FIELD STUDY

# Some effects of porcine circovirus on performance

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***Summary:*** *Ten sows positive for antibodies to porcine circovirus (PCV) and ten negative sows from an endemically infected herd were followed through farrowing to look for differences in production factors and changes in infection status. The ten sows that were seronegative at the start of the study seroconverted; however, there was no difference in the two groups for production factors. Three pigs from each litter, which did not have congenital tremors, were followed from weaning to market weight looking for production factors and evidence of infection. There was no significant difference in the production factors of selected pigs from sows infected prior to farrowing or from sows that became infected over farrowing. The pigs were infected at approximately 6 months as evidenced by antibody titer.*

Porcine circovirus (PCV), one of the smallest DNA viruses (17 nm), has a circular, single-stranded DNA genome of 1.76 kilobases, and codes for a single protein of 36,000 kd.[1] The prototype virus was discovered as a contaminant of the ATCC PK-15 cell line,[2] but has not been isolated from a naturally occurring infection.

Antibodies to PCV have been demonstrated in swine populations in Germany,[3] Canada,[4] and in the United States[3] using an indirect-fluorescent antibody (IFA) test. Tischer, et al.,[3] infected seronegative pigs in Germany but were unable to produce a disease.

This study was intended to compare the performance of sows with antibody titers to PCV, indicating recent infection, to sows without antibody titers to PCV at farrowing. The offspring of these sows were tested to determine the age of infection as determined by the antibody response, and to determine whether infection altered growth.

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## Materials and methods

### Detecting PCV antibodies

PK-15 cells permanently infected with PCV were used as antigen for testing of swine serum by IFA. Cells were grown on glass coverslips that had been cleaned, flame sterilized, then rinsed in sterile phosphate buffered saline (PBS) (pH 7.4) before use. Coverslips were placed in 30 × 10-mm tissue culture dishes (Falcon, Corning), then seeded with 70,000 cells per $cm^3$ in 2 mL of medium. Cultures were incubated for 24 hours, then fixed with cold acetone (4°C) for 15 minutes. Coverslip cultures were washed in PBS for 15 minutes, then porcine serum was placed on the coverslips, incubated for 30 minutes, and washed off with distilled water. Rabbit anti-pig IgG FITC conjugate (Sigma Immuno Chemicals) was placed on the coverslips, incubated for 30 minutes, washed off with PBS (pH 7.4), and mounted on glass slides using a one-to-one glycerin-saline (pH 7.2) mounting fluid. Fluorescence was detected using a fluorescent light microscope. Nuclear fluorescence in infected cells indicated that the serum had PCV antibodies. For determining antibody titer, twofold dilutions were made of the serum. The titer was expressed as the last dilution of serum that exhibited fluorescence.

### Comparing the performance of PCV-positive and -negative sows

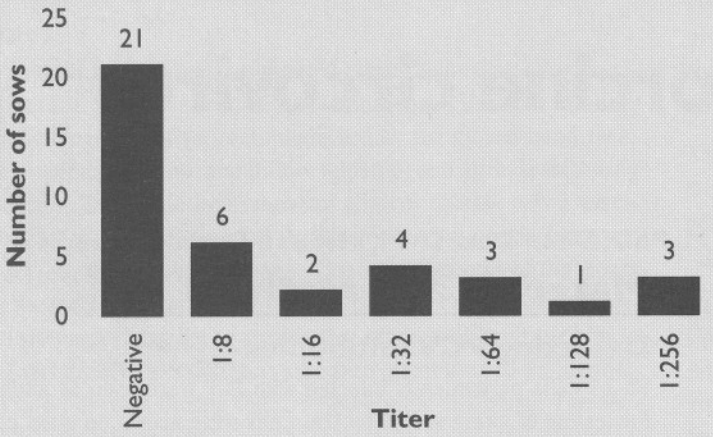
Ten seronegative and ten seropositive sows were selected and evaluated from the farrowing through the nursing period. Serum titers to PCV were determined at a week before gestation and at weaning. In order to select participants for the sow study, blood was collected from 40 sows in a gestation barn. The gestation crates, arranged in a long line, confined the sows so that they could not turn around. From the 40 sows, the first 10 seronegative sows and the 10 seropositive sows with the highest titer to PCV were selected for the study.

The change in PCV antibody titer of the sows was evaluated over the course of the nursing period. The litters were evaluated at the time of weaning for PCV antibody titers, pig weight, litter size, and litter weight.

### Comparing the performance of pigs from seronegative and seropositive sows

One purpose of this study was to determine the age at which pigs became infected with PCV using seroconversion as an indicator of infection. Three pigs from each litter were selected at 1 day of age for the study. We picked only pigs that were of average size for the litter and appeared healthy. They were weighed, bled, and the an-

**Figure 1**




Anti-PCV IgG titers of sows at the beginning of the study. Forty sows were screened to be selected for the study. The antibody titers are depicted in this figure. The study included ten seronegative sows and the ten sows with the highest titers.

tibody titer was determined at weaning and then at monthly intervals until market weight. The pigs were not separated from other pigs of the same size and sex. After they were selected, four pigs from positive sows and one pig from a negative sow died of crushing injuries; thus, 55 pigs started the study as weanlings.

### Swine

The swine used in this study were from a commercial hog farm in north central Georgia with an endemic infection of PCV. The farm was a 500-sow, total-confinement, farrow-to-finish operation. The sows were from one DeKalb crossbred line, while the boars were from a different DeKalb crossbred line.

### Cell culture

The PK-15 (ATCC CCL 33) cells were in the 172d passage. The cell culture medium used was M199 with Hanks salt (pH 7.2-7.4) (GIBCO), to which was added L-glutamine (0.2 mM) (GIBCO), nonessential amino acids (GIBCO), sodium pyruvate (0.1 mM) (MEM sodium pyruvate, GIBCO), gentamicin sulfate (0.05 mg per mL) (Gentocin, Shering-Plough), Amphotericin B (2.5 µg per mL) (Fungazone, Squibb), and 10% fetal bovine serum (GIBCO). Cells were cultured in 75 $cm^2$ cell culture flasks (Falcon, Corning) in a carbon dioxide incubator (Hotpack) at 37°C, with 5% $CO_2$.

## Results

### Survey of the gestating sows

Nineteen of the 40 sows were positive (47.5%), and 21 were negative (Figure 1). The sows with positive titers were geographically clustered in clumps of three to four separated by several negative sows.

**Table 1**

Field study sow data. Anti-PCV titers were determined a week prior to farrowing (Titer 1) and at weaning (Titer 2). The table lists the number of pigs born alive (Live), stillborn (Stilbrn), mummified (Mummies), and weak (Weak), as well as the number of pigs weaned (Weaned). It also lists the litter weights at birth (L WT 1) and at weaning (L WT 2).

| Sow | Titer 1 | Titer 2 | Live | Stilbrn | Mummies | Weak | Weaned | L Wt 1 | L Wt 2 |
|---|---|---|---|---|---|---|---|---|---|
| 2725 | neg | 1:128 | 11 | 2 | 0 | 0 | 8 | 40 | 100 |
| 2634 | neg | 1:128 | 11 | 1 | 0 | 1 | 10 | 32 | 143 |
| 3113 | 1:64 | 1:32 | 13 | 0 | 1 | 3 | 6 | 33 | 77 |
| 76262 | neg | 1:32 | 12 | 0 | 2 | 0 | 10 | 41 | 132 |
| 64049 | neg | 1:256 | 12 | 0 | 0 | 4 | 10 | 31 | 130 |
| 72421 | neg | 1:128 | 11 | 0 | 0 | 0 | 9 | 42 | 160 |
| 41763 | 1:32 | 1:32 | 10 | 0 | 0 | 1 | 8 | 28 | 103 |
| 41788 | neg | 1:128 | 9* | 1 | 0 | 1 | 9 | 30 | 125 |
| 41632 | neg | 1:64 | 11 | 0 | 0 | 0 | 9 | 35 | 133 |
| 59236 | neg | 1:32 | 12 | 1 | 0 | 1 | 11 | 35 | 126 |
| 72601 | neg | 1:256 | 5 | 0 | 0 | 0 | 5 | 20 | 64 |
| 63474 | neg | 1:32 | 12 | 0 | 0 | 0 | 9 | 44 | 156 |
| 72597 | 1:128 | 1:256 | 5 | 0 | 0 | 0 | 5 | 13 | 87 |
| 71351 | 1:64 | neg | 13 | 0 | 0 | 1 | 11 | 41 | 170 |
| 72307 | 1:256 | 1:512 | 10 | 0 | 0 | 0 | 9 | 32 | 132 |
| 3206 | neg | cull | 2 | 1 | 0 | 0 | 2 | 8 | 28 |
| 76255 | 1:32 | 1:16 | 10 | 1 | 0 | 0 | 8 | 30 | 111 |
| 52105 | 1:256 | 1:512 | 10 | 0 | 0 | 0 | 10 | 43 | 134 |
| 99123 | 1:64 | 1:256 | 13 | 0 | 0 | 0 | 9 | 40 | 127 |
| 64041 | 1:32 | 1:128 | 10 | 1 | 1 | 0 | 9 | 25 | 121 |

* Some of the pigs in this litter had congenital tremors.

**Table 2**

Average pig weights by litter at birth and at weaning. The table is divided with litters from seropositive sows on the right and litters from seronegative sows on the left. The weights are given in lb.

Seronegative Sows

| At birth | | At weaning | |
|---|---|---|---|
| Litter # | Weight | Litter # | Weight |
| 1 | 3.6 | 1 | 12.5 |
| 2 | 2.9 | 2 | 14.3 |
| 3 | 3.4 | 3 | 13.2 |
| 4 | 2.6 | 4 | 13.0 |
| 5 | 3.8 | 5 | 17.8 |
| 6 | 3.3 | 6 | 13.9 |
| 7 | 3.2 | 7 | 14.8 |
| 8 | 2.9 | 8 | 11.5 |
| 9 | 4.0 | 9 | 12.8 |
| 10 | 4.0 | 10 | 14.0 |

Seropositive Sows

| At birth | | At weaning | |
|---|---|---|---|
| Litter # | Weight | Litter # | Weight |
| 11 | 2.5 | 11 | 12.8 |
| 12 | 2.8 | 12 | 12.9 |
| 13 | 3.7 | 13 | 17.3 |
| 14 | 2.6 | 14 | 17.4 |
| 15 | 3.2 | 15 | 15.5 |
| 16 | 3.2 | 16 | 14.7 |
| 17 | 3.0 | 17 | 13.9 |
| 18 | 4.3 | 18 | 13.4 |
| 19 | 3.1 | 19 | 14.1 |
| 20 | 2.5 | 20 | 13.4 |

## Comparison of infected and negative sows

The most significant observation of the study was that all of the negative sows became infected (Table 1).

The negative sows farrowed 96 pigs, with 83 (86.46%) surviving to weaning, and the positive sows farrowed 106 pigs, with 84 (79.25%) surviving to weaning. The negative sows had six stillborn, two mummified, and seven weak pigs, collectively. The positive sows had two stillborn, two mummified, and five weak pigs, collectively. There was no statistically significant difference between the two groups by Chi-square analysis.

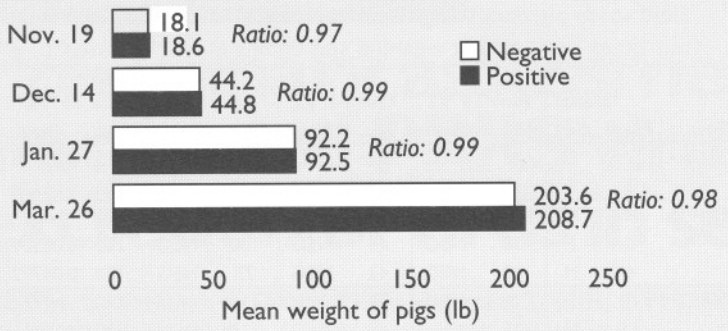
The average pig weight was determined for each litter at birth and again at weaning (Table 2). The average pig weights by litter at birth were compared between seropositive and seronegative sow groups. A two-sample analysis found no statistical difference ($P > 0.05$) between groups using a 95% confidence interval.

**Table 3**

Field study pig data. Weights and anti-PCV titers were taken four times beginning at weaning and ending at market weight.

| | | Date: | Nov 19 '92 | | Dec 14 '92 | | Jan 27 '93 | | Mar 26 '93 | |
|---|---|---|---|---|---|---|---|---|---|---|
| Sow | Pig # | Sex | Wt. | Titer | Wt. | Titer | Wt. | Titer | Wt. | Titer |
| 2725 | 2 | F | 25 | NEG | 42 | NEG | 85 | NEG | 215 | 0.05278 |
| | 3 | F | 18 | NEG | 48 | NEG | 105 | NEG | 200 | 0.05278 |
| 2634 | 4 | M | 18 | NEG | 44 | NEG | 80 | 0.04444 | 200 | NEG |
| | 5 | F | 18 | NEG | 44 | NEG | 100 | NEG | 185 | 0.06389 |
| | 6 | M | 20 | NEG | 42 | NEG | 75 | NEG | 180 | 0.04722 |
| 76262 | 10 | F | 18 | NEG | 48 | 0.04444 | 110 | 0.04444 | 190 | NEG |
| | 11 | M | 25 | NEG | 46 | 0.04722 | 110 | 0.04444 | 230 | NEG |
| | 12 | M | 18 | NEG | 46 | NEG | 75 | NEG | 245 | NEG |
| 64049 | 13 | M | 25 | NEG | 46 | 0.04722 | 70 | NEG | 215 | NEG |
| | 14 | F | 20 | NEG | 46 | 0.04722 | 105 | NEG | 175 | NEG |
| | 15 | F | 20 | NEG | 50 | NEG | 110 | 0.04444 | 190 | 0.05278 |
| 72421 | 16 | F | 20 | NEG | 46 | NEG | 95 | NEG | 215 | NEG |
| | 17 | F | 24 | NEG | 50 | 0.04444 | 110 | 0.04444 | 220 | 0.04444 |
| | 18 | F | 18 | NEG | 45 | 0.04722 | 95 | NEG | 190 | NEG |
| 41788 | 22 | F | 22 | NEG | 38 | 0.04722 | 90 | 0.04722 | 185 | 0.05278 |
| | 23 | F | 20 | NEG | 42 | NEG | 100 | NEG | 200 | NEG |
| | 24 | F | 20 | NEG | 45 | NEG | 100 | 0.04722 | 190 | NEG |
| 41632 | 25 | F | 25 | NEG | 46 | NEG | DIED | 0 | 0 | 0 |
| | 26 | F | 15 | NEG | 32 | 0.04722 | 80 | 0.04444 | 175 | NEG |
| | 27 | F | 22 | NEG | 46 | NEG | 110 | 0.04722 | 200 | 0.04722 |
| 59236 | 28 | F | 16 | NEG | 35 | NEG | 85 | NEG | 175 | NEG |
| | 29 | M | 13 | NEG | 40 | 0.04444 | 85 | 0.04722 | 215 | 0.05278 |
| | 30 | M | 13 | NEG | 38 | NEG | 85 | NEG | 210 | NEG |
| 72601 | 31 | M | 15 | NEG | 45 | NEG | 80 | NEG | 230 | NEG |
| | 32 | M | 15 | NEG | 48 | 0.04722 | 110 | 0.04722 | 225 | 0.05278 |
| | 33 | F | 18 | NEG | 48 | 0.04722 | 105 | NEG | 200 | NEG |
| 63474 | 34 | F | 20 | NEG | 45 | NEG | 95 | NEG | 190 | NEG |
| | 35 | M | 25 | NEG | 55 | NEG | 100 | NEG | 230 | NEG |
| | 36 | M | 16 | NEG | 36 | NEG | 85 | 0.04722 | 225 | NEG |
| 72597 | 37 | M | 18 | NEG | 40 | 0.04722 | 85 | 0.04722 | 235 | NEG |
| | 38 | M | 16 | NEG | 40 | NEG | 75 | NEG | 195 | NEG |
| | 39 | M | 18 | NEG | 46 | 0.04722 | 100 | NEG | 220 | NEG |
| 71351 | 40 | M | 20 | NEG | 45 | 0.04722 | 95 | 0.04444 | 235 | NEG |
| | 41 | M | 20 | NEG | 50 | 0.04722 | 110 | 0.04444 | 240 | NEG |
| | 42 | F | 18 | NEG | 46 | NEG | 105 | NEG | 190 | NEG |
| 72307 | 43 | M | 18 | NEG | 50 | NEG | 100 | NEG | 235 | 0.05278 |
| | 44 | F | 20 | NEG | 46 | NEG | 105 | 0.04722 | 190 | NEG |
| | 45 | M | 13 | NEG | 45 | 0.04444 | 70 | 0.04722 | 165 | NEG |
| 3206 | 46 | M | 18 | NEG | 44 | NEG | 80 | 0.04444 | 220 | 0.05278 |
| | 47 | M | 16 | NEG | 44 | NEG | 70 | NEG | 200 | NEG |
| | 48 | NA | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 76255 | 49 | F | 18 | NEG | 46 | 0.04722 | 105 | 0.04444 | 180 | NEG |
| | 50 | M | 20 | 0.04722 | 46 | 0.04722 | 95 | 0.04444 | 230 | NEG |
| | 51 | M | 23 | NEG | 48 | NEG | 105 | NEG | 230 | NEG |
| 52105 | 52 | M | 20 | NEG | 42 | NEG | 90 | 0.04444 | 240 | 0.05278 |
| | 53 | F | 16 | NEG | 30 | NEG | 75 | 0.04444 | 180 | NEG |
| | 54 | M | 18 | NEG | 42 | NEG | 80 | 0.04444 | 221 | 0.05278 |
| 99123 | 55 | F | 20 | 0.04722 | 46 | NEG | 100 | NEG | 180 | NEG |
| | 56 | M | 18 | 0.04722 | 48 | NEG | 100 | NEG | 225 | NEG |
| | 57 | F | 25 | NEG | 48 | NEG | 105 | NEG | 200 | NEG |
| 64041 | 58 | F | 20 | NEG | 45 | NEG | 105 | 0.04444 | 200 | 0.05278 |
| | 59 | M | 18 | 0.04722 | 45 | NEG | 75 | NEG | 230 | 0.05278 |
| | 60 | F | 20 | NEG | 45 | NEG | 95 | 0.04444 | 195 | 0.04722 |
| 41763 | 19 | M | 15 | NEG | 45 | NEG | 80 | NEG | 240 | NEG |
| | 20 | F | 18 | 0.04722 | 46 | 0.04722 | 100 | 0.04722 | 180 | 0.05278 |
| | 21 | F | 20 | NEG | 48 | NEG | 100 | NEG | 170 | 0.06389 |

**Figure 2**




Mean weights of pigs by group. The mean weights were determined for every test period for each group of pigs. There was no significant difference in the mean weights of the offspring of the seronegative and seropositive sows.

The average pig weight by litter for each pig at weaning were compared as above. A two-sample analysis found that there was no statistical difference ($P > 0.05$) between groups, using a 95% confidence interval.

Some of the pigs from one sow in the seronegative group had classic symptoms of congenital tremors. Pigs with symptoms of congenital tremors were not used in the pig study.

### Comparing the performance of pigs from seronegative and seropositive sows

Table 3 lists the antibody titers and weights of each pig at the time of each bleeding. Figure 2 compares the mean weights for each group. The ratio of the mean weights between the two groups of pigs approaches 1, indicating that there was no significant difference between the two.

All titers of 1:8 or less are considered nonspecific reactions. The only specific antibody reactions occurred at approximately 6 months of age when 15 of 54 exceeded a 1:8 titer.

## Discussion

After the discovery of the virus, Tischer determined that it infected many of the swine herds in Germany.[1,2] She used the virus first to inoculate month-old, then newborn pigs. Although she could recover the virus, she did not find a disease.

We have focused on naturally occurring infection in an endemically infected herd. Since the studies were conducted in a commercial swine herd, they were designed around the normal management practices of production. In the herd we used, production records on individual sows were limited. Because obvious disease caused by the virus has not been found, we have attempted to determine whether the virus affects growth or other production factors in the endemic infected herd, and have found that it does not.

There was no significant difference between the positive and negative sows for the:

- number of pigs farrowed,
- number of pigs weaned,
- weaning weights, or
- number of weak or stillborn pigs.

We selected apparently healthy pigs for the study that were of average size for the litter; therefore, the few pigs that had congenital tremors were not selected for the study. There was no difference in the growth of selected pigs from the two groups. If we had considered pigs with congenital tremors in the study, the results would surely have been different; however, this study focused on average or normal pigs.

When we evaluated the antibody titers of the pigs, we found that titers of 1:16 or greater only occurred at about 6 months of age, and we concluded that viral infection occurs then. Titers of 1:8 or less decreased in 21 cases, increased in 13 cases, remained the same in two cases, and decreased then increased in one case. We concluded that the antibody titers of 1:8 or less were not specific for infection. Our conclusion concurs with the findings of Dulac and Afshar.[4]

We observed the infectivity of the virus to be low. The survey conducted in the gestation house where the sows were in a fixed geographic location within the house found pockets of infection. If the virus was spread by aerosol, the pattern of infected animals would have followed the air flow. The fact that positive and negative sows were found side by side with only pipe barriers between them suggests that natural infectivity in healthy animals is low.

If we have virus with low infectivity, why did all of the negative sows become infected over farrowing? We don't know. We hypothesize that the stress of farrowing makes the sows more vulnerable to infection. The virus may replicate in the placenta, the cells of the amniotic fluid, or in the fetus, and thus be in greater concentrations in the farrowing house. These questions remain to be answered.

Also still to be answered is the significance of congenital tremors in the pigs. The sow whose pigs had congenital tremors began the study in the seronegative group, but became infected as evidenced by her antibody titer. The focus of further investigation is the relationship of PCV to congenital tremors.

## Implications

- We could determine no production differences between sows seropositive and seronegative for porcine circovirus.
- Although the infectivity of porcine circovirus appears to be low, by the end of weaning, all the negative sows had become positive. We hypothesize that this was due to the stress of farrowing and lactation.

# References

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