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National reduction in porcine circovirus type 2 prevalence following introduction of vaccination

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ABSTRACT

Porcine circovirus type 2 (PCV2), a small, single-stranded circular DNA virus and the causative agent of porcine circovirus associated disease (PCVAD), was first observed in the mid-1990s in pigs with a post-weaning wasting disease. In 2006 the number of PCVAD cases greatly increased, marking it as an important viral pathogen for the United States (US) swine industry. PCV2 vaccines were introduced to the US in 2006 in response to widespread outbreaks of PCVAD. These vaccines were effective in preventing disease, but did not eliminate virus from the animals. In 2006, prior to vaccine use, a study of PCV2 prevalence in pig herds across the US was performed in conjunction with the US National Animal Health Monitoring System. In 2012, 6 years after widespread PCV2 vaccination, this study was repeated. Since the introduction of PCV2 vaccines in 2006, viral presence and viral loads have greatly decreased, and a genotypic shift dominated by PCV2b has occurred. Antibody levels have decreased in the pig population, but approximately 95% of sites continue to be antibody-positive. Widespread vaccination has controlled PCVAD and decreased PCV2 prevalence to the point that viremia is not detected on many sites. Thus, continued vaccination may lead to PCV2 elimination in the national herd over time.

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1. Introduction

Porcine circovirus type 2 (PCV2) is a small, single-stranded circular DNA virus. PCV2 is the causative agent of porcine circovirus associated disease (PCVAD), but requires additional factors for disease to manifest (Allan and Ellis, 2000). PCVAD was first observed in the mid-1990s as a wasting disease in pigs (Allan and Ellis, 2000). In 2006, the number of PCVAD cases appeared to increase, marking PCV2 as an important viral pathogen for the US swine industry (Gillespie et al., 2009; Opriessnig et al., 2007). PCV2 vaccines were introduced to the US in 2006, in response to the increasing number of PCVAD cases and its impact on the swine industry. PCV2 vaccination is effective in preventing PCVAD, inducing high levels of anti-PCV2 antibodies and reducing the level of PCV2 in serum, but does not eliminate virus from the animal (Fort et al., 2008; Opriessnig et al., 2008a, 2010).

In 2006, prior to vaccine availability, a biologic sampling was performed in pig herds across the US and the prevalence of PCV2 virus and antibodies was examined (Puvanendiran et al., 2011). Here, we re-examined the prevalence of PCV2 virus and immunity in pigs and on sites in the US in 2012 to assess the potential impact of intensive vaccination. We observed substantial decreases in viral load and the frequency of positive results at both the pig and site levels. The PCV2 vaccination program implemented in 2006 has controlled PCVAD and appears to have successfully decreased the prevalence of virus in the swine population.

2. Material and methods

2.1. Serum samples

Serum samples were obtained from 177 grower/finisher or sow swine operations from 12 states throughout the US in the 2012 National Animal Health Monitoring System (NAHMS) fifth swine health survey (http://www.aphis.usda.gov/animal_health/nahms/swine/). Serum was collected by venipuncture in clot tubes from approximately 30 animals per site for 3933 grower/finisher swine (20 weeks or older, noted hereafter as finishers or from

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finisher sites) and 1772 sows or gilts (noted hereafter as sows or from sow sites, parity 0–5+). Sera were separated, aliquoted, and frozen at −20 °C until testing. Subsets of the serum samples were used for the analysis of PCV2-specific antibodies (2044 finisher, 958 sow) and viremia (1097 finisher, 407 sow).

2.2. Antigen preparation and ELISA procedure

The full length PCV2 replicase antigen (Genbank ID JF290418) was PCR amplified using forward 5′CGCGGATCCATGCCCAGCAAGAAGAATG3′ and reverse 5′CCGCTCGAGGTAATTTATTTCATATGGA3′ primers. The PCR product was then purified and cloned into a modified pET24b vector (Novagen, Madison, WI) as described previously for the PCV2 capsid antigen (Puvanendiran et al., 2011). The PCV2 replicase (Rep) and capsid (Cap) antigens were then prepared as previously described (Johnson et al., 2007; Puvanendiran et al., 2011), except that HisPur cobalt agarose resin was used for purification by affinity chromatography (Thermo Fisher Scientific Inc., Waltham, MA).

Detection of antibodies to PCV2 Cap and Rep antigens was performed by indirect ELISA as previously described (Puvanendiran et al., 2011) with the plates coated with 100 ng/well of either the Cap or Rep antigen, test sera diluted 1:50 in phosphate-buffered saline with 0.05% Tween-20 (PBST) containing 5% nonfat dry milk (NFDM), pH 7.4, and horseradish peroxidase (HRP)-labeled goat anti-swine IgG (Bethyl Laboratories, Inc., Montgomery, TX) diluted 1:100,000 in PBST containing 5% NFDM, pH 7.4. Positive results were read as absorbance value >0.24, as determined by background, and positive and negative controls run on each plate.

2.3. DNA extraction, viral copy number, and viral genotype determination

Total DNA was isolated from serum using the QIAamp DNA blood mini kit (Qiagen, Inc., Valencia, CA) following the manufacturer's protocol, with the addition of 10 μg poly dA per 200 μl AL buffer, and an elution in 50 μl of EB buffer (Dvorak et al., 2013a, b). Positive and negative control samples were run with each set of DNA isolations.

PCV2 viral DNA copy number was determined using a SYBR green quantitative PCR assay as previously described (Dvorak et al., 2013a,b). The assay does not detect PCV1 DNA.

In cases of ambiguous genotype determination (4.7% of samples) a probe-based quantitative TaqMan PCR was run, with PerfeCTaqPCR FastMix II, Low ROX (Quanta Biosciences, Inc., Gaithersburg, MD) containing 250 nM of each primer (forward 5′ CAGGGCCAGAATTCAACCT3′ and reverse 5′ AGGAAAAATGGCATCTTCAAC3′), 200 nM of probe (either PCV2a probe 5′/56-FAM/RGAKATTTT/ZEN/GTTGGTCCCCC/3IABkFQ/3′ or PCV2b probe 5′/56-FAM/CTCAAACCC/ZEN/CCKCWCTGTGC CC/3IABkFQ/3′), and 5 μl of isolated DNA in a 20 μl reaction. Reactions were run in a Stratagene MX3005P machine (Agilent Technologies, Santa Clara, CA) with the following cycling conditions; 95 °C for 2 min, followed by 40 cycles of 95 °C for 15 s and 58 °C for 1 min. A standard curve for PCV2b, PCV2a, and positive and negative controls were present on every plate.

2.4. Statistical methods

Viral copy numbers, genotypes and prevalence results were described and compared to that of the NAHMS 2006 data and a correlation analysis was used to compare parity to viremia and antibody prevalence using GraphPad Prism software (Version 5.0a, GraphPad Software, Inc., La Jolla, CA).

3. Results

3.1. PCV2 viral presence before and after widespread vaccination

A total of 1097 finisher sera from 73 sites and 407 sow sera from 27 sites (approximately 15 samples each per site) were analyzed for PCV2 viral presence using qPCR. PCV2 viremia was observed in 189 finishers (17.2%) and 36 sows (8.8%) (Table 1), greatly reduced from the 82.6% of viremic finishers in 2006 (Puvanendiran et al., 2011). Of the 73 finisher sites examined, 38 sites (52.1%) were negative for PCV2 in all animals examined and 18 of the 27 sow sites (66.7%) were negative (Table 1), whereas in 2006 only one finisher site (0.5%) was negative (Puvanendiran et al., 2011). The remaining finisher (47.9%) and sow (33.3%) sites contained both PCV2-positive and PCV2-negative animals (Table 1). In 2006 the majority of sites (98.9%) contained at least one positive animal (Puvanendiran et al., 2011). No sites had all animals test positive for PCV2 in 2012, but 17.3% (32 sites) were all positive in 2006 (Puvanendiran et al., 2011).

Examination of viral copy numbers in PCV2-positive animals showed that the great majority of positive animals (96.3% of finishers, 100% of sows) had levels of viremia below 10^6 viral copies/ml (Fig. 1A and B). There was a 22-fold decrease in median viral levels in finishers as compared to 2006 (Fig. 1B) (Puvanendiran et al., 2011). The highest viral titers in 2012 were observed in finishers at 4×10^7 viral copies/ml and only 3 finishing pigs had titers above 10^7 viral copies/ml. This amount was a 50-fold decrease from maximal 2006 viral levels, when viral titers $>10^8$ viral copies/ml were observed in 1.5% of positive animals (Fig. 1B) (Puvanendiran et al., 2011). In positive sows, there was no statistical association of parity with the level of viremia (data not shown).

3.2. PCV2 genotype distribution before and after widespread vaccination

The genotypes present in viremic pigs (i.e., above the assay sensitivity of 500 viral copies/ml) were determined using qPCR. PCV2b, in 80.4% of finishers and 75.0% of sows, was the genotype most commonly observed in 2012 (Fig. 2A). This was an increase in frequency from 50.7% of PCV2b positive finishers observed in 2006.

Table 1
PCV2 viral presence in 2012 at the sample and site levels.

	Finishers				Sows			
	Per Animal Presence		Per Site Presence		Per Animal Presence		Per Site Presence	
	N	%	N	%	N	%	N	%
Positive	189	17.2	35	47.9	36	8.8	9	33.3
Negative	908	82.8	38	52.1	371	91.2	18	66.7
Total	1097	100	73	100	407	100	27	100

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