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Abstract

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Utilizing Vaccination for Porcine Circovirus Type 2 as a Tool to Aid Elimination of PCV2 from Swine Populations[1,2]

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Summary

A total of 928 pigs from the Swine Teaching and Research Centers at Michigan State University (MSU) and Kansas State University (KSU) and a Kansas commercial farm were used during a 3-year study to determine whether circovirus vaccination influenced porcine circovirus type 2 (PCV2) circulation within a herd and could be used as a tool to eliminate PCV2 from PCV2-positive swine herds. Infection with PCV2 was confirmed in both university herds before circovirus vaccine introduction. After vaccination implementation, vaccinated barrows from consecutive groups were serially tested for viremia. Follow-up antibody and growth testing with vaccinated and non-vaccinated pigs was performed at the KSU farm. In a circovirus-vaccinated commercial herd, testing of non-circovirus-vaccinated pigs for viremia was completed. Environmental swab samples were collected from facilities at the KSU and commercial farms for PCV2 DNA detection.

Sera from 0 of 9 MSU vaccinated-cohorts and 3 of 10 KSU vaccinated-cohorts had detectable PCV2 DNA. From follow-up testing, a PCV2 antibody rise after vaccination was detected for vaccinated pigs with no detectable antibody rise for non-vaccinated pigs. Overall growth rate of non-vaccinated pigs tended ($P = 0.07$) to increase compared with vaccinated pigs. Non-vaccinated pigs became PCV2 viremic at the commercial farm. Viral DNA was detected in the environment of the commercial farm but not in the KSU facilities.

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Therefore, circovirus vaccine can affect viral circulation on farms but would need to be used in conjunction with other management practices to eliminate PCV2 from most swine populations.

Key words: circovirus, disease elimination, PCV2, swine, vaccine

Introduction

Infection with porcine circovirus type 2 (PCV2) can result in a multi-syndrome disease, porcine circovirus disease (PCVD).[7] Identified in diagnostic laboratory samples in the early 1990s, PCV2 has affected most U.S. swine herds. Despite a long history of PCV2 circulation within the swine population, vaccines against PCV2 have been commercially available only since 2006.[8] Initial studies evaluating the effects of circovirus vaccination on production parameters in PCV2-affected herds indicate that vaccination was effective at reducing finishing phase mortality and increasing pig growth rate.[9,10,11] In single-cohort studies, vaccination with commercial or experimental vaccines against PCV2 reduced viremia[10,11] and decreased viral shedding in nasal secretions and feces,[12,13] but data evaluating the effects of vaccination on PCV2 viral circulation within a herd over time are limited. Our goal was to monitor PCV2 viral circulation in swine herds after implementing a circovirus vaccination program for growing pigs. The short-term objective of this project was to determine whether circovirus vaccination could be used to affect viral circulation within 2 farrow-to-finish herds. The long-term objective of the project was to understand whether use of circovirus vaccines over time in PCV2-positive swine herds could provide a tool to eliminate PCV2 from these herds.

Procedures

Procedures used in these studies were approved by the Kansas State University and Michigan State University Institutional Animal Care and Use Committees.

Herd History. The MSU and KSU Swine Teaching and Research Centers were single-location farrow-to-finish operations. Pigs were moved through the KSU farm in an

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all-in, all-out manner in nursery, grower, or finisher rooms. In the MSU farm, about half of the pigs placed in a nursery, grower, or finisher room were moved in and out at a time. Pigs were born (farrowed) at each farm approximately every 4 (MSU) or 5 (KSU) wk, which resulted in growing pig populations of about 300 pigs in each age group. Both herds were negative for porcine reproductive and respiratory syndrome virus, and the MSU herd was negative for *Mycoplasma hyopneumoniae (M. hyo)*. Pigs at the KSU farm were vaccinated at weaning for *M. hyo* (RespiSure-ONE; Pfizer Animal Health, New York, NY), which, along with other management procedures, contributed to low levels of clinical disease. Prior to the start of our study, both farms had been closed to live animal introductions, but semen was introduced from outside sources. In October 2007, the KSU farm began to bring replacement gilts from an outside source into the herd approximately every 9 wk.

Clinical history. The KSU farm did not have any clinical signs of PCVD noted before the baseline testing and subsequent implementation of a circovirus vaccination program, although prior to baseline testing, histopathologic evaluation on tissues of one pig documented lymphoid depletion lesions consistent with PCVD. The MSU farm had evidence of moderate clinical PCVD (10 to 15% nursery mortality) prior to baseline testing.

Phase 1: Baseline testing procedures. In early 2007, a cross-sectional survey was conducted of both university herds to verify the presence of PCV2 and to characterize patterns of PCV2 infection and seroconversion. At the MSU farm, blood was collected from 101 pigs across a total of 5 growing pig populations (6 to 10, 11 to 15, 16 to 20, 21 to 25, and 26 to 30 wk of age). Within the KSU farm, 141 pigs were sampled across 5 growing pig populations (4, 9, 14, 19, and 24 wk of age). Serum was pooled (MSU: 21 pools, and KSU: 27 pools) within age group and analyzed using the Kansas State Veterinary Diagnostic Laboratory (KSVDL) PCV2 PCR assay for detection of PCV2 nucleic acid. Viral template quantities for each serum pool were $\log_{10}$ transformed and transformed results were averaged for pools within each age range to characterize the changes in viral load. For the detection of PCV2 antibodies, individual serum samples were tested using the 96-well format KSVDL PCV2 indirect fluorescent antibody (IFA) assay with serial 1:2 dilutions beginning with a 1:20 serum to phosphate-buffered saline dilution and ending with a 1:2,560 ratio. The titration endpoint was calculated as the reciprocal of the last serum dilution that gave a positive result.

All IFA titers were $\log_2$ transformed to approximate a normal distribution prior to descriptive analysis. For samples that did not have antibody detected at the most concentrated dilution (1:20), the $\log_2$ of 10 was used in the analysis. For samples that were strongly positive at the least concentrated dilution (1:2,560), the $\log_2$ of 5,120 was used. This approach allowed results to be weighted differently than samples with antibody detected with a normal level of fluorescence at the 1:20 and 1:2,560 dilutions.

Infection and antibody profiles obtained from the baseline testing were considered when deciding on sampling times for the Phase 2 study on each farm.

Phase 2: Trial procedures. In the spring of 2007, both MSU and KSU initiated circovirus vaccination programs. A 2-dose circovirus vaccine (Circumvent PCV; Intervet/Schering-Plough, Millsboro, DE) was administered as an intramuscular injection

(2 mL per dose) to all growing pigs in each weaning group with 3 to 5 wk between vaccine doses. Pigs were weaned and vaccinated with the first dose of circovirus vaccine at approximately 3 wk of age at the KSU farm, but weaning age and timing of first vaccination at the MSU farm varied (range: 2 to 6 wk).

From 2007 through 2008, barrows from consecutive weaning cohorts at the MSU (9 groups) and KSU (10 groups) farms were monitored for PCV2 viremia. A minimum of 12 barrows per group from different litters were randomly selected, ear-tagged, and serially bled at 4 time points: weaning or just before vaccination, entry-to-finishing, mid-finishing, and end-of-finishing. After completion of data collection in 2008, individual serum samples for pigs with complete serum sets (4 serum samples per pig) were tested by the KSVDL PCV2 PCR assay for detection of PCV2 nucleic acid. An average of 40 cycles was run with a cycle time threshold of 0.05 for classification of PCV2 nucleic acid-containing (positive) samples.

Phase 3: Follow-up monitoring procedures. Beginning in the spring of 2009, a total of 372 pigs (186 non-vaccinated control pigs and 186 circovirus-vaccinated pigs) across 3 weaning groups were used in a Phase 3 growth and PCV2 antibody follow-up study at the KSU farm. At the start of the Phase 3 study, the KSU farm had been vaccinating pigs against PCV2 for the previous 2 years. During that time there had been no evidence of clinical disease. A first objective of this follow-up study was to document the effects of circovirus vaccination on PCV2 antibody titers and to determine whether there was evidence of PCV2 exposure. A second objective of this Phase 3 study was to evaluate the effects of circovirus vaccination on growth rate of pigs in the KSU herd.

Three groups of pigs were used in the Phase 3 study. Groups 1 and 2 had 7 pigs per nursery pen. A total of 18 barrow pairs (36 pigs; 1 pair in each of 18 pens) for group 1 and 30 barrow pairs (60 pigs; 1 pair in each of 30 pens) for group 2 were utilized. Within a pen, a pair of barrows was selected with one barrow per pair randomly allotted to a vaccinated treatment and the pen-mate barrow assigned to the non-vaccinated control treatment. Barrows assigned to the vaccinated treatment were injected intramuscularly with a 2-dose circovirus vaccine (Circumvent PCV) at approximately 3 and 6 wk of age. All other pigs in the weaning group not enrolled in the follow-up study were vaccinated with the same 2-dose circovirus vaccine.

Throughout the entire study, pairs of barrows remained penned together. Barrows were individually weighed and bled at 4 time points: d 0 (pre-vaccination), entry-to-finisher, mid-finishing, and end-of-finishing. From these data, ADG was calculated for 3 periods: nursery and grower, finisher, and overall nursery to finisher. Removals and mortalities were recorded and weighed and their gain and time on test were included in performance calculations.

For group 3, 138 barrow or gilt pairs (276 pigs) were randomly allotted to treatments (vaccinated or non-vaccinated control) at the time of weaning with procedures similar to those used for groups 1 and 2. For group 3, 6 or 8 pigs were assigned to each nursery pen (3 or 4 pairs within a pen) and all pigs were placed on test. Pigs assigned to the vaccinated treatment were injected intramuscularly with a 2-dose circovirus vaccine (Circumvent PCV) at approximately 3 and 9 wk of age. Weighing and penning procedures for each pair were similar to those used for groups 1 and 2. A subset of 20 barrow

pairs (40 pigs) from 20 different pens distributed throughout the nursery were bled at the time of weighing. Pairs of barrows were selected and, within each pair, one barrow was randomly assigned to a vaccinated treatment and the pen-mate barrow assigned to the non-vaccinated control treatment. For group 3, removals and mortalities were recorded and weighed and their gain and time on test were included in performance calculations.

Individual serum samples for groups 1, 2, and 3 were tested for PCV2 antibodies using the KSVDL IFA assay. Test procedures used were similar to those used in Phase 1; however, an initial serum to phosphate-buffered saline dilution of 1:40 was used with subsequent serial 1:3 dilutions for group 1, 2, and 3 samples. Testing was performed over 7 d (2 d for group 1, 3 d for group 2, and 2 d for group 3), and pairs of pigs were balanced across IFA days within each study.

Group 1, 2, and 3 IFA titers were $\log_3$ transformed to approximate a normal distribution prior to statistical analysis. For samples that did not have antibody detected at the most concentrated dilution (1:40), the $\log_3$ of 13.3 was used in the analysis, whereas the $\log_3$ of 262,440 was used for analysis for samples that were strongly positive at the least concentrated dilution (1:87,480). This approach allowed these samples to be weighted differently than positive samples with normal level fluorescence at 1:40 and 1:87,480.

Group 1, 2, and 3 IFA data were analyzed by repeated measures analysis using the GLIMMIX procedure in SAS version 9.1.3 (SAS Institute, Inc., Cary, NC). Fixed effects in the model included treatment, time, and their interaction. Group and IFA day were used as random effects. Differences between treatments were determined using least squares means ($P < 0.05$). Log_3 transformed least squares means were transformed back to the original scale for presentation as geometric mean titers (GMT).

Growth data were analyzed using the GLIMMIX procedure in SAS version 9.1.3. The interaction with gender and treatment was determined to be non-significant for group 3, and growth data were pooled across the genders for subsequent analysis of the treatment effect. Thus, growth data for all 3 groups were analyzed using a single model. Treatment was a fixed effect and group was included as a random effect. Differences between treatments were determined using least squares means ($P < 0.05$).

Phase 4: Monitoring for PCV2 under commercial conditions. A commercial farm in Kansas that was determined to have had severe PCVD before circovirus vaccine became available was selected as a herd for an additional monitoring study (Phase 4) because of proximity and clinical history. Prior to the introduction of circovirus vaccine, post-weaning mortality had ranged from 5% to 19%. After implementation of a circovirus vaccination program (Circumvent PCV), the herd had less apparent clinical disease (mortality: 4 to 9%). The circovirus vaccination program had been in place for a year before our Phase 4 study began. In addition to the history of PCV2 infection, porcine reproductive and respiratory syndrome virus and *M. hyo* also contributed to the health challenges in the nursery and finishing phases of production. Pigs were weaned from a sow farm in western Kansas and moved to eastern Kansas to be placed at a nursery-finishing site with 2 nursery barns with 4 rooms each and 8 finishing barns. Pigs were moved all-in, all-out by nursery room and finishing barn.

A total of 85 pigs (1.7 to 3.1 wk of age) from a 1,100-pig weaning group were ear-tagged and bled just prior to weaning. These 85 pigs were not vaccinated against PCV2 and were monitored for 9 wk. All other pigs in the weaning group were vaccinated according to standard farm protocol with a 2-dose circovirus vaccine (Circumvent PCV). The 85 non-vaccinated sentinel pigs were initially penned in 4 pens in the nursery room that also contained pens of circovirus-vaccinated pigs. If pigs were removed from their initial pens because of illness or injury, they were moved to a sick pig pen but were still monitored. After approximately 8 wk in the nursery, pigs were moved to a single finisher barn at the same farm location and were placed in pens according to their vaccination status. Pigs were bled approximately every 3 wk for a total of 4 sampling times (sampling time age ranges: 1.7 to 3.1, 4.9 to 6.3, 7.9 to 9.3, and 10.9 to 12.3 wk of age). The objective of this monitoring effort was to determine whether non-vaccinated pigs housed in barns with pigs vaccinated against PCV2 became viremic with PCV2 after circovirus vaccine was used in the herd for a year.

Serum samples were pooled (5 samples per pool) within age range and were analyzed by the KSVDL PCV2 PCR assay for presence of PCV2 nucleic acid. Genotype of PCV2 (PCV2a or PCV2b) was determined for samples with detectable PCV2 nucleic acid.

Phase 5: Monitoring for PCV2 in the environment of swine barns. As pigs involved in all previous phases of this study were exposed to different environments and pigs over time, we wanted to determine whether documentable sources of PCV2 exposure existed. The objective for this phase of monitoring was to demonstrate applicability of swabbing and PCV2 PCR testing as a method for monitoring PCV2 levels on environmental surfaces in swine production facilities.

Swab samples were collected from the nursery and finisher rooms at both the KSU farm and the commercial farm in eastern Kansas that was used in the Phase 4 study. Cotton swabs were used to sample the floor slats, gating, waterers, feeders, fans and heaters in the nursery or finishing rooms. Swabs were placed in vials containing enriched media. For each farm, samples were pooled within nursery or finishing production phases (2 KSU nursery or finishing pools and 16 commercial farm nursery or finishing pools). A uniform amount of this pooled suspension was tested by KSVDL PCV2 PCR for detection of PCV2 nucleic acid.

Results

Phase 1. Baseline PCV2 IFA testing of the serum collected from pigs from the MSU herd demonstrated that passively acquired antibody declined by 15 wk of age (Figure 1). Higher levels of antibody were apparent in pigs 16 to 20 wk of age or older. PCV2 nucleic acid was detected by PCR in serum samples from pigs 11 to 15 wk of age and older (Figure 2).

In the baseline analysis of the KSU herd (Phase 1), passively acquired antibody in growing pigs declined by 19 wk of age with higher levels of antibody detected following this decline (Figure 3). Viremia was detectable only in populations consisting of pigs that were 19 and 24 wk of age (Figure 4). The 19-wk-old pigs were viremic but did not have antibody levels suggestive of seroconversion.

Phase 2. After introduction of circovirus vaccination, PCV2 PCR testing of serum samples collected over time from 9 MSU and 10 KSU cohort groups showed a different infection pattern on each farm compared with baseline PCR profiles. From the MSU farm, PCV2 PCR testing on sera collected from 86 barrows at 4 sampling points (pre-vaccination, entry-to-finishing, mid-finishing, and end-of-finishing) failed to detect PCV2 nucleic DNA (Table 1).

From the KSU farm, testing by PCV2 PCR on serum samples from 111 barrows failed to detect nucleic acid (PCV2 PCR negative) in samples collected at any time from pigs in groups 1, 2, 4, 7, 8, 9, and 10 (Table 2). Serum samples with detectable PCV2 DNA (PCV2 PCR positive) were found in group 3 (10%, 1/10 samples from mid-finishing), group 5 (25%, 3/12 samples from weaning; 25%, 3/12 samples from entry-to-finishing; 8.3%, 1/12 samples from mid-finishing; and 8.3%, 1/12 samples from end-of-finishing), and group 6 (8.3%, 1/12 samples from entry-to-finishing). For serum samples with detectable DNA, viral template quantity ranged from 5 to 379 viral template copies per reaction. In only 1 (group 5) of the 10 groups (10%) did a pig remain viremic for longer than 1 testing interval. Overall, no PCV2 viral DNA was detected in samples from 7 of the 10 groups (70%) monitored over a time period of greater than 1 year.

Phase 3. After 2 years of vaccinating growing pigs against PCV2 at the KSU farm, subsamples of pigs were allocated to a circovirus-vaccinated treatment or a non-vaccinated control treatment in a growth and PCV2 antibody follow-up study (Phase 3). An interaction ($P < 0.001$) between treatment and time occurred for antibody level (Table 3). With the exception of the initial bleed (d 0; during the wk of weaning) when control and vaccinated pig antibody levels were similar ($P = 0.41$), vaccinated pigs had increased ($P < 0.001$) PCV2 antibody levels compared with controls at all other sampling times. The magnitude of the antibody responses varied over time for control and vaccinated pigs, as did the pattern of antibody production or decay. By the time the pigs were placed into the finisher, control pig antibody levels had declined ($P < 0.001$) compared with their respective d 0 levels; however, control pig antibody levels remained similar ($P \geq 0.61$) throughout the finishing period. In contrast, compared with their respective d 0 antibody levels, vaccinated pigs had an increase ($P < 0.001$) in PCV2 antibody titer by the time of entering the finisher, which decreased ($P < 0.001$) by each of the subsequent sampling points.

During the nursery and grower periods, vaccinated pigs had decreased ($P = 0.005$; Table 4) ADG compared with non-vaccinated control pigs. Vaccinated and control pigs had similar ($P = 0.30$) finishing ADG, although growth rates for vaccinated pigs continued to be numerically less than control pig growth rates. Overall, a tendency ($P = 0.07$) was observed for vaccinated pigs to have decreased ADG compared with control pigs. These growth rate differences resulted in control pigs entering the finisher 2.6 lb heavier ($P = 0.03$) than vaccinated pigs. When pigs were taken off test at the end of the finishing period, control pigs had a numeric weight advantage ($P = 0.16$) of 4.4 lb over vaccinated pigs.

Phase 4. Results obtained from the commercial farm with a 1-year history of circovirus vaccination differed from those observed in the KSU farm. From a serial sampling of 85 non-vaccinated sentinel pigs, no PCV2 DNA was detected in the weaning pools (0/17 pools; Table 5). In contrast, PCV2 nucleic acid was detected in pooled samples at each

of 3 subsequent sampling ages (4.9 to 6.3 wk of age: 1/17 pools; 7.9 to 9.3 wk of age: 6/16 pools; and 10.9 to 12.3 wk of age: 12/16 pools). Genotype was reported for each pool. PCV2a was detected in all but 1 pool (4.9 to 6.3 wk of age: 1/17 pools; 7.9 to 9.3 wk of age: 6/16 pools; and 10.9 to 12.3 wk of age: 11/16 pools), but PCV2b was not detected in any of the pools until 10.9 to 12.3 wk of age (2/16 pools).

Phase 5. Environmental swabbing and testing by PCV2 PCR (Figure 5) detected PCV2 DNA in samples from 8 commercial nursery and 8 commercial finisher barns. In contrast, the presence of PCV2 DNA was not detected by PCV2 PCR testing of environmental swab samples from the KSU farm.

Discussion

This was a first study to evaluate the effects of circovirus vaccination on viral circulation at the herd level. Our study was designed to begin to evaluate the hypothesis that circovirus vaccination programs in herds would affect viremia and subsequent viral shedding into the environment. Over time, a reduction in environmental contamination coupled with continued use of circovirus vaccine to build immunity in growing pigs prior to viral exposure would aid derivation of PCV2-free herds.

The MSU and KSU herds and management served as models for commercial multisite swine production systems. Based on the Phase 1 baseline testing, PCV2 was detected in both swine populations, although viremia was not increased until after the nursery period. This testing provided evidence for primarily horizontal rather than vertical transmission. Both herds had PCV2-viremic pigs during finishing and showed evidence that pigs likely seroconverted after the documented time for onset of viremia (Figures 1, 2, 3, and 4).

Although both farms had evident viral circulation during finishing, the MSU pigs experienced an earlier onset of viremia than the KSU pigs. Both herds were considered good models in which to monitor the effects of circovirus vaccination long-term because baseline results from both non-vaccinated populations indicated viral presence and seroconversion-supporting antibody profiles.

Circovirus vaccination programs were started in each herd in the spring of 2007, and monitoring of barrows from each farrowing group began. In the MSU herd, viremia was not detected in serum collected at any sampling point from circovirus-vaccinated barrows (Table 1). During the same time, there were no reports of clinical PCVD from the farm, but some pigs may have become transiently viremic between sampling points; however, the MSU farm baseline testing indicated onset of viremia early in the finishing phase and infection appeared to be detectable in a portion of the population throughout finishing. Thus, the MSU vaccinated pig PCR data demonstrate that vaccination had an effect on the viral circulation within this farm by either shortening the duration of viremia or preventing it altogether.

In the KSU herd, 3 groups had at least 1 pig with detectable PCV2 DNA in the serum. These groups (3, 5, and 6; Table 2) were not consecutive groups, nor were the ages at the time of detectable viremia consistent among groups. In addition, only 1 group had pigs testing positive for PCV2 at more than 1 sampling point. Although the viral load

levels between sampling points were not known, the PCV2 viral loads detected in the positive serum samples among the 4 bleeding times were 379 template copies per reaction or less. Additionally, none of the viremic vaccinated pigs or their group-mates had been identified as PCVD suspects. Evidence of PCV2 problems was restricted to PCR detection of transient viremia. Although PCV2 was intermittently detected among vaccinated pigs, because no naïve pigs were in the population, the virus was not able to transmit readily, propagate within groups, and establish widespread infection within the herd; therefore, the KSU herd results indicate immunization by circovirus vaccination affected viral circulation by controlling the spread of virus and shortening the duration of viremia or by preventing the infection entirely.

The follow-up study (Phase 3) was performed at the KSU farm to verify that circovirus vaccination had affected within-farm viral circulation patterns and to determine the farm's new PCV2 status. Results indicate a change in the herd PCV2 antibody profile. Pigs for this follow-up study were born primarily from dams that were vaccinated against circovirus as weaned pigs; however, gilts or sows were not vaccinated against circovirus prior to breeding or during gestation. Before vaccine introduction into the herd, pigs had antibody decay until mid-finishing followed by high levels of antibody in late-finishing, so the pattern after 2 years of continuous vaccination was different. Antibody levels at the time of weaning were similar and low for pigs assigned to the control or vaccinated treatments (Table 3). After vaccination, vaccinated pigs had a rise in antibody by the beginning of the finishing period that then decreased throughout finishing. In contrast, control pigs had decay in antibody levels through the beginning of finishing and never had a rise in antibody levels. The lack of antibody rise suggests that control pigs were not exposed to the PCV2 virus during the time period for sampling. Residual PCV2 virus shed from previously infected pigs and present in the environment did not appear to stimulate an immune response in these control pigs, nor did it appear that there was exposure to PCV2 virus transmitted from vaccinated but infected pigs within the groups. These follow-up KSU results indicate that the virus had either been eliminated from the herd and farm facilities, or had fallen below the threshold that could trigger stimulation of the immune system.

Growth rate has been used as an indicator of disease and was therefore included as a response for this study. In our study, circovirus vaccination negatively affected growth rate during the nursery and grower periods (Table 4). This resulted in vaccinated pigs 2.6 lb lighter than non-vaccinated control pigs at the beginning of the finishing period.

During the finisher phase and for the overall study, vaccinated pigs had numerically reduced ADG compared with control pigs. At the time pigs were taken off test, control pigs had a 4.4 lb numeric weight advantage compared with vaccinated pigs, but the lack of positive growth rate response due to vaccination may be explainable by low or no natural PCV2 challenge in the KSU herd.

In our study, vaccinated pigs during finishing did not demonstrate greater ADG compared with non-vaccinated control pigs. Vaccinated pigs were not able to compensate for or overcome the negative effects of vaccination in the nursery. Thus, the immunity built in the nursery and grower period did not provide any benefit during finishing because PCV2 was not present as a challenge to the immune system of the pigs. Therefore, the lack of serologic evidence for PCV2 exposure coupled with the tendency for

vaccinated pigs to have poorer overall growth performance than control pigs suggests that PCV2 was not a pathogenic threat for growing pigs in the KSU herd during the follow-up testing.

The results that indicated PCV2 was no longer an apparent natural challenge for pigs in the KSU farm could not be replicated in a commercial farm in Kansas despite both farms having implemented long-term circovirus vaccination programs. At the time the data were collected, the commercial farm had been continuously vaccinating pigs for 1 year—slightly less time than the KSU farm. Clinical disease had decreased during the time the vaccine was being used in the commercial herd. The commercial farm moved pigs all-in, all-out from their nursery and finisher rooms and used a disinfectant similar to that of the KSU farm; however, the period of downtime between batches of pigs for cleaning and disinfection of rooms was longer at the KSU farm compared with the commercial farm.

In the commercial farm, the non-vaccinated pigs did become viremic after movement into the nursery (Table 5) and exhibited clinical signs of PCVD. The clinical disease in these pigs was apparent even though they constituted a relatively low percentage of the population, and herd immunity did not appear to prevent propagation of the infection; therefore, the belief that housing environment contributed a significant source of PCV2 virus in this population led us to perform the environmental evaluation. We acknowledge that pig-to-pig transmission from viremic pigs could also play a role in the dynamics of the infection, but we believe this was less likely. At each time point, more serum pools had detectable DNA, which indicated that more pigs were becoming infected. In addition, PCV2a was detected first, followed by PCV2b, so the infection profile also changed over time. Whether this differential pattern has biologic significance is yet to be determined.

To understand why non-vaccinated pig results differed between the KSU herd and the commercial farm, it was important to identify sources of viral exposure. Pigs at both farms were seemingly weaned free of PCV2, implicating PCV2 in the environment as a primary source of exposure. Swabs were collected in all nursery and finishing rooms at the commercial farm. Nursery and finishing rooms at the KSU farm that had housed study pigs at some point through the 3-year study were also sampled. Although PCR detection of PCV2 nucleic acid does not provide any information about whether the viral material is infectious, it does allow measurement of environmental viral loads that could potentially contain infectious material.

In the commercial facility, PCV2 DNA was found in every room and barn. In contrast, at the KSU farm, PCV2 nucleic acid was not detected in either the nursery or finishing facility. Although the infectivity status of the PCV2 DNA detected at the commercial site was not known, any residual infectious material present in the environment could explain why non-vaccinated pigs placed in this facility became viremic shortly after movement into the facility. Complete inactivation of PCV2 was difficult by disinfection under laboratory conditions.[14] Therefore, in our study, with viral material detected in the environment, some infectious virus likely remained. Further investigation of this

[14] Royer, R. L., P. Nawagitgul, P. G. Halbur, and P. S. Paul. 2001. Susceptibility of porcine circovirus type 2 to commercial and laboratory disinfectants. J. Swine Health Prod. 9:281-284.

environmental virus-based route of transmission is warranted to determine the importance of this potential risk.

In conclusion, results from this 3-year investigation indicate that circovirus vaccination did affect viral circulation in swine herds. Success in lowering levels or eliminating the virus as a pathogenic threat was achieved at a university research herd, but other exposure risk factors, such as residual PCV2 in the environment, appeared under commercial conditions and inhibited viral elimination efforts. Therefore, circovirus vaccine provides a tool to affect viral circulation on farms but needs to be used in conjunction with other management practices to eliminate PCV2 from most swine populations.

Table 1. Detection of porcine circovirus type 2 (PCV2) nucleic acid in serum samples serially collected from barrows across 9 consecutive weaning groups enrolled in a post-circovirus-vaccination implementation monitoring program at the Michigan State University Swine Teaching and Research Center[1]

		Sampling[2]			
Item	Pigs, no.[3]	d 0 (wean wk)	Entry-to-finishing	Mid-finishing	End-of-finishing
Group 1	10				
Interval, wk[4]		---	6.1	14.0	17.1
PCV2 DNA detected[5]		no	no	no	no
Group 2	9				
Interval, wk[4]		---	7.1	15.1	18.0
PCV2 DNA detected[5]		no	no	no	no
Group 3	9				
Interval, wk[4]		---	5.9	12.0	18.0
PCV2 DNA detected[5]		no	no	no	no
Group 4	9				
Interval, wk[4]		---	7.0	13.0	18.1
PCV2 DNA detected[5]		no	no	no	no
Group 5	11				
Interval, wk[4]		---	5.9	12.9	16.9
PCV2 DNA detected		no	no	no	no
Group 6	10				
Interval, wk[4]		---	6.0	11.1	18.0
PCV2 DNA detected[5]		no	no	no	no
Group 7	10				
Interval, wk[4]		---	8.1	15.0	20.0
PCV2 DNA detected[5]		no	no	no	no
Group 8	9				
Interval, wk[4]		---	6.3	13.1	18.0
PCV2 DNA detected[5]		no	no	no	no
Group 9	9				
Interval, wk[4]		---	6.0	13.0	18.0
PCV2 DNA detected[5]		no	no	no	no

[1] A total of 86 barrows (4 samples per barrow) were serially bled and serum was analyzed by PCR for detectable PCV2 DNA. All pigs were vaccinated intramuscularly with 2 doses (2 mL per dose) of Circumvent PCV (Intervet/Schering-Plough Animal Health, Millsboro, DE) after the d 0 blood sample was collected (during the week of weaning).

[2] Sampling points were during weaning week (d 0; single pre-vaccination serum sample), after entry to the finisher, during mid-finishing, and at the end of the finishing period.

[3] An average of 12 barrows were randomly selected across 9 consecutive farrowing groups, ear-tagged, and monitored for their lifetime. Only serum samples from barrows with complete serum sets (4 serum samples per pig) were tested by PCR for detectable PCV2 nucleic acid. Number of pigs reported in the table represents the number of pigs with complete serum sets.

[4] Interval indicates the amount of time in weeks that had elapsed since the previous sampling point. The d 0 sample was collected during weaning week.

[5] All serum samples were individually tested by PCR for presence of PCV2 nucleic acid. Results are reported as "yes" if a sample had detectable PCV2 nucleic acid for the indicated group and sampling point, and "no" if no samples had detectable PCV2 nucleic acid.

Table 2. Detection of porcine circovirus type 2 (PCV2) nucleic acid in serum samples serially collected from barrows across 10 consecutive weaning groups enrolled in a post-circovirus-vaccination implementation monitoring program at the Kansas State University Swine Teaching and Research Center[1]

		Sampling[2]			
Item	Pigs, no.[3]	d 0 (wean wk)	Entry-to-finishing	Mid-finishing	End-of-finishing
Group 1	11				
Interval, wk[4]		---	8.7	15.0	21.9
PCV2 DNA detected[5]		no	no	no	no
Group 2	10				
Interval, wk[4]		---	9.9	14.9	20.0
PCV2 DNA detected[5]		no	no	no	no
Group 3	10				
Interval, wk[4]		---	9.3	14.4	19.1
PCV2 DNA detected[5,6]		no	no	yes	no
Group 4	8				
Interval, wk[4]		---	10.1	14.9	20.0
PCV2 DNA detected[5]		no	no	no	no
Group 5	12				
Interval, wk[4]		---	9.9	15.0	19.9
PCV2 DNA detected[5,6]		yes	yes	yes	yes
Group 6	12				
Interval, wk[4]		---	10.3	15.3	19.8
PCV2 DNA detected[5,6]		no	yes	no	no
Group 7	12				
Interval, wk[4]		---	10.2	14.0	19.5
PCV2 DNA detected[5]		no	no	no	no
Group 8	12				
Interval, wk[4]		---	9.7	14.7	17.1
PCV2 DNA detected[5]		no	no	no	no

Table 2. Detection of porcine circovirus type 2 (PCV2) nucleic acid in serum samples serially collected from barrows across 10 consecutive weaning groups enrolled in a post-circovirus-vaccination implementation monitoring program at the Kansas State University Swine Teaching and Research Center[1]

Group 9	12				
Interval, wk[4]		---	9.7	15.0	19.6
PCV2 DNA detected[5]		no	no	no	no
Group 10	12				
Interval, wk[4]		---	10.3	14.9	20.3
PCV2 DNA detected[5]		no	no	no	no

[1] A total of 111 barrows (4 samples per barrow) were serially bled and serum was analyzed by PCR for detectable PCV2 DNA. All pigs were vaccinated intramuscularly with 2 doses (2 mL per dose) of Circumvent PCV (Intervet/Schering-Plough Animal Health, Millsboro, DE) after the d 0 blood sample was collected (during the week of weaning).

[2] Sampling points were during weaning week (d 0; single prevaccination serum sample), after entry to the finisher, during mid-finishing, and at the end of the finishing period.

[3] An average of 12 barrows were randomly selected across 10 consecutive farrowing groups, ear-tagged, and monitored for their lifetime. Only serum samples from barrows with complete serum sets (4 serum samples per pig) were tested by PCR for detectable PCV2 nucleic acid. Number of pigs reported in the table represents the number of pigs with complete serum sets.

[4] Interval indicates the amount of time in weeks that had elapsed since d 0 (day of vaccination). The d 0 sample was collected during weaning week and was collected before the vaccine was administered.

[5] All serum samples were individually tested by PCR for presence of PCV2 nucleic acid. Results are reported as "yes" if a sample had detectable PCV2 nucleic acid for the indicated group and sampling point, and "no" if no samples had detectable PCV2 nucleic acid.

[6] Viral template quantities ranged from 5 to 379 template copies per reaction across serum samples with detectable PCV2 nucleic acid. Within group 5 pigs and 2 barrows had serum samples with detectable nucleic acid at more than 1 sampling point.

Table 3. Effect of circovirus vaccination and time on indirect fluorescent antibody (IFA) geometric mean titer (GMT) in pigs produced at a farm that had been vaccinating growing pigs against porcine circovirus type 2 (PCV2) continuously for 2 years[1]

		Treatment[2]								
		Control				Vaccinate				Probability, $P <$
Item	Time:	d 0 (wean wk)	Entry-to-finishing	Mid-finishing	End-of-finishing	d 0 (wean wk)	Entry-to-finishing	Mid-finishing	End-of-finishing	Treatment × time
Samples, no.		68	68	68	68	68	66	66	66	---
GMT[3]		35.9[a]	15.2[b]	14.8[b]	13.6[b]	43.6[a]	52789.3[c]	13841.2[d]	3729.8[e]	<0.001

[a,b,c,d,e] Means without a common superscript letter differ ($P < 0.05$).

[1] A total of 136 barrows (68 control and 68 vaccinated pigs) across 3 farrowing groups were ear-tagged and monitored from weaning through finishing at the Kansas State University Swine Teaching and Research Center. Pigs were serially bled on d 0 (within a wk of weaning), after entering the finisher (time elapsed since d 0 range: 8.4 to 8.9 wk), mid-finishing (time elapsed since d 0 range: 13.4 to 13.9 wk), and at the end of the finishing period (time elapsed since d 0 range: 18.0 to 19.4 wk). Antibody levels against PCV2 were determined by IFA testing on individual serum samples. Individual pig IFA titer data were $\log_3$ transformed and were analyzed by repeated measures analysis using the GLIMMIX procedure in SAS version 9.1.3 (SAS Institute, Inc., Cary, NC). Fixed effects in the model included treatment, time, and their interaction. Group and IFA day were included as random effects.

[2] Treatments were non-vaccinated control or vaccinated. Vaccinated pigs were injected intramuscularly with 2 doses (2 mL per dose) of Circumvent PCV (Intervet/Schering-Plough Animal Health, Millsboro, DE) after the d 0 blood sample was collected (during the week of weaning).

[3] Geometric mean titers were calculated by taking the mean of the $\log_3$ transformed IFA titer values then converting the resulting transformed mean back to the original scale for presentation.

Table 4. Effect of circovirus vaccination on growth rate of pigs produced at a farm that had been vaccinating growing pigs against porcine circovirus type 2 (PCV2) continuously for 2 years[1]

	Treatment[2]			
Item	Control	Vaccinate	SEM	Probability, $P <$
Pigs started on test, no.	186	186	---	---
ADG, lb				
Nursery-grower[3]	1.24	1.18	0.013	0.005
Finisher[4]	2.41	2.39	0.029	0.30
Overall[5]	1.88	1.85	0.023	0.07
Weight, lb				
d 0	13.9	14.0	0.44	0.97
Entry-to-finishing	89.0	86.4	1.99	0.03
End-of-finishing (off test)	262.8	258.4	5.33	0.16

[1] A total of 372 weanling pigs (186 control and 186 vaccinated pigs) across 3 farrowing groups were ear-tagged and monitored from weaning through finishing at the Kansas State University Swine Teaching and Research Center. Pigs were individually weighed on d 0 (within the weaning week and the day of vaccination), after entering the finisher, and at the end of the finishing period to calculate ADG. Growth and on-test time data from mortalities and removed pigs were included in growth and period length calculations. Individual pig growth data were analyzed using the GLIMMIX procedure in SAS version 9.1.3 (SAS Institute, Inc., Cary, NC). The interaction with gender and treatment was determined to be non-significant for group 3, and growth data were pooled across the genders for subsequent analysis. Growth data for all 3 groups were analyzed using a model that included treatment as a fixed effect and group as a random effect.

[2] Treatments were non-vaccinated control or vaccinated. Vaccinated pigs were injected intramuscularly with 2 doses (2 mL per dose) of Circumvent PCV (Intervet/Schering-Plough Animal Health, Millsboro, DE).

[3] Nursery-grower ADG and period length include data from mortalities and removed pigs. The nursery period length did not differ ($P = 0.15$) between control (59.7 ± 1.48 d) and vaccinated (59.1 ± 1.48 d) pigs.

[4] Finisher ADG and length include data from mortalities and removed pigs. The number of days for the finisher period did not differ ($P = 0.94$) between control (71.7 ± 1.13 d) and vaccinated (71.6 ± 1.14 d) pigs.

[5] Overall ADG and length include data from mortalities and removed pigs. The number of days for the overall trial did not differ ($P = 0.96$) between control (132.1 ± 2.67 d) and vaccinated (132.1 ± 2.67 d) pigs.

Table 5. Detection of porcine circovirus type 2 (PCV2) nucleic acid in serum samples serially collected from pigs not vaccinated for PCV2 in a monitoring program at a commercial farm[1]

	Age, wk[2]			
Item	1.7 to 3.1 (wean wk)	4.9 to 6.3	7.9 to 9.3	10.9 to 12.3
Pig survival, %	100.0	97.6	91.8	91.8
Interval, wk[3]	---	3.2	6.2	9.2
PCV2 PCR results				
Pools for PCR, no.[4]	17	17	16	16
PCV2 DNA detected[5,6]	no	yes	yes	yes
Pools with detectable PCV2 DNA, %	0	5.9	37.5	75.0

[1] A total of 85 pigs were serially bled and serum was analyzed by PCR for detectable PCV2 DNA. Pigs were not vaccinated for PCV2 at any time during this monitoring period on this commercial farm.
[2] Pigs were bled initially during the wk of weaning when pig ages ranged from 1.7 to 3.1 wk of age. Pigs were serially bled every 3 wk (on average) thereafter until pigs were 10.9 to 12.3 wk of age.
[3] Interval indicates the amount of time in weeks that had elapsed since the initial sampling point. The initial sample was collected during weaning week.
[4] A total of 5 serum samples were included in a single pool for testing by PCV2 PCR.
[5] All serum samples were individually tested by PCR for presence of PCV2 nucleic acid. Results are reported as "yes" if a sample had detectable PCV2 nucleic acid for the indicated group and sampling point, and "no" if no samples had detectable PCV2 nucleic acid.
[6] For serum pools with PCV2 DNA detected, cycle time (Ct) values ranged from 27.7 to 40.7.

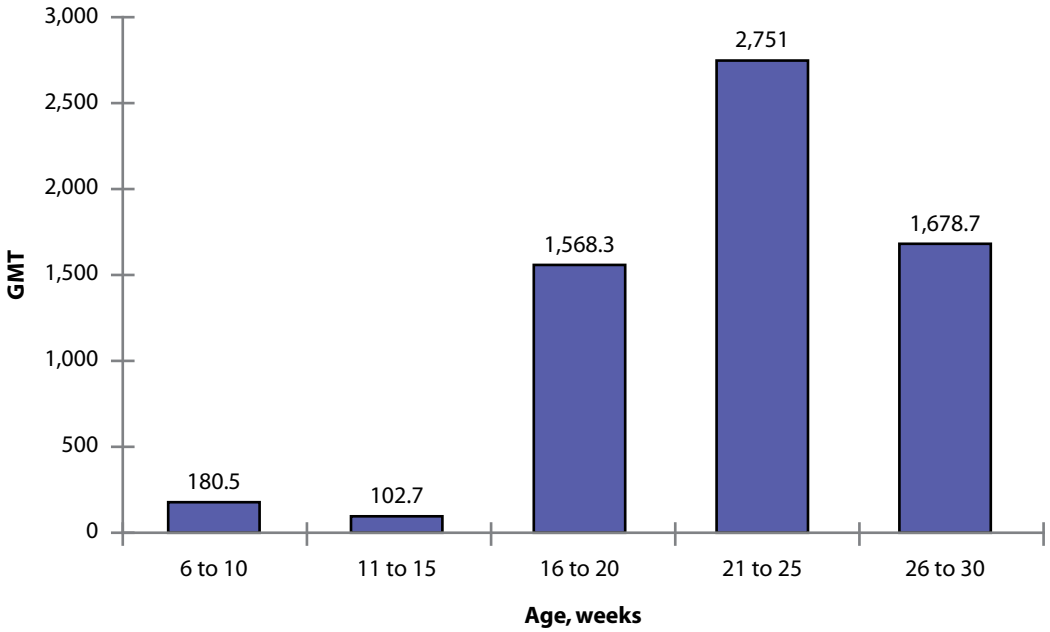

Figure 1. Characterization of the porcine circovirus type 2 (PCV2) antibody profile of the Michigan State University (MSU) Swine Teaching and Research Center herd prior to implementation of a circovirus vaccination program.
At the MSU farm, a total of 101 pigs were sampled across 5 growing pig populations (6 to 10, 11 to 15, 16 to 20, 21 to 25, and 26 to 30 wk of age) using a cross-sectional design. Serum samples from individual pigs were tested by the Kansas State University Veterinary Diagnostic Laboratory PCV2 indirect fluorescent antibody (IFA) assay for detection of PCV2 antibodies. All IFA titers were $\log_2$ transformed to approximate a normal distribution prior to descriptive analysis. Resulting transformed means were transformed back to the original scale for presentation as geometric mean titers (GMT).

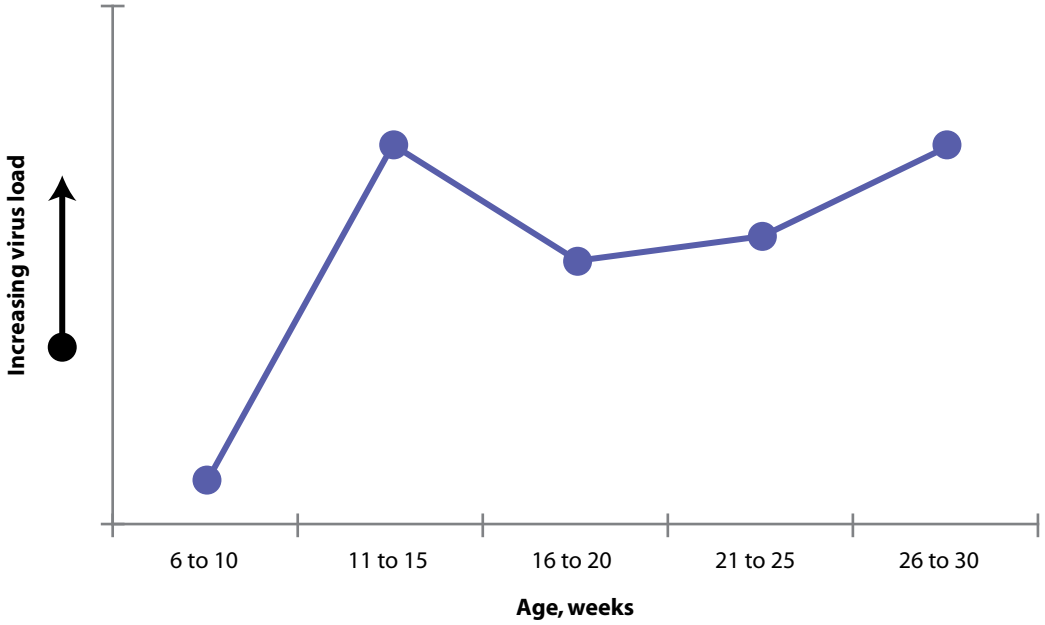

Figure 2. Characterization of the porcine circovirus type 2 (PCV2) infection profile of the Michigan State University (MSU) Swine Teaching and Research Center herd prior to implementation of a circovirus vaccination program.
Serum was pooled (MSU: 21 pools) within age group and analyzed using the Kansas State University Veterinary Diagnostic Laboratory PCV2 PCR assay for detection of PCV2 nucleic acid. Pooled results were $\log_{10}$ transformed and transformed results were averaged within age ranges to characterize patterns for viral load.

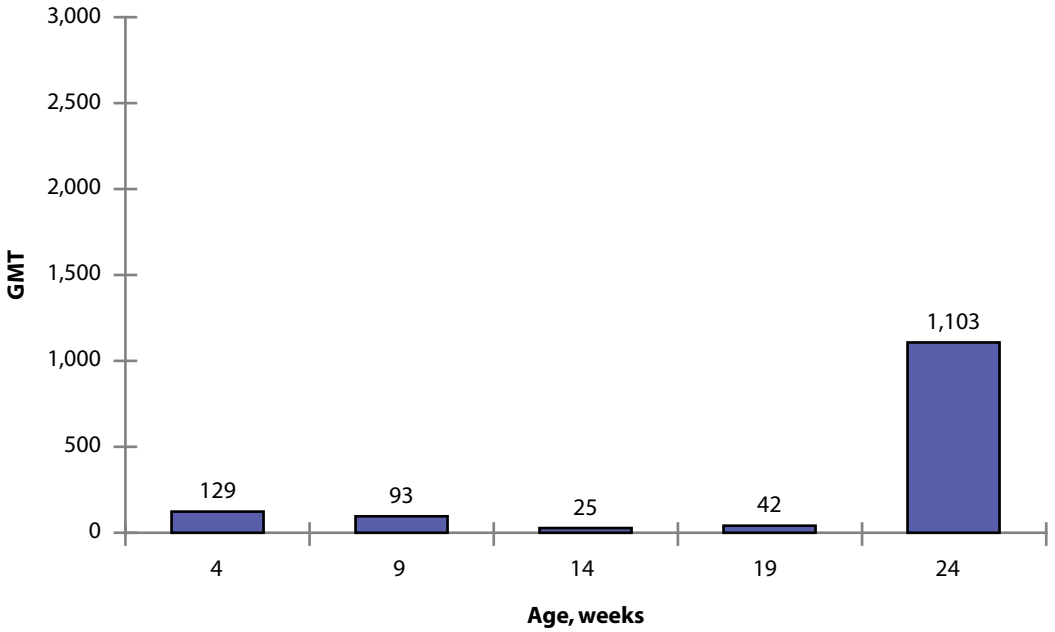

Figure 3. Characterization of the porcine circovirus type 2 (PCV2) antibody profile of the Kansas State University (KSU) Swine Teaching and Research Center herd prior to implementation of a circovirus vaccination program.
At the KSU farm, a total of 141 pigs were sampled across 5 growing pig populations (4, 9, 14, 19, and 24 wk of age) using a cross-sectional design. Serum samples from individual pigs were tested by the Kansas State University Veterinary Diagnostic Laboratory PCV2 indirect fluorescent antibody (IFA) assay for detection of PCV2 antibodies. All IFA titers were $\log_2$ transformed to approximate a normal distribution prior to descriptive analysis. Resulting transformed means were transformed back to the original scale for presentation as geometric mean titers (GMT).

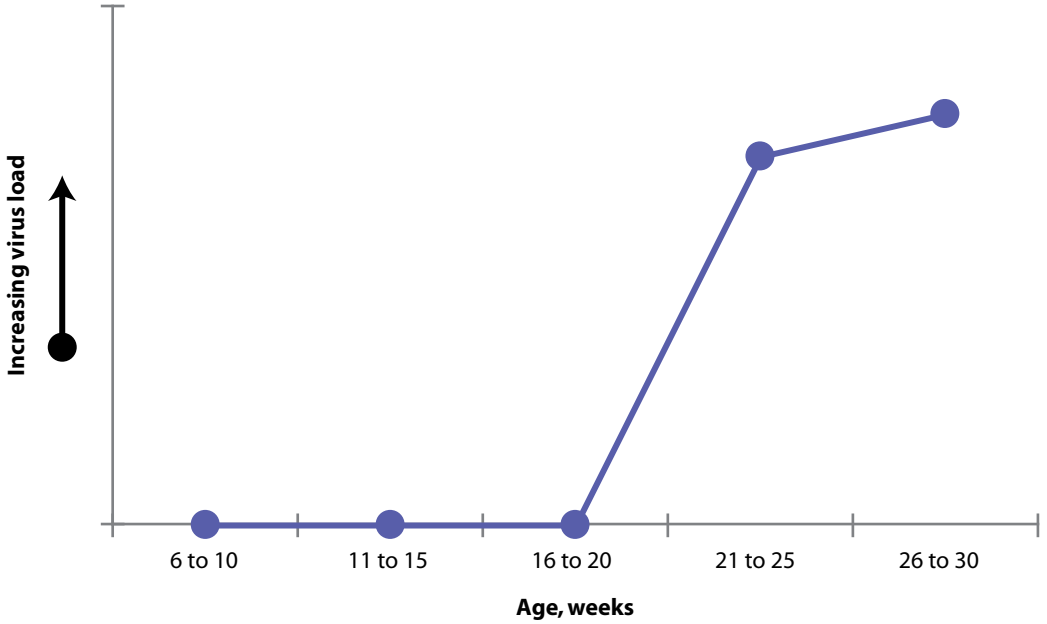

Figure 4. Characterization of the porcine circovirus type 2 (PCV2) infection profile of the Kansas State University (KSU) Swine Teaching and Research Center herd prior to implementation of a circovirus vaccination program.
Serum was pooled (KSU: 27 pools) within age group and analyzed using the Kansas State University Diagnostic Laboratory PCV2 PCR assay for detection of PCV2 nucleic acid. Pooled results were $\log_{10}$ transformed and transformed results were averaged within age ranges to characterize patterns for viral load.

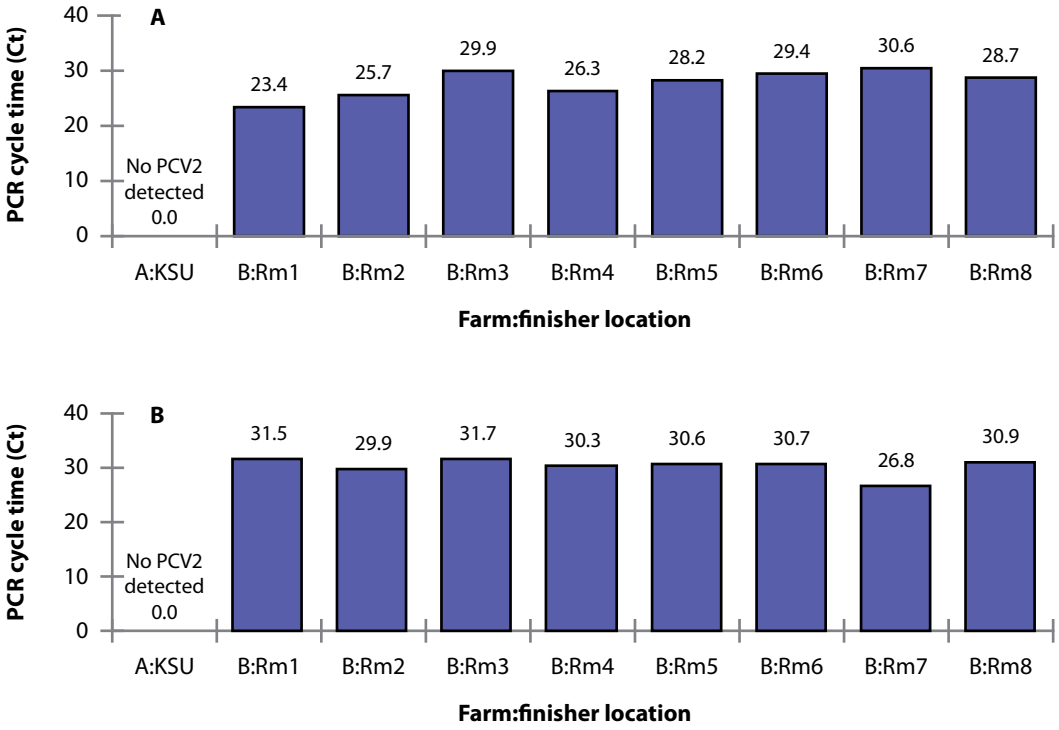

Figure 5. Detection of porcine circovirus type 2 (PCV2) nucleic acid in the environment of nursery and finisher facilities at the Kansas State University (KSU) Swine Teaching and Research Center and a commercial farm. Effect of farm and nursery location on environmental PCV2 DNA detection (A) and effect of farm and finisher location on environmental PCV2 DNA detection (B) are shown below.

Porcine circovirus type 2 (PCV2) PCR results for environmental swabs of Farm A (KSU farm) and Farm B (commercial farm) nursery and finisher locations. Cycle time (Ct) values are reported as 0.0 (no PCV2 DNA detected) or greater than 0.0 (PCV2 DNA detected) with the lower positive Ct values indicative of more PCV2 viral DNA.