Unusual central nervous system lesions in slaughter-weight pigs with porcine circovirus type 2 systemic infection

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ABSTRACT

Porcine circovirus type 2 systemic infection was diagnosed in 2 slaughter-weight pigs based on postmortem examination. The infection was associated with unusual central nervous system lesions characterized by a multifocal lymphohistiocytic to granulomatous meningoencephalomyelitis with giant cell formation. The role of these nervous lesions in the development of the clinical signs observed in these pigs remains uncertain.

RÉSUMÉ

Lésions inhabituelles au système nerveux central chez des porcs au poids d'abattage atteints d'une infection systémique par le circovirus porcin type 2.

Une infection systémique par le circovirus porcin type 2 fut diagnostiquée chez 2 porcs au poids d'abattage suite aux examens postmortem. L'infection était associée à des lésions inhabituelles touchant le système nerveux central et caractérisées par une méningoencéphalomyélite multifocale, lymphohistiocytaire à granulomateuse avec formation de cellules géantes. Le rôle de ces lésions nerveuses dans le développement des signes cliniques observés chez ces animaux demeure incertain.

Porcine circovirus type 2 (PCV2) infection is highly prevalent in the swine population worldwide and is often subclinical. However PCV2, under certain circumstances, has been known to cause or to be associated with various disease syndromes collectively named either porcine circovirus disease (PCVD) or porcine circovirus-associated disease (PCVAD) (1,2). These disease syndromes include namely a systemic infection with wasting known as postweaning multisystemic wasting syndrome (PMWS), reproductive failure, respiratory disease, porcine dermatitis and nephropathy syndrome (PDNS) and enteritis (2). The purpose of this report is to describe an unusual pathological manifestation of PCVD in slaughter-weight pigs.

CASE DESCRIPTION

Pigs involved in this report originated from 2 breeding herds and an off-site nursery that were porcine reproductive and respiratory syndrome virus (PRRSV) and *M. hyopneumoniae* negative. Sows were not vaccinated for PCV2 but the piglets had received a 1-dose commercial vaccine at weaning. Eight weeks after weaning, 887 pigs were moved to an all-in all-out finisher barn. For the first 9 weeks after transfer, growth performances were normal and the mortality rate was at 2.0%. Clinical signs compatible with an affection of the locomotor/nervous system began on week 10 after transfer. Some pigs were suddenly unable to stand by themselves and were in lateral decubitus or were able to move but only with their forelimbs. These pigs became easily dyspneic when attempting to stand. The condition in most of these animals evolved quickly and pigs were completely paralysed or died within 48 hours. Ceftiofur and ampicillin injections were tried but were not effective. Sudden death, PDNS, redness of the skin of the ears and hindlimbs were also observed within the group. All affected pigs were in good body condition and previously healthy.

Pigs were sent to slaughter between week 12 and 17 after placement. Most of the mortality occurred on week 16 while only 346 pigs were remaining in the barn: 18 pigs died during that week, with the previously described clinical signs. By the end of the finishing period, mortality had reached 8.1%, with 4.7% of the mortality caused by sudden death, locomotor/nervous problem, or PDNS between week 12 and 17. The remaining 1.4% were euthanized for unspecified reasons.

Necropsy was performed on 3 pigs of approximately 6 month of age and that presented the above mentioned locomotor/nervous signs. Two of these pigs (pig #1 and #2) had similar pathological findings reported herein while the third animal had a focal degenerative leucomyelopathy of undetermined etiology (focal ischemic myelopathy?) and subacute degenerative myocardial and vascular lesions suggestive of vitamin E/selenium deficiency.

At necropsy, pig #1 had lesions of interstitial pneumonia and the tracheobronchial and superficial inguinal lymph nodes were hypertrophied. There was no evidence of bone fracture, vertebral abcess, arthritis or osteochondrosis. The animal was in good body condition. Microscopic examination revealed a lymphohistiocytic interstitial pneumonia with multifocal alveolar, peribronchial and perivascular granulomatous inflammation showing several multinucleated giant cells. In one lung section there was a necrohemorrhagic area of about 1 cm in diameter associated with lesions of necrotizing vasculitis. There was also a lymphohistiocytic to granulomatous hepatitis and interstitial nephritis. In lymphoid tissues including lymph nodes, tonsils, spleen and Peyer’s patches, there was a mild to moderate lymphoid depletion and tissue replacement by histiocytes and multinucleated giant cells as well as multiple small and irregular fibrinonecrotic foci with scattered neutrophils. These latter necrotic lesions were often more prominent in and around the histiocytic cellular infiltrates. In the sections of skeletal muscle examined (from the

hindlimbs) there was a mild to moderate polyphasic myonecrosis. Unusual lesions were found in the central nervous system (CNS). In the brain and throughout the spinal cord there were multiple perivascular and parenchymal foci of lymphohistiocytic to granulomatous inflammation with multinucleated giant cell formation. These lesions were found in the meninges as well as in the gray and white matter of the CNS. Few apoptotic cells and polymorphonuclear cells were found within some of these foci (Figures 1 and 2).

Gross lesions found in pig #2 included a lobular bronchopneumonia, a mild splenomegaly and focal ulceration of the nonglandular part of the gastric mucosa. Both kidneys had multiple whitish foci of variable diameter. There was no evidence of wasting. Microscopic lesions observed in this pig were qualitatively similar to those found in pig #1. Compared to pig #1 the lymphoid and renal lesions were more severe while the hepatic and CNS lesions were of lesser severity. In the lymphoid tissues, particularly within the spleen and lymph nodes, larger areas of necrosis were observed and were occasionally associated with thromboses of small caliber blood vessels. Few histiocytic cells contained intracytoplasmic basophilic inclusions typical of PCV2.

Pool of lung and lymphoid tissues were found positive in both pigs for PCV2 and negative for PRRSV using a fluorescent antibody test. Frozen and formalin-fixed paraffin-embedded sections of central nervous tissues were found negative for PCV2 with the immunofluorescence and immunoperoxidase techniques using a specific anti-PCV2 porcine serum (3).

In addition, several PCR/RT-PCR assays were performed with the spinal cord and tissue homogenates of lung and lymph nodes of pig #2 for the detection of swine pathogens like porcine reproductive and respiratory syndrome virus (PRRSV), PCV2, coronaviruses, swine influenza virus (SIV), porcine parvovirus (PPV), swine torque teno virus (swTTV), swine hepatitis E virus (swHEV) and encephalomyocarditis virus (EMCV) as previously described (4-8). Only the PCV2

multiplex real-time quantitative PCR (mrtqPCR) assay (5) gave positive results with the spinal cord and tissue homogenates of lung and lymph nodes. Interestingly, the mrtqPCR indicates that an amount of 6.60 X 10^7 and 2.07 X 10^{12} copies of PCV2 viral genome/gram of spinal cord and tissue homogenates of lung and lymph nodes, respectively, (which correspond to an amount of 3.75 X 10^1 and 1.18 X 10^6 $TCID_{50}$ infectious virus/gram of spinal cord and tissue homogenates of lung and lymph nodes, respectively, when using the conversion formula reported by Gagnon et al.(5) were found in pig #2. Furthermore, an amount of 4.88 X 10^9 copies of PCV2 viral genome/gram of spinal cord, which correspond to an amount of 2.77 X 10^3 $TCID_{50}$ infectious virus/gram of spinal cord, was found in pig #1. In addition, the mrtqPCR results indicate that the virus genotype found in the tissues of pigs #1 and #2 was the PCV2b genotype, which is the genotype usually considered to be the most virulent compared to PCV2a (9). Furthermore, the PCV2b viral genome obtained from the tissue samples of pigs #2 was sequenced as previously described (4). Overall, the PCV2b viral sequence was 99.2 to 99.8% similar to other PCV2b references strains such as the Quebec reference strain FMV05-6302, Genbank accession number DQ220739, and only 96,1% similar to PCV2a stoon-1010 reference strains (data not shown). The PCV2b viral genome length was found to possess 1767 nucleotides (nt) and 3 nt substitutions were found compared to FMV05-6302 (position 39: T to C, 804: C to T and 1132: C to T).

DISCUSSION

The 2 finishing pigs involved in this report had systemic lesions typical of PMWS but without wasting. The precise cause of the locomotor/nervous signs presented by these pigs remains uncertain but could potentially be attributed to the lymphohistiocytic to granulomatous meningoencephalomyelitis or to the skeletal muscle necrosis observed. Although these muscular

lesions could be secondary to the decubitus they could also be associated with a deficiency of vitamin E and selenium. Although normal levels of vitamin E and selenium were labelled as such in the feed, a deficiency (absolute or relative) still could not be excluded in light of the findings of the third pig necropsied that had subacute degenerative cardiovascular lesions compatible with vitamin E/selenium deficiency. Pigs necropsied had no evidence of bone fracture, vertebral abscess, arthritis or osteochondrosis, selenium toxicosis, fibrocartilaginous emboli or lesions compatible with other endemic viral infections such as teschovirus (enterovirus) infection.

Clinical signs involving the central nervous system have rarely been described in weaned pigs with PCV2 associated diseases. In one report, PCV2 associated cerebellar vasculitis was described in several PMWS affected pigs (10). Acute hemorrhages and edema of cerebellar meninges and parenchyma due to a necrotizing vasculitis resulted in degeneration and necrosis of the gray and white matter. Affected pigs, aged between 6 weeks and 2 months, showed wasting, coughing, opisthotonus, nystagmus and convulsions (10). CNS lesions in pigs with classical PMWS are infrequently observed and have been described as a mild perivascular mononuclear leptomeningitis or meningoencephalitis (11-12) occasionally accompanied by degenerative vascular lesions (10,13). The severity and extent of the CNS lesions found in the 2 pigs of this report are unusual and the presence of multinucleated giant cells within the lesions have not, to our knowledge, been reported within the nervous tissue of PCV2 infected pigs. Although PCV2 was not detected by immunohistochemistry within the CNS, large amounts of PCV2 DNA were detected in the spinal cord by mrtqPCR. In addition, the histologic appearance of the lesions in CNS and in non-nervous tissues was similar (granulomatous). Therefore, these results strongly suggest a causal relationship between PCV2 and the lesions in the CNS. The PCV isolate from

this case was of genotype 2b, the most prevalent type found in our area at this time (14). Results from sequencing did not suggest that it represents a new type of strains that could be potentially more neurotropic.

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FIGURE LEGENDS

Figure 1. Photomicrograph of the thoracic spinal cord of pig # 1 showing 2 distinct foci of granulomatous inflammation within the gray matter. HEPS stain. Bar = 70 um.

Figure 2. Closer view of a portion of Figure 1 showing a focal granulomatous myelitis with multinucleated giant cell formation. HEPS stain. Bar = 50 um.