Porcine circovirus: A serological survey of swine in the United States

Raymond K. Hines, DVM, PhD; and Phil D. Lukert, DVM, PhD

Summary: *An indirect-fluorescent antibody procedure was used to assay swine serum for the presence of porcine circovirus (PCV) antibodies. We tested sera from 11 Georgia swine herds, one herd from North Carolina and one herd from Iowa. Antibodies against PCV were found in an average of 53% of the samples tested. The incidence varied from a low of 27% to a high of 100%. The antibody titers on one of the farms varied from 1:8 to 1:256. A titer of 1:8 or greater is considered to be positive. The significance of PCV infections has not been determined and further studies are needed to determine the possible role this virus might have in disease.*

Because porcine circovirus (PCV) infections in the United States have not been documented, we conducted a serological survey to determine whether these infections also occur in this country. Serological surveys in Germany[1] and Canada[2] indicate that the virus is indigenous to those countries.

Porcine circovirus is an extremely small virus that contains a circular single-stranded DNA. First characterized in 1982,[3] PCV was originally isolated as a contaminate of the PK-15 cell line obtained from the American Type Culture Collection (Cat.# ATCC CCL33). The virus is 17 nm in diameter, and the single stranded circular DNA contains about 1760 nucleotide bases.[3] The virus is very similar to the beak-and-feather disease virus (PBFDV) of psittacine birds and also closely resembles the chicken anemia virus (CAV) of chickens.[4]

The results of the serological survey in Germany[1] demonstrated that 85% of swine from three slaughter houses were positive for PCV antibodies. A breeding group of mini-pigs had a positive incidence rate of 36%, and five of eight wild boars (62%) were positive. Dulac and Afshar[2] reported an incidence of 55% in commercial herds and 26% from a slaughterhouse. They found that a specific-pathogen-free (SPF) herd was negative for PCV antibodies.

RKH, PDL: Department of Medical Microbiology, College of Veterinary Medicine, University of Georgia, Athens, Georgia 30602.

Supported by funds from the Georgia Veterinary Medical Experiment Station.

After Tischer et al.[1] infected 9-month-old seronegative pigs with PCV, no overt disease was evident. The virus could be recovered from nasal swabs for 6 days and from feces for 2 weeks. The infected pigs produced anti-PCV antibodies as a result of the infection.

To determine whether PCV infections were common in the United States and especially in the Southeast, we tested sera from one swine herd in Iowa, one herd in North Carolina, and 11 herds in Georgia.

Methods

To definitely show that the antibodies we were assessing were for PCV, we isolated the virus from the PK-15 cells that we had obtained from ATCC.

Cell cultures

The PK-15 (ATCC CCL33) cell line was used as the porcine circovirus-infected cell. The cells were grown in M199 with Hank's salts, to which was added L-glutamine (0.01mM), nonessential amino acids, sodium pyruvate (0.1 mm), gentamicin sulfate (0.05 mg per mL), amphotericin B (2.5 mg per mL), and 10% fetal bovine serum. Cells were maintained in 75 cm^3 cell culture flasks and incubated at 37°C in a 5% CO_2 atmosphere. Cells used in the indirect fluorescent antibody (IFA) tests were grown on sterile 22 × 22 mm glass coverslips placed in 30 × 10 mm culture dishes and seeded with 67,000 cells per cm^2.

Indirect fluorescent antibody (IFA) test

Antibodies to the PCV were detected and quantified by the IFA test. The PK-15 cells grown on coverslips were fixed in cold acetone for 15 minutes and washed in phosphate buffered saline (PBS) (pH 7.2) for 15 minutes. Cells were covered with test serum and incubated for 30 minutes in a humidified atmosphere at 37°C. After cells were rinsed three times in PBS, fluorescein isothiocyanate (FITC) conjugated rabbit anti-pig IgG (Sigma Immunochemicals) was added. After a 30-minute incubation at 37°C the cells were rinsed three times in PBS. Then the coverslip was mounted on a glass slide using 1 part glycerin to 1 part PBS mounting fluid. Fluorescence was detected using an epifluorescent UV illumination microscope.

Table 1

Porcine circovirus:
Indirect fluorescent antibody test results

Farm location	# Tested	# Positive	% Positive
GA	12	11	92
GA	12	12	100
GA	12	7	58
GA	36	19	53
GA	37	23	62
IA	20	11	55
GA	15	14	93
GA	16	7	44
GA	30	17	57
GA	28	19	68
NC	40	24	60
GA	30	17	57
GA	100	27	27
Totals	**399**	**208**	**53**
		Median:	**58**
		Mean:	**63**

Table 2

Distribution of porcine circovirus antibody titers
by IFA assays of 27 positive sera

IFA Titer	1:8	1:16	1:32	1:64	1:128	1:256
No. of sera	7	8	4	2	5	1

Serum samples

We tested 328 swine serum samples from 11 farms in Georgia, 20 samples from a farm in Iowa, and 40 samples from a farm in North Carolina. All were screened at a dilution of 1:8. Those that exhibited fluorescing cells at that dilution were considered positive for PCV antibodies. Positive serum samples from one farm were titered to determine the levels of antibody that existed in swine from a population positive for PCV.

Virus purification

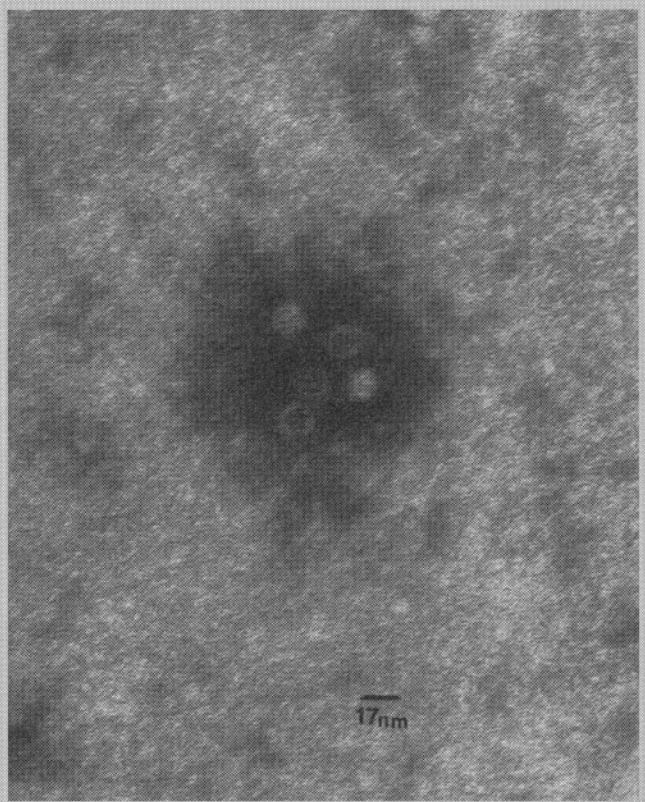
To verify that the PK-15 cell line used in this study was truly infected with PCV, the virus was purified and observed by electronmicroscopy and immune electronmicroscopy. The DNA from the virus was electrophoresed on a 2% agarose gel to verify that the DNA was similar to that described for PCV. The procedures for virus purification and DNA characterization have been described previously.[5] Electronmicroscopy and immunoelectronmicroscopy were performed as previously described.[4]

Results

The virus was identified by electronmicroscopy and DNA extracted from the virus was similar to the previously described DNA of PCV.

Figure 1

Electronmicrograph of porcine circovirus (PCV) purified from PK-15 cells and clumped with PCV antibodies (original magnification × 255,000).



Serological survey

The overall percentage of positive serum samples from the Georgia farms was 53% (range, 27%–100%). The percentage of positives from the Iowa and North Carolina farms was 55% and 60%, respectively (Table 1). The titers of 27 positive sera selected from one herd ranged from 1:8 to 1:256 (Table 2).

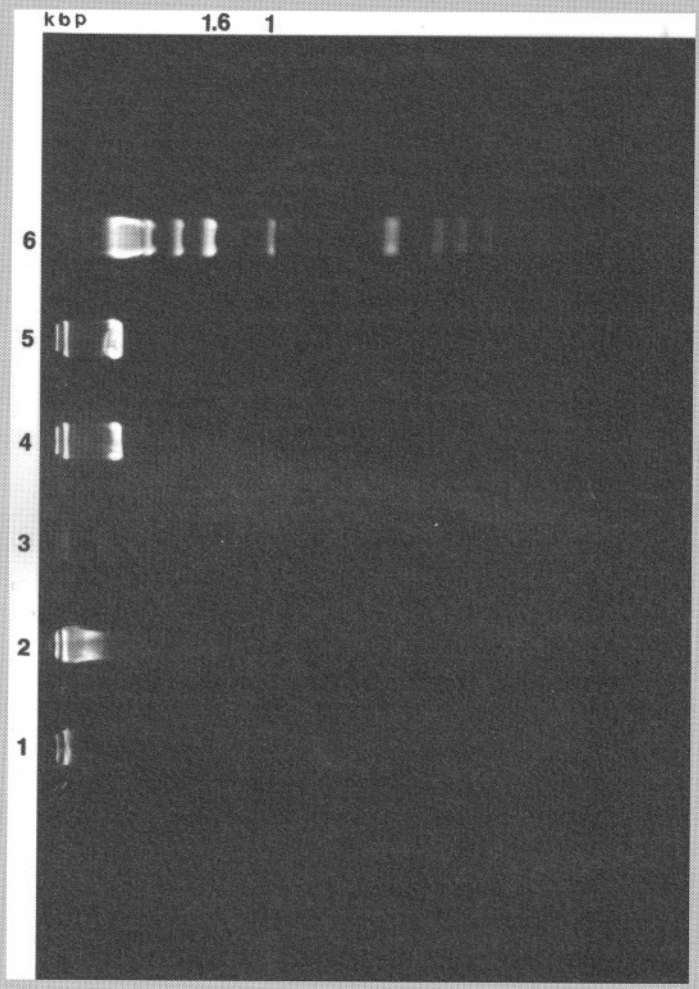
Isolation and characterization of PCV

An electronmicrograph of virus-antibody complexes indicated that the virus was indeed PCV (Figure 1). Further proof of the identity of the virus is that the DNA was of the same size as described for PCV. Figure 2 is a photograph of the agarose gel electrophoresis of the 1700 kb PCV-DNA extracted from infected PK-15 cells. It is compared to similar extracts from noninfected primary pig kidney, embryonic swine kidney, and noninfected PK-15 cells.

Discussion

We found that PCV is indigenous to the United States, as has been reported for Germany[1] and Canada.[2] While there was considerable variation in the prevalence of positive sera among herds, it was evident that animals in all herds tested had been infected. Because we did not sample any SPF herds we cannot say whether

Figure 2



Agarose gel electrophoresis of purified DNA from porcine circovirus (PCV) infected PK-15 cells. Lanes 1 and 2 were extracted from primary porcine kidney cells, lane 3 from ESK porcine kidney cells, lane 4 from PCV infected PK-15 cells, and lane 5 from PK-15 cells free of PCV. Lane 6 is a 1 Kb DNA ladder. Lane 4 has a single band of DNA at approximately 1.7 Kb that is not found in the other sample lanes.

there are swine units that are free of the virus. The incidence varied from 27%–100%, with an average of 53%. Most of the herds fit in the 40%–60% range.

Implications

- Porcine circovirus is a ubiquitous virus and infected herds are common in Europe, Canada, and the United States.
- Practitioners should be aware of this virus as a potential pathogen.
- Our laboratory has isolated this virus from a pig with congenital tremors and porciñe circovirus may be one of the causes of this condition.

References

1. Tischer I, Mields W, Wolff D, Vagt M, Griem W. Studies on epidemiology and pathology of porcine circovirus. *Arch. of Virol.* 1986; 91: 271-276.

2. Dulac GC, Afshar A. Porcine circovirus antigens in PK-15 cell line (ATTC CCL-33) and evidence of antibodies to circovirus in Canadian pigs. *Can. J. of Vet. Res.* 1989; 53: 431-433.

3. Tischer I, Gelderblom H, Vetterman W, Koch MA. A very small porcine virus with circular single-stranded DNA. *Nature* 1982; 295: 64-66.

4. Todd D, Creelan JL, Mackie DP, Rixon F, McNulty MS. Purification and biochemical characterization of chicken anemia agent. *J. of Gen. Virol.* 1990; 71: 819-823.

5. Ritchie BW, Niagro FD, Lukert PD, Steffens WL, Latimer KS. Characterization of a new virus from cockatoos with psittacine beak and feather disease. *Virology* 1989; 171: 83-88.