RESEARCH REPORT
SWINE HEALTH

Title: Comparison of porcine circovirus type 2 (PCV2) vaccine efficacy in a PCV2 positive production environment with concurrent porcine reproductive and respiratory syndrome virus (PRRSV) circulation – **NPB #09-164**

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Industry summary:
Objectives. The first objective was evaluation of the effectiveness of three porcine circovirus type 2 (PCV2) vaccines available commercially. Criteria used for satisfying this objective included measurement of pig growth from the nursery to finisher phase of growth, determination of antibody responses to vaccination and determination of the prevalence of circovirus in the serum of pigs in the study. The second objective was to evaluate whether vaccination against PCV2 had an impact on porcine reproductive and respiratory syndrome (PRRS) virus circulation and development of clinical disease. Criteria used for satisfying this objective included determination of antibody responses indicating virus exposure and determination of the prevalence of PRRS virus in the serum of pigs in the study.
Experimental plan. The study was completed in a wean-to-finish facility that was supplied by a sow farm that satisfied the case definition for porcine circovirus associated disease (PCVAD) and where PRRS virus had been previously recovered from a pig co-infected with PCV2. Approximately 1023 weaned pigs were assigned to four experimental treatments, which consisted of the three commercial vaccines and a non-vaccinated Control (Ingelvac Circoflex®, Circumvent™PCV and Suvaxyn® PCV2). Pigs were weighed four times (21, 63, 103 and 144 days of age) during the study to calculate average daily gain and blood was collected from ten pigs in each pen a total of four times (21, 42, 63 and 144 days of age) during the study for analysis of antibody production and to determine the presence of live virus by polymerase chain reaction (PCR).
Results. With the exception of bodyweights obtained at 63 days of age, pig growth data was unremarkable. At 63 days of age, pigs assigned the Circumvent™PCV treatment had lower bodyweights than pigs assigned the Control and Ingelvac Circoflex® treatments. Bodyweights obtained at 21, 103 and 144 days of age were not different. Analysis of the blood for PCV2 indicated that vaccinated pigs had significant increases in the amount of PCV2 antibody in the serum at 42 days of age compared to the non-vaccinated Control pigs. Antibodies were decreased at 63 and 144 days of age for pigs vaccinated with the Ingelvac Circoflex® and Suvaxyn® PCV2 vaccines. Pigs vaccinated with the Circumvent™PCV vaccine, the only two-dose preparation, maintained consistently high antibody levels from 42 days of age to 63 days of age, after which the amount of antibody was reduced at 144 days of age. Viable PCV2 virus was detected among pigs assigned to all experimental treatments. The Circumvent™PCV vaccinated pigs had the greatest percentage of PCV2 virus positive pigs at 21 days of age. By 42 days of age the non-vaccinated Control group had the greatest percentage of PCV2 virus

positive pigs and the percentage of PCV2 virus positive pigs at 63 and 144 days of age was similar for all treatments. Analysis of the blood for PRRS virus yielded highly variable results. The presence of serum antibodies indicated that 35 pigs at 21 days of age, 21 pigs at 42 days of age, 16 pigs at 63 days of age and 234 pigs at 144 days of age had been exposed to PRRS virus. Although antibodies were detected at all sampling time points, attempts to detect viable PRRS virus in the serum of the pigs was unsuccessful.
Discussion/Conclusion. Based on the results of the study, the three commercial vaccines did not have a stimulatory effect on average daily gain because the growth of the vaccinated pigs was similar to the growth of the non-vaccinated Control pigs. The vaccines did stimulate satisfactory antibody production against PCV2 in the vaccinated pigs and although virus was detected in the serum of vaccinated pigs, clinical disease indicative of PCVAD did not develop. The level of PCV2 virus in the environment was perceived to be insufficient to provide a challenge to the non-vaccinated pigs because none showed clinical signs indicative of PCVAD. The level of PRRS virus in the environment was sufficient to stimulate an antibody response that could be measured but live virus could not be detected and clinical signs indicative of active PRRS infection were not observed.

Keywords: porcine circovirus type 2 (PCV2), porcine reproductive and respiratory syndrome (PRRS) virus, viremia, polymerase chain reaction (PCR), serology

Scientific Abstract:

Objectives. The first objective of the study was to evaluate the efficacy of approved porcine circovirus type 2 (PCV2) vaccines based on growth performance, antibody response to vaccination and prevalence of circovirus viremia. The second objective was to investigate the impact of vaccination against PCV2 on porcine reproductive and respiratory syndrome (PRRS) virus circulation in a commercial finishing facility that has satisfied the case definition for porcine circovirus associated disease (PCVAD) and has a documented history of PRRS virus infection.
Introduction. In 1996, swine veterinarians in Western Canada reported a disease syndrome where nursery pigs exhibited generalized lymphadenopathy, poor body condition and/or wasting, interstitial pneumonia, diarrhea, and jaundice. The agent responsible was eventually identified as porcine circovirus type 2 (PCV2). After documented spread around the world, PCV2 resurfaced in Canada affecting late nursery and grower/finisher pigs and clinical signs included generalized lymphadenopathy, poor body condition and/or wasting, interstitial pneumonia, diarrhea, jaundice and skin lesions. To recognize the many clinical expressions that can result from infection with PCV2, the name porcine circovirus associated disease or PCVAD was adopted and diagnostic criteria for case definition established in 2006. Vaccines were developed by Boehringer Ingelheim Vetmedica, Fort Dodge Animal Health and Intervet/Schering Plough Animal Health and controlled studies utilizing infectious disease challenge models have demonstrated that the vaccines effectively stimulate satisfactory antibody responses, reduce viremia of infected pigs and reduce gross and histologic lesions. Evaluation of these vaccines in the commercial production environment is warranted to determine their ability to promote health and satisfactory performance of vaccinated pigs. The purpose of the research project was to evaluate the three commercially available vaccines utilizing average daily gain, serum antibody titers and viremia to PCV2 and PRRS virus.
Materials/Methods. The study was completed in a 26-pen, slotted-floor, wean-to-finish facility with 1023 pigs enrolled in the study. The pigs were split-sex fed with food and water provided *ad libitum*. Twenty four pens were used for the trial with a stocking density of 41 pigs per pen at the beginning of the study. Pigs were assigned to four treatment groups (3 vaccinated: 1 non-vaccinated) designated by ear tag color and based on a randomized complete block experimental design with 6 replicates. Pigs were placed at weaning (3 weeks old) with bodyweight data being collected from all animals at 21, 63, 103 and 144 days of age. Blood samples were collected from 10 pigs per pen at 21, 42, 63 and 144 days of age to obtain serum for serologic testing and polymerase chain reaction (PCR) to assess viremia for PCV2 and PRRSV. Data was analyzed using STATA Data Analysis and Statistical Software. Growth data was subjected to ANOVA and the Bonferroni test was used as the mean separation procedure. The PCV2 serology data was subjected to log transformation, analyzed by ANOVA and the Bonferroni test was used as the mean separation procedure. Chi square analysis was used

to compare the percentage of PCV2 positive pigs in each treatment on successive sampling days. For all tests, $p<0.05$ was considered to be statistically significant.
Results. Bodyweight data over the course of the study revealed only one significant difference ($P<0.05$) in bodyweight. Pigs assigned the Circumvent™PCV treatment had lower bodyweights than the Control and Ingelvac Circoflex® treatments at 63 days of age. Indirect fluorescent antibody testing demonstrated that the vaccinated pigs experienced a significant increase ($P<0.05$) in titer at 42 days of age. Antibody titers decreased over the next two test dates for the pigs assigned the one-dose vaccines. The Circumvent™PCV treatment group, which received two doses of vaccine maintained constant titer levels from 42 days of age to 63 days of age, after which titers were significantly reduced at 144 days of age. The Circumvent™PCV treatment group had the highest ($P<0.05$) percentage of PCV2 positive pigs at 21 days of age. At 42 days of age the Control group had the highest ($P<0.05$) percentage of positive pigs compared to the Suvaxyn® PCV2 treatment group. Differences ($P>0.05$) in viremia were not detected when the pigs were tested at 63 and 144 days of age. Seroconversion to PRRS was detected at all sampling points and by day 144 of the study, all pigs exhibited antibodies to PRRS by IDEXX ELISA. Although we were able to confirm exposure to PRRS virus via serology, the amount of viremia from PRRS virus was low at all sampling points to the extent that virus amplification was deemed to be unsuccessful and the PCR assays were of minimal value to the overall scope of project.
Discussion. Based on the results obtained during the study, vaccination against PCV2 did not have a stimulatory effect on pigs achieving heavier bodyweights than non-vaccinated pigs. The growth of the non-vaccinated Control pigs remained similar to the vaccinated pigs throughout the study. Vaccination of pigs with the two-dose PCV2 preparation promoted persistently higher titers compared to the one-dose vaccines. Vaccination did not completely eliminate PCV2 viremia but a steady reduction in the number of viremic pigs was observed and may have coincided with the increase in immune status.

Introduction:
Belying its small size, porcine circovirus type 2 (PCV2) has emerged as an important pathogen of swine worldwide. Porcine circovirus was first identified by German researchers in 1974 as a contaminant of porcine kidney cell tissue culture (cell line PK-15).[1] The contaminant was later identified as porcine circovirus type 1 (PCV1) and is not considered to be pathogenic to swine.[2] In 1996, swine veterinarians in Western Canada reported a disease syndrome where nursery pigs exhibited generalized lymphadenopathy, poor body condition and/or wasting, interstitial pneumonia, diarrhea, and jaundice.[3] Investigation of the Canadian disease outbreak uncovered a circovirus isolate that was antigenically and genetically different from PCV1. The isolate from the disease outbreak was later named PCV2 and was recognized as the agent responsible for post-weaning multisystemic wasting syndrome or PMWS, the complex of clinical signs associated with PCV2 infection.[2] After dissemination around the world, PCV2 resurfaced in Canada affecting late nursery and grower/finisher pigs and clinical signs included generalized lymphadenopathy, poor body condition and/or wasting, interstitial pneumonia, diarrhea, jaundice and skin lesions.[4] Numerous clinical presentations are associated with PCV2 infection and the name porcine circovirus associated disease or PCVAD was adopted to incorporate the clinical features of the condition and establish the criteria that would be used for diagnosis.[5] In an effort to establish control over this emerging pathogen, Boehringer Ingelheim Vetmedica, Fort Dodge Animal Health and Intervet/Schering Plough Animal Health developed vaccines for administration to post-weaned pigs. Although the vaccines have received full licensure by the United States Department of Agriculture (USDA) information related to their capacity to stabilize pig health and promote satisfactory pig performance in commercial swine operations is still needed. Controlled studies utilizing infectious disease challenge models have demonstrated that the available vaccines are effective at stimulating satisfactory antibody responses, reducing viremia of infected pigs and reducing gross and histologic lesions.[5,6] However, a marginal number of studies have been published where the efficacy of licensed vaccines have been evaluated in a commercial production setting.[7,8] Although few in number, these studies suggest that vaccination against PCV2 is beneficial to overall pig health, pig performance (weight gain) and survivability in commercial swine production environments. The proposed research project will provide critical information related to vaccine efficacy and applications in commercial

swine herds that are challenged with PCV2 by evaluating the three licensed vaccines concurrently. Moreover, the proposed research project will provide critical information relative to the impact of PCV2 vaccination on the dynamics of PRRSV circulation in swine herds challenged with PCV2 and PRRSV concurrently.

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Objectives:

The first objective of the completed research was to evaluate the efficacy of the three licensed PCV2 vaccines utilizing growth performance, antibody response to vaccination and prevalence of circovirus viremia as response criteria in a commercial swine herd satisfying the case definition for PCVAD. In addition to protection against PCV2 infection, improvements in pig performance (average daily gain) have been described as a beneficial effect of circovirus vaccination. The purpose of this objective was to investigate the promotion of improved performance as well as determine the ability of the vaccines to promote immunity to PCV2 and minimize virus circulation in the production environment. The second objective of the completed research was to evaluate the impact of vaccination against PCV2 on PRRSV circulation in a commercial swine herd satisfying the case definition for PCVAD and a documented history of PRRSV infection. During the process of confirming PCVAD at the site where the study was completed, PRRS virus was recovered from tissues submitted to the diagnostic laboratory. Up to that point, the site was considered to be free of PRRS and the virus had never been recovered on previous blood and tissue submissions. The purpose of this objective was to determine if the risk of disease among the study population was heightened by the presence of PRRS virus in conjunction with PCV2.

Materials and Methods:

The study was completed in a 26-pen, slotted-floor, wean-to-finish barn that was equipped with zone heating and tunnel ventilation. A randomized complete block design was used for the experimental design with a total of 6 replicate blocks. The weaned pigs were assigned to four (Non-vaccinated Control, Ingelvac CircoFLEX, Circumvent^{TM}PCV, Suvaxyn$^{®}$PCV2 One Dose) treatment groups and housed in 24 pens. The pigs were approximately 3-weeks of age at the beginning of the study, a total of 1023 pigs were initially enrolled in the study, the average stocking density was 42 pigs per pen and the room was filled with pigs over a two-week period. The pigs were split-sex fed with food and water provided *ad libitum*. Pigs were placed at weaning with bodyweight data collected from all animals at 21, 63, 103 and 144 days of age. Blood samples were collected from 10 pigs per pen at 21, 42, 63 and 144 days of age to obtain serum for serologic testing and polymerase chain reaction (PCR) to assess viremia for PCV2 and PRRSV. Indirect fluorescent antibody (IFA) testing was used to determine the amount of PCV2-specific antibody in the serum and enzyme–linked immunosorbent assay

(ELISA) was used to determine the presence of PRRS-specific antibodies in the serum. Quantitative real-time PCR was used to determine the number of viral copies of DNA in the serum. Statistical analysis was performed using STATA Data Analysis and Statistical Software. Means for bodyweight were calculated and subjected to analysis of variance (ANOVA) and the Bonferroni test was used as the mean separation procedure. Antibody titers to PCV2 were subjected to geometric mean calculation and the resulting means were subjected to logarithmic transformation to harmonize the data. The data was then subjected to ANOVA and the Bonferroni test was used as the mean separation procedure. Chi square analysis was used to compare the percentage of PCV2 positive pigs in each treatment on successive sampling days. For all tests, $p<0.05$ was considered to be statistically significant.

Results:

Objective 1 – Analysis of bodyweight data did not reveal major differences throughout the study. Bodyweight data collected on days 0, 103 and 144 days of age were similar ($p>0.05$) for all treatments. Differences in bodyweight data collected on day 63 were apparent as the pigs assigned the Circumvent™PCV treatment exhibited lower ($p<0.05$) bodyweights than the pigs assigned the Ingelvac CircoFLEX and non-vaccinated Control treatments. Bodyweight of the pigs assigned the Circumvent™PCV and Suvaxyn®PCV2 One Dose treatments were similar ($p>0.05$). Differences in serum antibody concentration were apparent at all time points measured. At 21 days of age, PCV2 antibody concentration was similar ($p>0.05$) for pigs assigned the non-vaccinated Control, Ingelvac CircoFLEX and Suvaxyn®PCV2 One Dose treatments. At 42 days of age, PCV2 antibody concentration was similar ($p>0.05$) for pigs assigned the Ingelvac CircoFLEX, Circumvent™PCV and Suvaxyn®PCV2 One Dose treatments. At 63 days of age, pigs assigned the Circumvent™PCV treatment had the highest ($p<0.05$) concentration of serum antibody. At 144 days of age, pigs assigned the Circumvent™PCV treatment had the highest ($p<0.05$) concentration of serum antibody followed by pigs assigned the Suvaxyn®PCV2 One Dose treatment. Pigs assigned the non-vaccinated Control and Ingelvac CircoFLEX treatments had similar ($p>0.05$) serum antibody concentrations. Differences in PCV2 viral DNA determined by PCR analysis were apparent at two time points. At 21 days of age, the percentage of PCV2 positive pigs was greatest ($p<0.05$) for pigs assigned the Circumvent™PCV treatment. At 42 days of age, the percentage of PCV2 positive pigs was similar ($p>0.05$) for pigs assigned the Ingelvac CircoFLEX, Circumvent™PCV and non-vaccinated Control treatments. At 63 and 144 days of age, the percentage of PCV2 positive pigs was similar ($p>0.05$).

Objective 2 – Antibody responses to PRRS was detected at all sampling points and by day 144 of the study, all pigs exhibited antibodies to PRRS by IDEXX ELISA. At 21 days of age, 35 pigs were positive on serology. At 42 days of age, 21 pigs were positive on serology. At 63 days of age, 16 pigs were positive on serology and at 144 days of age, all pigs tested had serum antibodies against PRRS virus. The serum concentration of PRRS virus DNA was negligent at all sampling time points. Virus amplification was consistently unsuccessful at all time points in spite of the detection of PRRS antibodies in the serum.

Discussion

Although we anticipated that the vaccinated pigs would outperform the non-vaccinated Control pigs, the PCV2 vaccines did not promote a stimulatory effect on average daily gain or bodyweight because the growth of the vaccinated pigs was similar to the growth of the non-vaccinated pigs. Moreover, contrary to our expectations, none of the non-vaccinated pigs in the study developed signs of disease consistent with PCVAD. These observations were viewed as clear indicators that the infectious challenge posed by PCV2 in the environment was low and was best described as negligible. This is probably the best explanation for the less than superb growth response to vaccination because the presence of circulating live virus in the environment was confirmed by PCR. Detection of live virus by PCR was not surprising considering that PCV2 is described as demonstrating resistance to some disinfectants and is viewed as an inhabitant of the swine production environment. However, it does not appear that an infectious dose of virus was achieved that would result in development of PCVAD. Other explanations for the lack of response to vaccination include the fact that pigs from all the experimental treatments had PCV2 antibodies in their serum at the beginning of the study, thereby

possibly conferring some protection to the pigs at the outset. The percentage of viremic pigs consistently decreased over the course of the study and we assume the pigs would have been less susceptible to PCV2 later in the study. In addition, antibody levels in the non-vaccinated pigs mirrored antibody levels in the vaccinated pigs and the antibodies persisted, possibly conferring some protection to these pigs later in the study even though they were not vaccinated. The vaccines stimulated measurable antibody responses against PCV2 and appeared to protect against the development of PCVAD because these pigs never showed signs of disease and were viremic based on PCR testing. However, the conclusion that protection from PCVAD resulted from vaccination is tenuous because the non-vaccinated pigs never developed signs of disease consistent with PCVAD either. Therefore, as previously stated, the level of infectious challenge was probably insufficient to cause disease in the non-vaccinated pigs. Based on the results of the study, PRRS virus did not appear to have any impact on the health of the pigs during the completion of the study. Moreover, it is easily concluded that the virus that circulated among the study group was of exceptionally low virulence. First, there was evidence that pigs were exposed to virus during the lactation period because 35 out of 240 pigs had serum antibodies at the start of the study. Given the fact that virus circulation was apparent in such young pigs, we anticipated that the numbers of sick pigs would be very high due to PCV2 and PRRS virus, especially among the non-vaccinates. However, this was not observed and the pigs remained in satisfactory health throughout the study. The number of pigs that were seropositive for PRRS decreased over the next two sampling periods after the start of the study. The reason for this observation is not clearly understood because we expected the number of seropositive pigs to increase after the initial sampling. However, on the final sample day at 144 days-of-age all pigs bled were serologically positive for PRRS. The PCR analyses were difficult to interpret also because the sera from the respective sampling days were subjected to numerous attempts at virus amplification without success. The final conclusion was that the virus lacked virulence characteristics and may have been derived from an attenuated vaccine strain. Attempts to recover virus from sera for sequencing analysis were unsuccessful so we were unable to determine the genetic composition of the virus.