection and effect on growth in young pigs	Dr. Tanja Opriessnig Iowa State University
3:20 p.m.	<i>Break</i>	
3:30 p.m.	Efficacy of Ingelvac® CircoFLEX™ in Early and Late PCVAD Situations	Dr. Marc Eichmeyer BIVI, USA
3:50 p.m.	Preliminary results with Ingelvac® CircoFLEX™ to protect multiple ages of Quebec pigs against PCVAD	Dr. Robert Desrosiers BI-Canada
4:10 p.m.	Case report from Canada	Dr. Zenon Forster, Big Sky Farms, Saskatchewan
4:15	Case report from USA	Dr. Ron White, Iowa Select Farms, USA
4:20	Q&A	
4:30	Closing	



Investigating PCVAD – Results from the MAGIC™ Program

Dr. Kent Schwartz, Iowa State University VDPAM
Dr. John Kolb, Boehringer Ingelheim Vetmedica

Introduction

Porcine circovirus type 2 (PCV2) has emerged as a major contributor to disease and mortality in swine, with reports from around the globe. In the last decade, there has been an epidemic of a new manifestation and magnitude of disease associated with PCV2 in Europe and North America. While porcine circovirus (PCV) has been present in swine populations for decades, emergence of a cluster of the virulent phenotypic variants are now referred to as PCV2.

PCVAD Definitions

Disease associated with PCV2 (PCVAD) has been challenging to quantify and originally was met with some skepticism. However, the recent epidemic, along with sufficient experimental evidence, has confirmed a role for PCV2 as a pathogen. Definitions, perceptions, and nomenclature continue to evolve. Original descriptions of PCVAD were targeted toward the post-weaning multi-systemic wasting syndrome (PMWS) where clinical signs, typical lesions, and demonstration of the presence of PCV2 were required for diagnosis. PCV2 appears to cause and contribute to disease in a number of ways. Clinical disease (pneumonia, enteritis, septicemia, nephropathy, wasting, CNS) manifestations are magnified, if not caused, by the presence of virulent PCV2. Definitive diagnosis of PCVAD in an individual animal, in the author's opinion, requires demonstration of PCV2 antigen within a compatible lesion. Yet clinical disease (fever, malaise, inflammation) may be caused by PCV2 in the absence of definitive lesions or ability to demonstrate PCV2 by IHC. Dogmatically clinging to contrived definitions may be warranted for clarity in individual animal diagnosis but is usually not the goal of a diagnostic investigation. Immunohistochemistry is not particularly sensitive in detecting PCV2, yet is quite useful for confirmation of PCVAD in affected individuals when sufficient lesions and virus are present. PCR is quite sensitive to confirm the presence of PCV2, but is not sufficient for diagnosis of lesions or disease. Since disease severity is loosely correlated with viral load and lesion severity, quantitative PCR can be useful for diagnosis of clinical disease in groups of pigs.

Diagnosis of PCVAD on a herd basis relies on the best estimate of the contribution of PCV2 (within the context of the contributions by other endemic and epidemic agents) to overall clinical morbidity and mortality in the herd. Herd diagnosis is necessarily complicated and inexact because of confounders, perceptions, and the fact that a population is made up of unique individual animals which range from severely affected to not affected. Herd diagnosis(es) become important because of the range of interventions and economic resources that can be applied. Practitioners, hopefully, will continue to debate these issues.

Goals of MAGIC

The MAGIC™ project, defined as a “Monitoring Assignment for Global Insight of Circovirus disease”, was designed just as the PCVAD epidemic was beginning in the US. At that time, the exact expression of disease had yet to be revealed, and a comprehensive approach to understand the role of both PCV2 and co-infections was important in PCVAD. The main points the project sought to investigate included:

1. To better define the role of PCV2 and cofactor dynamics in disease-associated periods of high mortality in commercial swine populations,
2. To identify common co-infections or risk factors in periods of high mortality which could immediately provide control options,
3. Provide the optimal recommendations on use of PCV2 vaccines once they became available.

The Magic program initially targeted 15 herds in a pilot project. It rapidly expanded as the incidence of disease likewise increased in the industry over the course of 2006. A total of 59 herds fully completed the initial phase of MAGIC in 2006.

Design of MAGIC

The design of the project was based on both the definitions proposed by Sorden (2000) for PCVAD in individual pigs, and by the European and American Association of Swine Veterinarians (AASV) PCVAD committees for a herd level diagnosis (Segales 2001). These include:

Individual Animal Case Criteria

1. Clinical signs consistent with PCVAD, including rapid weight loss, inappetance, icterus, respiratory disease and failure to respond to conventional treatments.
2. Lymphoid depletion with or without disseminated granulomatous inflammation in those same tissues (e.g. spleen, thymus, intestines, lymph nodes (sternal, bronchial, inguinal and mesenteric), lung, kidney, liver, tonsil, etc.).
3. Detection of PCV2 antigen with typical lesions, by immunohistochemistry (IHC) or in situ hybridization.

Herd Case Criteria

4. Elevated mortality, expressed either as average mortality greater than 1.67sd the historical mean, or approximately double the baseline pre-outbreak mortality
5. Demonstration of PCVAD as the primary cause of the elevated mortality.

The diagnostic protocol consisted of either a cross sectional or longitudinal serum sampling, and targeted post mortem examinations. This standard diagnostic protocol was developed to identify the presence of swine pathogens. The majority of cases were performed in a cross sectional approach, and completed the full protocol. Clinical signs for the group, and both clinical signs and gross lesions of pigs selected for post mortem

were recorded. Tissues were submitted to Iowa State University under this standard protocol.

Serum was collected from the breeding herd (20 animals) and growing herd (50 animals, 10 per age group at approximately 4, 10, 14, 18 and 22-24 weeks of age), with sample sized based on detecting at least one positive sample when the estimated prevalence of disease is at least 10%. Tissue diagnostics were designed to identify those primary pathogens associated with PCVAD, and collect tissues to allow for identification of any novel pathogens. These tissues were collected at the period of peak mortality/clinical signs, three to four weeks prior to the peak mortality, and five to six weeks prior to peak.

The time frames for tissue selection were chosen to match onset of PCV2 circulation, viral build up and peak impact of the virus, respectively, based on models in controlled challenge studies (Allen 2000). Based on other reported challenge models, co-infections commonly identified with PCV2 would also be successfully detected from the three to four weeks pre-peak, and peak samples (Porcine Reproductive and Respiratory Syndrome (PRRS), *Mycoplasma hyopneumoniae* and *Salmonella*). Four case pigs and one control pig were targeted at each time point, though in some cases not all pigs selected fit the clinical definitions. The AASV case definition (at least double mortality vs. baseline) gave an estimated prevalence of 50% of total mortalities due to PCVAD. From this, the sample size determination of five pigs per tissue sampling was made (to detect at least one positive pig with 90% confidence from the population of dead pigs).

Table 1 – Tissue Sample Collection Schedule

RESP.	ENTERIC	SYSTEMIC	LYMPH
Cranial lobe	Mid Jejunum	Liver	Tonsil
Middle lobe	Proximal ileum	Spleen	Resp. Nodes
Caudal lobe	Distal ileum	Kidney	Mesen. Nodes
Hilar section	Cecum		Subiliac Nodes
	Spiral Colon		

Testing methods included serum antibody tests for PRRS, *Mycoplasma hyopneumoniae* and *Salmonella* species. PCR was performed on hilar lung samples and serum samples to detect PCV2, PRRS and SIV. Immunohistochemistry was performed on lung, and a portion of lymphoid tissues for PCV2, and selectively on other tissues. IHC for *Lawsonia intracellularis* was performed when suspicious lesions were encountered. Bacterial culture was performed to identify *Salmonella* from intestinal or lymphoid samples, and aerobic bacteria from lung, liver and other tissues, based on the discretion of the attending diagnostician.

Herd Results

The evaluation that follows used herd results as the basis for calculations. That is, one herd could have three tissue submissions and a cross sectional or serial serology submission to determine the final herd diagnosis. The diagnosis rates were based on the number of farms submitting samples at each post mortem time point. Fifty-nine farms were sampled in the MAGIC diagnostic process in 2006, and submitted at least one set of post mortem tissues, with final results. Lesions consistent with PCVAD, including lymphoid depletion and multifocal granulomatous inflammation of lymphoid tissues, liver, intestine and lungs were detected in at least one set of tissues from all farms, with concurrent positive detection of PCV2 antigen.

At five to six weeks pre-peak, 37% of sampled cases had at least one pig diagnosed positive for PCVAD. At 3-4 weeks prior to peak clinical disease, 74% of cases had at least one positive. At peak mortality, 90% of cases had at least one positive pig detected. Those cases where no pigs were identified at peak were all confirmed with one or more pigs diagnosed at the 3-4 wk pre-peak sampling point. Hence, all farms were confirmed positive for PCVAD at one or more time points of the clinical episode.

PRRS virus was the second most common pathogen identified as a concurrent infection during clinical PCVAD. Seventy-two percent of cases had an identified co-infection with PRRS virus concurrent with PCVAD. Diagnosis was made based on positive PCR from tissue or serum samples at the same time of PCVAD diagnosis in tissue, or on seroconversion from none positive to two more serum antibody positive pigs at the time of PCVAD tissue diagnosis.

Salmonella species were identified as the second most common co-infection. Diagnosis was made based on concurrent detection of group seroconversion (increase of two more positive pigs), bacterial isolation, or presence of lesions consistent with intestinal salmonellosis (ulcerative colitis). Fifty-eight percent of cases had concurrent Salmonellosis. *Salmonella typhimurium* was the most common serovar isolated from intestinal samples.

Swine influenza was present in 30 % of cases, as indicated by positive PCR from hilar lung samples. Serology was discontinued after the initial phase, due to the difficulty in differentiating antibodies due to vaccination and the variable serologic response to differing SIV isolates.

Mycoplasma hyopneumoniae was identified as a concurrent infection in 18% of MAGIC cases. A positive diagnosis was made when pigs were PCR positive from consolidated lung tissue accompanied by typical microscopic lesions. Additionally, post mortem results for all positive herds had clinical signs and gross lesions consistent with *M. hyo*-associated pneumonia. One additional case had positive lung tissue PCR accompanied by typical clinical signs, gross lesions and seroconversion in the cross sectional tested pigs.

The criteria for co-infection with PRRS or other pathogens were made to be as specific as possible to prevent diagnosing pathogens as cofactor infections when their circulation was not present at the time of the PCVAD outbreak. As a result, the role of a given pathogen may be underestimated for the period of the entire finishing phase.

Additionally, circulation of pathogens may have occurred at other time points during the nursery or finishing phases than did PCV2 circulation. These infections may be clinically important on their own, even if they do not directly contribute to the severity of PCVAD.

Observations from MAGIC

MAGIC provided a unique opportunity to examine lesions present in pigs (typically four clinically affected and one clinically unaffected) during periods of peak mortality. The clinically unaffected animals had very similar lesions to those clinically affected. These animals may have simply been preclinical, not yet fully affected, or conversely in the process of clearing viral infection at the time of sampling.

Animals with concurrent disease generally had more PCV2 antigen and compatible lesions associated with concurrent disease process. *The variation in PCV2-associated lesions and intensity of IHC staining may be exacerbated within tissues with concurrent or previous insult. Don't limit diagnostic opportunities by selecting a limited number of tissues for submission.*

Lesions vary considerably in tissue location and severity between individuals within groups, amongst groups, between timing of selection, and between farms. *Submission of a full set of tissues for diagnostic examination is important to rule out a role for PCV2. Samples collected early in the disease process, at 5-6 six weeks pre-peak, are significantly less likely to contain antigen compared to peak samples, and their interpretation must be made with this in mind.*

Specific lesions and locations of interest:

Enteritis: The gut of swine is a dynamic organ important in immune surveillance. Microscopic changes of nonsuppurative infiltrates and reactive lymphoid tissues are fairly common. In this study, nonsuppurative to macrophagic enteritis was a common finding, albeit not specific, in cases of PCVAD. In some cases, there was severe lymphoid depletion and granulomatous inflammation in Peyer's patches and the lymphoglandular complexes of colon. In some animals, microscopic lesions and positive IHC staining were found only in the enteric system.

A decade ago, it was unusual to find lesions in kidneys of growing swine in routine submission to ISU VDL. In this study, the kidneys frequently had multifocal to locally extensive nonsuppurative inflammation. Mild lesions were characterized as multifocal, mild to moderate nonsuppurative cuffing of small blood vessels, particularly at the cortico-medullary junction. In more severe cases, there was severe diffuse nonsuppurative interstitial nephritis. In the most severe,

there was accompanying fibrosis and glomerulopathy with distention of tubules with protein and features of end-stage kidney disease.

Respiratory disease is very common in commercial swine operations, and it was not unexpected that lungs were frequently diseased in this study. The lesions of PCV2 in the absence of other detected pathogens in lung were usually not severe. Mild nonsuppurative interstitial pneumonia, nonsuppurative bronchiolitis, peribronchiolar cuffing, perhaps with fibrosis is a feature in some cases. Occasionally, necrotizing bronchiolitis was associated with PCV2, but this lesion is a common feature with swine influenza virus as well. In some cases, dramatic interstitial and interlobular edema was present, which is generally associated with PCV2. It is noteworthy that lung lesions are not often specific for a particular etiology in naturally occurring porcine respiratory disease complex (PRDC). Microscopic lesions in lung in PRDC are often mixed, chronic-active, and irregularly distributed.

Spleens are not consistently affected with lesions, but when present, either had macrophagic to granulomatous inflammation with lymphoid depletion or had acute necrotizing vasculitis, hemorrhages, or infarctions.

Livers frequently had increased periportal mixed inflammatory infiltrates. Occasionally, there were multifocal aggregates of nonsuppurative inflammation, sometimes with frank hepatocyte necrosis. While neither consistent nor specific, these lesions in PCVAD pigs were sometimes quite notable and are not commonly associated with other specific disease processes.

Lymph nodes and lymphoid tissues were as previously described and expected. In pigs that are not terminal, the inflammation and lymphoid depletion may not be dramatic. There often was wide variation in severity of lesions and intensity of IHC staining between lymphoid organs in a single pig as well as between pigs in the cohort. Tonsils were sometimes unremarkable with some or all of lymph nodes severely affected or visa versa.

Implications & Interpretation

Animals with concurrent disease generally had more PCV2 antigen and compatible lesions associated with concurrent disease process. *Variation in PCV2-associated lesions and intensity of IHC staining may be exacerbated within tissues with concurrent or previous insult. It is important to rule out a role for concurrent infectious diseases.*

Lesions vary considerably in tissue location and severity between individuals within groups, amongst groups, between timing of selection, and between farms. *Submission of a full set of tissues for diagnostic examination is important to rule out a role for PCV2. Most practitioners would necropsy sufficient numbers of pigs to establish a trend in observations; that number is a minimum of 3 to 5. A standard diagnostic workup for diagnosis of PCVAD should include:*

1. In 10% formalin (3.7% formaldehyde solution), collect one-half inch thick slices to be used for histopathology and IHC for PCV2 antigen.
 - a. Lung: 2-3 portions (1" x 2" x ½" slices) from the range of gross lesions observed
 - b. Tonsil: ½" slice
 - c. Lymph nodes: ½" slices (larger slices are better)
 - d. Ileum: 2 portions, each 2" long (contains Peyer's patches)
 - e. Colon: 1 portion, 2" segment (feces removed enhances fixation)
 - f. Kidney: one slice
 - g. Liver: one slice
 - h. Other gross lesions: ½" slices
 - i. Brain is required if CNS: ½ in formalin
 - j. Synovium required if arthritis

2. Fresh tissues: Syndrome-driven but will likely include
 - a. Lung (bronchoalveolar lavage or parenchymal swabs can work as well)
 - i. PCR for PRRSV, SIV, M hyo
 - ii. Bacteriology
 - b. Lymph nodes
 - i. For PCV2 isolation or characterization (optional)
 - ii. PCR, Sequence, RFLP, isolation (optional)
 - c. Enteric disease, if present:
 - i. 6" ileum, affected portions of small intestine
 - ii. 6" colon, affected portions of large intestine
 - iii. Feces from ACUTE pigs for PCR for TGE if suspected
 - iv. Gross examination only for gastric ulcers: pars esophagea
 - d. CNS disease, if present: Bacteriology, Virology, PCR
 - i. ½ brain on ice
 - ii. Spinal cord segments if paresis/paralysis
 - e. Joints if arthritis present: Bacteriology and PCR
 - i. Joint fluids, joint swabs
 - ii. *M hyosynoviae*, *Erysipelothrix*, *Hemophilus*, *Strep*, *Actino*
 - f. Other tissues as indicated by clinical signs or gross lesions

These samples would best be collected approximately four weeks pre-peak clinical disease, and again at peak disease. Those critical co-infections or farm practices would be most likely detected at these points.

Conclusions

PCVAD presents the practitioner and diagnostician with the opportunity and necessity to perform a thorough diagnostic evaluation of production systems. Only a complete diagnostic approach can be expected to routinely detect the critical co-infections that may help drive the clinical severity of PCV2 associated disease. The MAGIC project allowed for correct diagnosis and a unique investigation of tissue pathology throughout the course of disease.

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References

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Efficacy of Ingelvac® CircoFLEX™ in Early and Late PCVAD Situations

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Porcine circovirus type 2 (PCV2) has been associated with disease around the world. Recently there have been reports of varying levels of virulence associated different isolates³. Virulence may vary by isolate but it is still not understood why some animals are clinically unaffected while others within a herd experience clinical signs and mortality¹. Problems with Porcine Circovirus Associated Disease (PCVAD) have also been reported in a wide variety of scenarios. With the development of Ingelvac® CircoFLEX™, a PCV2 vaccine by Boehringer Ingelheim, there was a need to evaluate the effectiveness of this PCV2 vaccine in both early and late PCVAD field situations.

Piglets typically acquire maternal antibodies against PCV2. As these maternal antibodies wane, the pigs become susceptible to PCV2 infection. PCV2 infection can be detected by monitoring the level of viremia and it has been reported that the level of PCV2 viremia can be correlated with PCVAD status. PCV2 levels of 10^4 to 10^6 genomic copies are associated with a sub clinical infection, while levels of greater than 10^6 has been reported as indicative of clinically apparent PCVAD infection with PCV2².

Herds have experienced differences in PCVAD but this may be explained by the complexity of the disease and differences in cofactors involved. One consistent aspect is that PCV2 infections result in one of two manifestations, acute or chronic PCV2 infections. An acute PCV2 situation is one for which a sudden onset and identifiable cause can be identified (PCV2), this is observed with clinical disease. A chronic PCV2 situation is one in which the signs are less noticeable and the progression of PCVAD is gradual, this is commonly associated with a sub clinical infection. Both of these situations can and do exist within herds experiencing problems with PCVAD.

Two field efficacy studies were performed to evaluate the effectiveness of the new Boehringer Ingelheim PCV2 vaccine, Ingelvac® CircoFLEX™, in both early and late PCVAD situations. Studies were setup as negative controlled, double-blinded trials following Good Clinical Practices (GCP). Animals were vaccinated with either Ingelvac® CircoFLEX™ or with a placebo product containing no PCV2 antigen. The Ingelvac® CircoFLEX™ vaccine contains the immunogenic portion of PCV2 formulated as Purified Circovirus Antigen (PCA™) and a specially designed adjuvant. Administration was a single 1ml dose at approximately three weeks of age with the Ingelvac® CircoFLEX™ vaccine formulated at a minimum immunizing antigen inclusion level, as determined in previous studies. A study performed in Northern Germany was setup to evaluate the performance of Ingelvac® CircoFLEX™ in a herd which had been experiencing PCVAD-related problems late in the finishing unit for several years. A trial performed in the United Kingdom (U.K.) was performed to evaluate the performance of Ingelvac® CircoFLEX™ in a herd which had been experiencing early PCVAD problems.

In Northern Germany, the farm had a trend towards a late onset of PCV2 viremia. As animals became PCV2 positive, there was an increase in Porcine Respiratory Disease Complex (PRDC) and a slight increase in mortality and reduced weight gain. The frequency of animals suffering from clinically apparent PCVAD signs had not been higher than approximately 1.5% but mortality had increased by 3-5% during finishing.

In an effort to monitor the effectiveness of Ingelvac® CircoFLEX™ in the Northern Germany trial, several parameters were assessed. Through quantitative PCR (qPCR) the onset of PCV2 viremia was monitored. Onset of viremia was defined as the time point at which 50% of the control animals became PCV2 positive

(>10⁴ genomic copies). The onset of viremia in the control animals occurred at 18 weeks of age during the trial. The animals that received the Ingelvac® CircoFLEX™ vaccine had a lower frequency of PCV2-positive animals (60-84% reduction), a significantly shorter duration of PCV2 viremia (p < 0.0001), fewer PCV2 positive sampling days (p < 0.0001), and a lower proportion of animals with either clinically relevant (>10⁶) or sub clinical (10⁴-10⁶) viral loads compared to the placebo-treated animals. A significant difference in mortality was observed after the onset of viremia (p = 0.0127) reducing mortality by 59% in the Ingelvac® CircoFLEX™ treated group. Finally, there was a significantly higher mean weight gain difference for the Ingelvac® CircoFLEX™ treated animals (p < 0.0001) at the termination of the study.

The study performed in the U.K. was characterized by a recent history of an early onset of PCV2 viremia and a marked increase in PCV2-associated mortality. The herd had a relatively high health status and was negative for PRRS, SIV, and *M. hyopneumoniae*. Observed mortality due to PCV2 was around 15%. The situation was consistent with both chronic and acute forms of PCVAD.

The same parameters to evaluate vaccine effectiveness were utilized in the U.K. field study. Viremia was monitored through qPCR and the onset of viremia was demonstrated to occur at eight weeks of age. Once again, the Ingelvac® CircoFLEX™ treated group had a significant reduction in viremia (p < 0.0001) over the course of the study. The reduction in the percentage viremic animals ranged between 53% and 79% for study weeks 6 through 18. In addition, the Ingelvac® CircoFLEX™ treated group had a significant earlier end of viremia (p < 0.0001), shorter duration of PCV2 viremia (p < 0.0001), fewer positive samples days (p < 0.0001), and a lower proportion of animals with either clinically relevant (acute) or sub-clinically relevant (chronic) PCV2 viral loads compared to placebo-treated animals. A significant difference in mortality was observed after the onset of viremia (p < 0.0001) with a mortality reduction of 75% in the Ingelvac® CircoFLEX™ treated group. Finally, there was a significant difference in the weight gain difference in favor of the Ingelvac® CircoFLEX™ treated animals (p < 0.0001) at the termination of the study

To date, five GLP field efficacy studies have been performed; two in Germany, one in the U.K., one in France and one in Canada. All five of these field efficacy studies were performed with conventional animals that had different onsets of viremia, vaccination programs, concurrent infections and maternally derived antibody levels. Despite these differences, all studies performed have resulted in statistically significant differences in the key efficacy and economic parameters resulting from vaccination with Ingelvac® CircoFLEX™.

References

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Preliminary results with Ingelvac® CircoFLEX™ to protect multiple ages of Quebec pigs against PCVAD

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Introduction

Porcine circovirus type 2 (PCV2) is considered an essential element for the development of post-weaning multisystemic wasting syndrome, now called porcine circovirus disease (PCVD) in Europe, and porcine circovirus associated disease (PCVAD) in North America. This paper describes results obtained with a new PCV2 vaccine, Ingelvac® CircoFLEX™ in Quebec pigs that were vaccinated at different ages.

Materials and Methods

The system chosen for the study consisted of a 1300-sow herd, an offsite nursery and four different finishing units on other sites. The system was PRRS and *Mycoplasma hyopneumoniae* negative, but had consistently suffered PCVAD losses for about 18 months. These losses occurred exclusively in finishing units, and usually started about three to four weeks post-placement. The performance of the sows (27 pigs/sow/year) and nursery pigs (~ 1% mortality) was excellent.

The nursery site consisted of four identical buildings, each with four rooms of about 275 pigs. The pigs were allocated to receive either 1 mL of placebo (sterile water for injection) or 1 mL of the Ingelvac CircoFLEX vaccine. In each room, six pens contained control pigs and six pens contained vaccinated pigs. These pens alternated (controls, vaccinated, controls, vaccinated, etc.) so that potential exposure to microorganisms and environmental stresses would be similar for both groups. The injections were all administered on the same day (June 5, 2006), so pigs that were between 19 and 59 days of age were injected either with placebo or vaccine. An independent vaccination crew was hired to inject the pigs so that the entire staff of the farms involved and the clinical investigators (RD and DT) would be blinded to vaccine and control pens. Groups were identified as either A pigs (product A) or B pigs (product B). The pigs normally left the nursery at an average of 62 days of age, so those that were injected at 59 days of age were introduced into the first finishing unit only 3 days after treatment. The first finishing barn was filled with pigs that had been injected between 45 and 59 days of age (Barn 1). The other three were filled with pigs that had been injected respectively at 38 to 45 (Barn 2), 22 to 36 (Barn 3) and 19 to 22 (Barn 4) days of age. In all four barns, pens of group A pigs alternated with pens of group B pigs so that potential exposure would be similar. The first pen in the first barn received pigs from group A after it was determined through the toss of a coin. The second pen received pigs of group B, the third pigs of group A and so on and so forth. The same principle was used in the three other barns.

On the day of the injections, the vaccination crew evaluated the study pigs for post-injection reactions of any kind. The next three days following the injections, one of the investigators went on-site to evaluate if there were any reactions, local or systemic, in the study pigs. Afterwards, weekly visits were made by one of the investigators to evaluate both the safety and efficacy of the tested products.

The manager of the four finishing units included in the trial was advised not to euthanize pigs before the weekly investigator's visit, so that animals showing clinical signs representative of PCVAD could be necropsied. Ten tissues (cranial and caudal lung, tracheobronchial, mesenteric and superficial inguinal lymph nodes, tonsil, spleen, Peyer's patches, liver and kidney) of dead or euthanized pigs were submitted from about 100 pigs during the course of the study. These tissues were evaluated for histological lesions suggestive of PCVAD, and for quantification of the PCV2 load by immunohistochemistry (IHC). The pathologist in charge (TC) of the evaluation was also blinded as to which pigs had received the vaccine.

The pigs to be euthanized were selected in common by both the investigator and manager of the farms taking into consideration the likelihood that the animal would live, the suffering endured and the probability that it would be condemned at the abattoir if kept alive.

Results and Discussion

At the time of writing the trial had just been completed and some data were not completely validated, but the mortality results were available.

About 3,850 pigs were initially part of the study, of which approximately 50% received placebo and 50% the test vaccine. No local or systemic reactions of any kind were noted immediately following injection, or in the days and weeks that followed. The mortality rate in the nursery for the study pigs was only 0.4%, and the manager did not notice any signs of any significant disease while the pigs were there. Similarly, nothing abnormal on a health status basis was noted by the investigator during the weekly visits.

As was the case historically in this system, pigs began to show clinical signs of PCVAD about three to four weeks post placement in finishing (80 to 90 days of age), and this in all four finishing units of the study. Clinical signs included wasting, lack of response to conventional treatments, paleness, diarrhea and increased mortality. Only few pigs were observed with thumping but some (about 0.2%) showed skin lesions suggestive of porcine dermatitis and nephropathy syndrome (PDNS). Gross lesions included: enlarged lymph nodes, particularly the mesenteric; lungs that usually failed to collapse normally and that had significant interlobular edema in some cases; kidneys that were very enlarged or with multiple white spots; lesions of enteritis suggestive of ileitis or salmonellosis; and edema of the mesentery. A few pigs also had splenic infarcts, a lesion considered as almost pathognomonic of hog cholera, a reportable disease, and others had much enlarged spleens with thickened, hardened and dark red to black areas. The pigs with skin lesions suggestive of PDNS usually had enlarged kidneys, sometimes with

petechiae or white spots, and one had a spleen with lesions at one end similar to those described above (enlarged, thickened, hardened, dark red to black).

At the time of writing, the histological and IHC results were available for the first 81 necropsied pigs. About 90% of these pigs had lesions and antigenic loads that were compatible with a diagnosis of PCVAD, confirming that this was the main condition involved in the finishing problems of this system.

As mentioned above, the investigators and farm crew were blinded as to which pigs had received the vaccine and placebo. However, since PCVAD problems had been observed in virtually every batch of this system in the 12-18 months prior to the study, and since in each of the four barns classical PCVAD clinical signs and lesions were observed in many of the pigs that had received product A, and very few of the pigs that had received product B, it quickly became obvious that the latter were the vaccinated pigs. At the time of the preliminary analysis, due to the overt differences between groups A and B, the trial was un-blinded.

Table 1 shows the mortality results (dead + euthanized) for pigs injected at different ages and placed in the four different finishing units. For each of the finishing batches, the vaccinated pigs had a significant reduction in mortality compared with the unvaccinated pigs, viz: Barn one – 3.0% vs 9.6%; Barn two – 2.1% vs 8.1%; Barn three – 2.8% vs 10.6%; Barn four – 0.4% vs 7.6%, respectively.

Table 1: Mortality rate in the four finishing units where pigs under test were placed.

	Treatment	Number of pigs introduced	Age (days) at vaccination	Mortality ^a Percent	p value
Barn 1	Sterile water	647	45-59	9.6	< 0.001 ^b
	Vaccine	633	45-59	3.0	
Barn 2	Sterile water	260	38-45	8.1	= 0.002
	Vaccine	286	38-45	2.1	
Barn 3	Sterile water	745	22-36	10.6	< 0.001
	Vaccine	717	22-36	2.8	
Barn 4	Sterile water	275	19-22	7.6	< 0.001
	Vaccine	274	19-22	0.4	
Wtd Avg	Sterile water	1,927	19-59	9.5	< 0.001 ^c
	Vaccine	1,910	19-59	2.4	

^a Mortality includes the animals that died and those euthanized for necropsy because of their poor condition.

^b Within farm (individual barn-level) statistical analysis utilized the two-sample proportions test, where H₀: Vaccinates = Controls.

^c Overall statistical analysis utilized the one-way analysis of variance test, where H_0 : Vaccinates = Controls.

Barn four allowed making a second comparison since this barn was also filled with pigs that had been vaccinated with a two dose product (at three and six weeks post weaning) in the nursery. On July 18, 275 control pigs, 274 pigs vaccinated with Ingelvac CircoFLEX and 250 pigs vaccinated with the two dose product were introduced in that barn. On July 20 and 27 respectively, 420 and 285 more pigs vaccinated twice with the two dose product were introduced in that barn. Table 2 shows the results that were obtained in these three groups of pigs.

Table 2: Mortality and days to market in control pigs, pigs vaccinated twice with a two dose product and those vaccinated once with Ingelvac CircoFLEX.

	Controls	Two dose product	Ingelvac Circo FLEX
# pigs	275	955	274
Death loss (%)	7.6	1.4	0.4
Duration (days)*	105	118	99
Weight (Kg)**	94.3	95.2	96.0

* From the first pigs introduced in the barn to the last ones sent to slaughter

** Carcass weight, which represents about 80% of the live weight

The numbers of controls, vaccinated once and vaccinated twice pigs differ because the number of Ingelvac CircoFLEX doses that was allowed by the Canadian authorities to perform the trial was limited, and this was the last barn of the trial. Since the two dose product was available to the producer and he wanted to have his pigs protected, he decided to vaccinate the rest of the pigs to be introduced in that barn with this two dose product.

Conclusions

An excellent efficacy was obtained whether pigs were vaccinated as young as 19-22 days, or as old as 45-59 days of age. These results suggest that under the conditions of this study, pigs could effectively be vaccinated at an early age. The data also suggest that immunity was relatively quick to develop, since pigs vaccinated only about four weeks before clinical signs were initially observed seemed to be protected. However, more data are needed, under various field conditions, before definitive conclusions can be reached on the optimal timing of vaccination. In the only barn where a comparison could be made between the two types of vaccines, Ingelvac CircoFLEX performed favorably compared to the two dose product.

Effect of Subclinical PCV2 Infection on Growth in Young Pigs

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Porcine circovirus type 2 (PCV2), porcine reproductive and respiratory syndrome virus (PRRSV), and swine influenza virus are considered to be the most important viral pathogens in the U.S. pig population. Case trends from the Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL) indicate a marked increase in PCV2-associated disease or PCVAD in 2006. The number of PCVAD cases in 2006 has surpassed the previous peak reached in 2005. What is not monitored through cases submitted to diagnostic laboratories is the effect of subclinical infection with PCV2 on growing pigs.

Although case trends of clinical PCVAD are markedly up in North America, it remains true that a high percentage of apparently healthy pigs are known to be infected with PCV2. A diagnosis of “subclinical PCV2-infection” implies that although PCV2 is present in the pig, the pig is not exhibiting clinical disease nor is there histological evidence (i.e. low amounts of PCV2-antigen associated with no-to-minimal lesions) of PCV2-induced lesions.

As we expected, the introduction of commercial PCV2 vaccines to North American market in early 2006 has resulted in reports of substantial decreases in morbidity and mortality on many of the farms that utilize PCV2 vaccine. In addition to decreases in morbidity and mortality, many producers also report improvement in average daily gain and feed efficiency after use of the PCV2 vaccine. This further supports another of our long held hypothesis that subclinical PCV2 infection may be economically very important. We have documented this in several of our experimental inoculation studies. For example, in a recent study at Iowa State University, one group of 10 pigs was inoculated with PCV2 on Day 0 and another group of 10 pigs was kept as an uninoculated negative control group. The pigs in both groups were weighed every week.

Clinical disease was not observed in any of the pigs, although the PCV2-inoculated pigs had low levels of viremia as determined by PCV2-PCR on serum. There were no differences in average daily gain until 49 days post inoculation when there was a significant ($P = 0.0012$) increase in daily gain in the uninoculated control pigs compared to the PCV2-inoculated pigs. Although these results are interesting, they were generated through several small experimental studies in research facilities. We encourage practitioners to conduct blinded, randomized clinical trials to provide evidence to further support or refute these findings.

It has been demonstrated experimentally that subclinical PCV2-infection is associated with decreased vaccine efficacy (Opriessnig et al., 2006a). In a recent study done at Iowa State University, 2-week-old pigs were randomly assigned to one of seven groups of 9-10 pigs each. At 6 weeks of age, a portion of the pigs were inoculated intranasally with

PCV2 ISU-40895. At 8 weeks of age, some pigs were vaccinated with a MLV PRRSV vaccine. At 12 weeks of age, pigs in three groups were challenged with PRRSV-SDSU73. All pigs were necropsied 14 days post PRRSV challenge. PCV2-infected, PRRSV-vaccinated and PRRSV-challenged pigs had significantly ($P < 0.05$) more severe macroscopic lung lesions compared to the PRRSV-vaccinated and PRRSV-challenged pigs that were not exposed to PCV2 prior to PRRSV vaccination. Nonvaccinated-PRRSV-infected pigs had significantly ($P < 0.001$) higher incidence of PRRSV-antigen in lungs compared to all other groups except the group infected with PCV2 prior to PRRSV vaccination and challenge. The nonvaccinated-PRRSV-challenged group and the group challenged with PCV2 prior to PRRSV vaccination and challenge had significantly ($P < 0.001$) lower average daily weight gain compared to controls and the vaccinated groups. The adverse effect of PCV2 infection on the development of protective immunity against PRRSV and other respiratory pathogens may be an important, and perhaps an underappreciated factor in controlling porcine respiratory disease complex (PRDC) and other diseases in pigs.

Practitioners and diagnosticians also need to understand that PCV2-associated lesions may be present in pigs but difficult to find. It is important to develop a system when necropsying pigs where multiple lymph nodes are submitted for examination. We commonly find in our experimentally-infected pigs that PCV2-associated lesions can be limited to one or two lymph nodes in a pig without causing any apparent clinical problems. Sometimes it may be a mesenteric lymph node, other times a tracheobronchial, an iliac, an inguinal or some other lymph node. For this reason we always collect at least five lymph nodes at necropsy.

PCV2-associated necrotizing lymphadenitis in individual lymph nodes in clinically healthy pigs at slaughter weight has also been described (Kim et al., 2005, Opriessnig et al., 2006b). The main lesion is follicular necrosis in the center of prominent lymphoid follicles and is most often restricted to one or two lymph nodes. The significance of this is unknown other than in cases where pigs with large lymph nodes are “condemned” at the slaughter house and submission of samples to diagnostic laboratories confirms necrotizing lymphadenitis.

PCVAD in growing pigs manifest as severe systemic disease (i.e. PMWS), pneumonia, and enteritis have been well documented and are now readily recognized by practitioners and diagnosticians. The remarkable success of the commercial PCV2 vaccines has made practitioners and researchers realize that use of the vaccine for control of subclinical PCV2 infection may also be a good return on investment. Although we do not fully understand the pathogenesis of PCVAD, we look forward to critical analysis of well designed field trials to further document the effects of PCV2 vaccine in controlling clinical and subclinical PCV2 infection in the pig population.

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