HISTOPATHOLOGICAL DIAGNOSIS OF POSTWEANING MULTISYSTEMIC WASTING SYNDROME

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Summary

Postweaning multisystemic wasting syndrome (PMWS) is a disease of pigs first recognised in North America in 1991 and subsequently reported worldwide that is caused by Porcine circovirus 2 (PCV2), a member of the family Circoviridae. The most consistent feature of PMWS is a generalized depletion of lymphocytes. Secondary infections with opportunistic organisms are common. There is evidence that the destruction of thymic lymphocytes has a central role in the pathogenesis of PMWS. The objective of this paper was to describe the microscopic and ultrastructural pathology associated with natural cases of PCV2, as well as PMWS, a key point in the diagnosis of PCV2 infection and associated diseases.

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Introduction

Since the first description of postweaning multisystemic wasting syndrome (PMWS) in Saskatchewan (Canada) in 1991 (Harding, 1997), this syndrome has now been described worldwide but Oceania (Allan and Ellis, 2000). The acronym PMWS was initially used to describe an apparently new disease which was clinically characterised by wasting, paleness of the skin and occasionally icterus in weaned pigs; affected pigs also had characteristic lesions in multiple tissues (multisystemic), mainly in lymphoid organs (Harding et al., 1998). In 1997, the presence of porcine circovirus (PCV) antigen was demonstrated in lesions of animals affected by PMWS (Clark, 1997). The final diagnosis of PMWS is established based on three criteria (Sorden, 2000; Quintana et al., 2001): (1) presence of compatible clinical signs (basically, wasting), (2) presence of characteristic histopathological lesions in lymphoid tissues, and (3) presence of PCV2 (antigen and/or nucleic acid) within the microscopic lesions. However, in a PMWS-affected farm, it is possible to find healthy pigs showing mild to moderate microscopic lesions typical of PMWS in lymphoid tissues, associated with low to moderate amounts of PCV2 nucleic acid (Quintana et al., 2001).

Material and methods

In the present study fifty-seven 8-week-old pigs were included. All these animals showed the PMWS compatible clinical signs. The animals had been euthanised and the sections from lungs, lymph nodes, thymus, liver, kidney, small intestine were collected in the form of slices of about 5 mm thick. The collected pieces were fixed for 48 hours in a Stieve mixture, included in paraffin and cut at 6 µm thick slices. The sections were colored employing the Goldner’s Trichrome method.
Results and discussion

Microscopical lesions attributable to PMWS were found in lymphoid organs (including lymph nodes, tonsil, Peyer’s patches and spleen), liver, kidney and varying degrees of lymphocellular depletion, affecting both lymphoid follicles and parafollicular zones, and progressive multifocal to diffuse infiltration of lymphoid tissue by large histiocytic cells (Fig. 2,4,6) were the characteristic lesions. Syncytial cells were seen frequently, especially in the lymph nodes, Peyer’s patches, and lamina propria of the intestinal villi (Fig. 1,6). Syncytial cells were occasionally present within lymph vessels of the villi. A prominent finding was the presence of sharply demarcated, spherical, basophilic, cytoplasmic inclusions in histiocytic cells (Fig.3). Inclusions were either large and single or smaller and multiple. Varying degrees and types of lymphoid tissue lesions were seen in the same animal. In lymph nodes, depletion was observed within lymphoid follicles or in the paracortical zones. In the follicles, large cells with abundant eosinophilic cytoplasm, probably follicular dendritic cells, were prominent (Fig.2). Occasionally, syncytial cells occupied the centre of the follicle. Lesions in the T-celldependent (paracortical) zone of the lymph node also included depletion of small lymphocytes. In these areas, the fibrovascular stroma was more evident. Large mononuclear cells and occasional mitotic figures were scattered between stromal components. Large histiocytic cells infiltrated the cortical sinuses of the lymph node. This infiltrate varied greatly in intensity, starting as small cell aggregates beneath the trabecular sinuses and extending through wide areas of the parafollicular zone in severe cases. Syncytial cells were mainly observed in the cortical sinuses, but also appeared in the paracortical zones. Lymphocellular depletion of the medullary cords and empty medullary sinuses were also observed, to varying degrees.

Changes in Peyer’s patches and tonsil consisted of depletion of lymphoid cells in the interfollicular area, depopulated follicles with stromal cells clearly evident and, sporadically, syncytial cells within the follicles (Fig. 4). Infiltration of the interfollicular area with histiocytic cells was also observed. Syncytia were seen at the periphery of the lymphoid tissue and in the stroma of the intestinal villi (Fig.5). Cytoplasmic inclusions in histiocytic cells were also observed in Peyer’s patches and tonsil (Fig.4,6).
DNA in the cytoplasm of tonsil macrophages. *In situ* hybridization x100.

The most common liver lesion was lymphohistiocytic infiltration of portal zones, to variable degrees of intensity (Fig. 8). Groups of mononuclear inflammatory PCV2 positive cells were also observed in liver sinusoids (Fig. 9).

Multifocal necrosis of single hepatocytes was observed in four cases. In one pig that died with icterus, periportal fibrosis, loss of most hepatocytes, and
mononuclear infiltrates were observed throughout the liver acini.

Lung lesions of PMWS-affected pigs were of variable severity. Mild multifocal interstitial pneumonia, with some macrophage-like cells in the alveoli, was observed in some lung sections (Fig. 10, 11).

Other lung changes were characterized by severe, diffuse interstitial pneumonia, with marked thickening of interalveolar walls and inflammatory cells (mononuclear cells and degenerative polymorphonuclear neutrophils) in the alveoli (Fig. 11).

In some lung sections, peribronchial and perivascular lymphohistiocytic infiltrates were present. Occasionally, syncytial cells were observed within the inflammatory infiltrates (Fig. 11).

In the kidney, a mild to intense multifocal interstitial nephritis was present. Lymphohistiocytic infiltrates were seen mainly within the renal cortex. In two pigs, acute exudative glomerulitis, with fibrin casts in the Bowman’s space and proximal tubular lumina, and severe interstitial nephritis were found (Fig. 13). Systemic necrotizing vasculitis was present in twenty pigs.
PCV nucleic acid was detected in all studied tissues of pigs (Fig.1,5,7,9,12,14). Labelling was found mostly in the cytoplasm of infected cells, and to a lesser extent in the nucleus. The amount of PCV nucleic acid in tissues from the same pig was highly variable.

This study describes the pathological findings in 55 natural cases of PMWS, together with the tissue distribution of PCV nucleic acid. Pigs were classified as cases of PMWS on the basis of macroscopical and microscopical lesions previously documented, and the detection of PCV antigen or nucleic acid (Daft et al., 1996; Harding and Clark, 1997; Segale´s et al., 1997).

Figure 12. Interstitial pneumonia. Positive hybridization signals for PCV2 DNA in macrophage and bronchiolar associated lymphoid follicles (BAL). In situ hybridization x100.

Figure 13. Nephropathy; tubular necrosis with hemorrhagic exsudate in Bowman space; hyaline cast in the renal tubules; polimorphe inflammatory cells infiltrate. TMGx200.

The study confirmed that lymphoid tissues are the main target of PCV in pigs with PMWS. Histiocytic infiltrates, formation of syncytia, and presence of cytoplasmic inclusions in histiocytic cells were the most prominent microscopical lesions.

The study also demonstrated that PCV was present in animals with PMWS and was regularly associated with lymphoid lesions, as observed by others (Harding and Clark, 1997).

Figure 14. Presence of PCV2 DNA in the interstitial macrophages, tubule epithelial and multinucleate giant cells. In situ hybridization x200.

The use of DNA in-situ hybridization helped to establish the cell tropism of PCV in cases of PMWS. PCV is unique in its tropism for a wide range of cells including monocyte-macrophage and antigen-presenting cell (APC) lineages.

Variation in intensity and distribution of lesions in lymphoid as well as other target organs in cases of PMWS probably depends on the stage of the disease in the affected pig. Most of our pigs were selected when showing initial clinical signs of disease and were killed for the pathological study.

These cases probably represented acute or subacute forms of the disease. The present study showed a close relationship between presence of lesions, PCV2 nucleic acid in PMWS.
References