Characterization of a New Disease Syndrome Associated with Porcine Circovirus Type 2 in Previously Vaccinated Herds

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Porcine circovirus-associated disease (PCVAD) encompasses a group of wasting syndromes linked to porcine circovirus type 2 (PCV2). This paper describes a new PCV2 disease syndrome, called acute pulmonary edema (APE), which, unlike other PCVAD syndromes, has a peracute onset and is associated with herds vaccinated for PCV2.

First described in Canada in the early 1990s, porcine circovirus-associated disease (PCVAD) impacts economically the swine industry worldwide (see references 1, 23, and 25 for review). PCVAD encompasses a group of multifactorial syndromes linked to a variety of infectious and noninfectious cofactors (2, 6, 7, 10, 12, 17, 20, 21). While porcine circovirus type 2 (PCV2) is considered necessary for disease (11, 22, 25, 27), the presence of PCV2 alone is not always considered sufficient for full-blown PCVAD. The most prevalent syndrome is porcine multisystemic wasting syndrome (PMWS), which is a slow and progressive disease (3, 27). The appearance of an outbreak of severe PCVAD in North America in 2005 was linked to the emergence of the PCV2b genotype (4, 13, 14, 15).

The PCV2 genome contains three open reading frames (ORFs). The 233-amino-acid capsid protein (CP), coded for by ORF2, forms the homopolymer capsid that surrounds the single-stranded, ambisense, circular, 1.7-kb DNA genome (11, 19). Subunit vaccines that possess only recombinant ORF2 protein are highly protective against PCVAD (15).

In late 2009, pigs from PCV2-vaccinated herds in the U.S. Midwest experienced a peracute syndrome, referred to in this report as acute pulmonary edema (APE), which primarily affected healthy nursery and younger finisher pigs. Mortality approached 20% in some affected groups. Clinical signs included the rapid onset of respiratory distress followed almost immediately by death. Often, pigs were found dead with no previous indication of disease signs.

The investigation of the etiology of APE began with clinical cases submitted to the Kansas State Veterinary Diagnostic Laboratory (KSVDL) as part of a routine infectious disease investigation by a private veterinarian. The pigs in the initial APE cluster were from eight farms located within a 200-mile radius, covering portions of Kansas and Nebraska. Table 1 summarizes the farms and sources of pigs. For investigative purposes, samples were also collected from 33 “nonclinical” pigs, from affected and nonaffected farms in the same region. APE first appeared in August 2009 on farm RV, affecting pigs 14 weeks of age, followed by the appearance of clinical signs on farms PH, HP, and PP (Table 1). Clinical signs were apparent in nursery pigs as young as 7 weeks. Gross examination of APE pigs revealed the accumulation of clear fluid in the thoracic cavity and diffusely wet and heavy lungs, with moderate to severe expansion of the interlobular septae. Most of the animals had cranioventral areas of lung lobe consolidation. Some animals had dorsal patchy areas of hemorrhage in the lungs (Fig. 1A). Microscopic examination of the lungs revealed diffuse distention of the intralobular septae, which was due to the presence of edema. There was also a diffuse interstitial infiltration of macrophages and lymphocytes. A common finding was fibrinoid necrosis of the blood vessel wall (Fig. 1B), with surrounding regions showing evidence of pulmonary edema (Fig. 1C). In most of the affected pigs, there was diffuse lymphoid depletion, and a few pigs had rhinitis.

Lymphoid depletion and pulmonary edema suggested a causative role for PCV2. PCR and immunohistochemistry (IHC), performed as diagnostic tests in the KSVDL, were used to assess PCV2 infection. Table 2 shows representative results for a single herd, PH, from which there were nearly equal numbers of APE and nonclinical pigs submitted for testing. All APE pigs were positive for PCV2 DNA in sera and tissues. The differential PCR test, which contains oligonucleotide probes specific for PCV2a and PCV2b, identified PCV2b as the pre-dominant genotype. The cycle threshold (C_T) values for PCR from pooled tissue homogenates of lungs, lymph nodes, and spleens were as low as seven, indicating massive quantities of PCV2 present in these animals. In contrast, only 2 of 13 serum samples from the nonclinical pigs were positive for PCV2 in serum. For PCV2, the difference between the APE and non-clinical pigs was highly significant (the odds ratio was equal to infinity; Fisher’s exact test P < 0.0001). A similar relationship was found when data from the other APE and nonclinical pigs were included in the analysis (data not shown). The presence of large quantities of PCV2 DNA in the APE pigs was con-
firmed by positive IHC staining in lungs and lymph nodes. Within the lung, PCV2 antigen was observed within the cytoplasm of mononuclear cells located within the blood vessels. Antigen was also observed in vascular endothelial cells (Fig. 1D). The localization of viral protein to the nucleus of endothelial cells suggests that this may be an active site of virus replication.

PCV2-specific antibodies were measured by indirect fluorescent-antibody assay (IFA) (15). Since all pigs were vaccinated against PCV2, IFA could not be used to distinguish vaccine antibody from antibody generated in response to PCV2 infection. However, there were important features related to antibodies in the APE pigs. For example, five of the nine serum samples from APE pigs possessed undetectable levels of antibody (IFA titers less than 40), indicating the absence of any PCV2-specific antibody, either from maternally derived antibody (MDA) or vaccine sources.

One possible explanation for the appearance of APE in vaccinated herds is the emergence of a PCV2 capsid variant that escaped protective antibody. PCV2 ORF2 peptide sequences from four APE pigs on farm PH were obtained by DNA sequencing PCR products directly amplified from tissues (8). All four APE-associated PCV2 ORF2 peptide sequences were identical, except for a conserved V-to-L change at position 30 in one sequence (data not shown). One APE-associated PCV2 ORF2 peptide sequence, D71923, was compared to 11 representative Kansas sequences obtained from the previous 2005-2006 PCVAD outbreak in the U.S. Midwest (14). As shown in Fig. 2, the D71923 peptide sequence was similar to sequences in this group and identical to four of the historical sequences, 237-A, 237-C, 237-D, and D-28-1. Therefore, the PCV2 viruses associated with APE are characteristic of viruses that have circulated in the region for some time.

Several viruses were investigated as possible APE cofactors. Porcine reproductive and respiratory syndrome virus (PRRSV) is a common cofactor associated with the onset and severity of PCVAD (6, 10, 12, 24). Even though farm PH had a history of

| Farm | Presence of PRRSV | No. of pigs |
|------|-------------------|-------------|
|      |                   | APE | Nonclinical | Total |
| PH   | +                 | 10  | 16          | 26    |
| JP   | -                 | 7   | 7           | 14    |
| HP   | +                 | 9   | 9           | 18    |
| DG   | -                 | 5   | 5           | 10    |
| RV   | +                 | 5   | 5           | 10    |
| PP   | -                 | 3   | 3           | 6     |
| OE   | -                 | 2   | 2           | 4     |
| CF   | +                 | 3   | 3           | 6     |
| Total|                   | 27  | 33          | 60    |

FIG. 1. Anatomical and microscopic features associated with APE. (A) Thoracic cavity from a pig that died acutely. The thorax contains a large amount of serosanguineous fluid, and the lungs are markedly edematous and possess dark red areas of consolidation. (B) Hematoxylin-eosin-stained thin section from a lung showing interstitial infiltration of macrophages, lymphocytes, and plasma cells and fibrinoid necrosis of the smooth muscle in the wall of an artery (arrows). Bar = 50 μm. (C) Hematoxylin-eosin-stained lung thin section with pulmonary edema, showing a marked distention of the tissue surrounding an artery (arrows). Bar = 50 μm. (D) IHC staining for PCV2 antigen in an affected lung. Note the cytoplasmic staining of many cells (mononuclear cells), which are free within the lumen of an artery. Also note the nuclear staining in an endothelial cell lining the artery (arrow). Tissue was counterstained with hematoxylin. Bar = 25 μm.
Nonclinical pigs are characterized by their clinical signs of influenza, which include fever, coughing, and nasal discharge. These symptoms are typically associated with the influenza virus, such as AAV (Aujeszky's disease virus), and can be exacerbated by secondary bacterial infections. Pulmonary edema is a common complication in pigs with influenza, as the virus damages the endothelial cells of the blood vessels in the lungs, leading to an acute inflammatory response.

The absence of clinical signs of influenza suggests that other factors, such as the presence of other viruses, may be contributing to the development of APE. The study found that the prevalence of PCMV in APE pigs was not significantly higher than in nonclinical pigs, indicating that this virus may not be a major contributor to the development of APE.

The presence of TTV in APE pigs was also investigated, as TTV has been identified as a cofactor in the development of other diseases. The study found no significant difference in the prevalences of PCMV among APE and nonclinical pigs, suggesting that TTV may not be a significant factor in the development of APE.

PCR results for pigs with APE and nonclinical pigs from farm PH are presented in Table 2. The table shows the PCR results for PCMV, PRRSV, TTV-1, and TTV-2, as well as the PCR results for PCV2. The table also includes the Ab titer for each pig.

PRRS, diagnostic PRRSV PCR and serological tests (data not shown) showed all APE pigs to be negative for PRRSV (Table 2). In addition, two of the four APE-affected farms were negative overall for PRRSV infection (Table 1). The presence of rhinitis indicated the possibility of porcine cytomegalovirus (PCMV) (28, 32). PCR for PCMV in serum samples and tissues, performed as described by Hamel et al. (9), showed no significant difference in the prevalences of PCMV among APE and nonclinical pigs (Fisher’s exact test P = 0.458). Recently, torque teno virus (TTV) has been identified as a cofactor in PCVAD (7, 16, 18, 29). PCR for TTV-1 and -2, performed as described by Segalés et al. (26), showed nearly equal percentages of TTV-positive samples in APE and nonclinical pigs (Fisher’s exact test P values of 0.709 and 0.392 for TTV-1 and TTV-2, respectively). The absence of clinical signs of influenza and negative results in nasal swabs from APE-affected herds ruled out the possibility of swine influenza virus (SIV) (data not shown). Similar results, indicating a lack of a relationship between other common viruses and APE, were obtained when data from the other APE and nonclinical pigs were included in the analysis (data not shown).

PCVAD is historically described as a group of slow and progressive syndromes in older pigs with the eventual wasting and death of the affected animal. Pulmonary edema is typically an end-stage outcome resulting from secondary bacterial or viral infections, a consequence of PCV2-induced immune depression or dysfunction (5, 23, 31). In contrast, the onset of morbidity/mortality in APE animals is rapid, affecting healthy-appearing pigs. APE is skewed toward younger pigs and, in this study, could not be linked to other suspected infectious disease cofactors. The data from this study also point to a likely mechanism. Pathogenesis begins with large quantities of virus replicating in mononuclear cells and endothelial cells lining the blood vessels in the lungs of young pigs, which may be more susceptible to infection and support higher levels of replication. Infection is established in young pigs prior to vaccination and in the absence of protective levels of MDA. Vascular endothelial cell damage combined with cytokine release by monocytes results in the loss of blood vessel wall integrity and outflow of vascular contents into the interstitium. APE-susceptible pigs lack sufficient levels of preexisting antibody and become infected prior to vaccination. PCV2 is a ubiquitous, highly environmentally stable agent, which means that pigs at all ages are exposed to virus (24). Breeding females receive the last PCV2 vaccine dose at 6 to 8 weeks of age. Antibody levels eventually decay, and by the time of farrowing, the low levels of maternal antibody in colostrum fail to adequately protect newborn pigs from PCV2 infection. Prior to the availability of
PCV2 vaccines, newborn pigs would be protected by MDA, produced in response to natural infection of the dam at some point prior to breeding. Therefore, APE is likely an unintended consequence resulting from the widespread use of PCV2 vaccines. One strategy to avoid APE in young pigs is to vaccinate earlier. Another approach is the monitoring of antibodies in breeding animals and boosting when PCV2-specific antibody levels become low.

After the initial reports of APE in Kansas and Nebraska, it has proved difficult to assess the overall prevalence and incidence of APE in the swine industry. One strategy to avoid APE in young pigs is to vaccinate earlier. Another approach is the monitoring of antibodies in breeding animals and boosting when PCV2-specific antibody levels become low.

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