Lesions Associated with Postweaning Multisystemic Wasting Syndrome in Pigs from Prince Edward Island, Canada

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Postweaning multisystemic wasting syndrome (PMWS) is a recently recognized swine disease originally reported in western Canada.[2,9] Retrospective postmortem studies revealed that this condition first appeared in 1991, but it was not recognized as a specific disease until 1996.[9] A similar syndrome has been described recently in pigs from the United States[4,11] and Europe.[1] The etiology of this syndrome is still under investigation, but porcine circovirus (PCV), a member of the family Circoviridae that includes chicken anemia virus, psittacine beak and feather disease virus, and a newly described pigeon circovirus,[15] is thought to play an important role in the pathogenesis of this condition. The purpose of this paper is to describe and illustrate the most common microscopic findings associated with PMWS in pigs and to report the presence of PMWS in swine herds from Atlantic Canada.

Six pigs, 5–12 weeks of age, from four swine herds located in Prince Edward Island were submitted to the Atlantic Veterinary College for postmortem examination. Five pigs were alive and one (pig no. 3) had been found dead in its pen. Pig nos. 2 and 3 and pig nos. 5 and 6 were herdmates. All pigs had a history of weight loss, dyspnea, and/or scouring, first noticed 1 or 2 weeks after weaning. Blood samples were obtained from live pigs prior to euthanasia. Complete postmortem examination was performed, and tissues were selected for histopathology, bacteriologic culture, and virologic analysis. Tissues for histopathology were fixed in 10% neutral buffered formalin, embedded in paraffin, cut at 5 μm, and stained with hematoxylin and eosin. In two cases, formalin-fixed tissue was fixed in 2% glutaraldehyde and postfixed in 1% osmium tetroxide, dehydrated in a graded series of ethanol, and infiltrated and embedded in epon/araldite for sectioning and examination by transmission electron microscopy. Thin sections were stained with a saturated solution of uranyl acetate and Sato lead stain. Tissues from five pigs (nos. 1, 2, 3, 5, and 6) were sent to the Veterinary Services Branch of Manitoba Agriculture for the detection of pathogenic PCV by polymerase chain reaction (PCR).[8] Porcine reproductive and respiratory syndrome (PRRS) serology was done on serum of two pigs by an indirect fluorescent antibody test (IFAT) that was developed in house. Gradual dilutions of the test serum were made and reacted with PRRS virus (PRRSV)-infected MARC cells, washed, mounted, and evaluated by fluorescent microscopy. Lungs from three pigs and lymph nodes from two others were tested for the presence of PRRSV antigen by fluorescent antibody test (FAT). The PRRS isolate used for this test originated from the National Veterinary Services Laboratory in Ames, Iowa. The antibody used (SDOW 17) was a monoclonal antibody obtained from Dr. Eric Nelson of the Veterinary Science Department, South Dakota University. Cryostat sections of lung were made, fixed in acetone, reacted with fluorescein-labeled monoclonal antibody against PRRSV, washed, mounted, and evaluated by fluorescent microscopy.

All pigs were in thin body condition with moderate pallor of the skin and variable degrees of muscle wasting. The main postmortem findings and serological and virological results are summarized in Table 1. One of the pigs (no. 4) had yellow discoloration of the skin, mucous membranes and depleted fat stores (icterus). In five of the pigs (nos. 1, 2, 3, 5, and 6) the lungs were moderately enlarged and heavy and failed to collapse. Lungs had patchy to diffuse grey-tan to red areas of consolidation that commonly involved at least 50% of the pulmonary parenchyma. Moderate amounts of froth and mucopurulent exudate were often seen within the trachea and bronchi. Mild to moderate enlargement of peripheral and visceral lymph nodes was present in all pigs. Mediastinal and tracheobronchial lymph nodes were particularly affected in the four animals with pneumonia. Pig no. 6 had marked peripheral and visceral lymphadenopathy, and its tracheobronchial lymph nodes were approximately four times normal size. Cut surfaces of affected lymph nodes were moist, white-gray, and homogeneous. Pig no. 4 also had, in addition to icterus, a large area of ulceration with raised, well-demarcated borders in the esophageal portion of the stomach. Semifluid dark tarry ingesta was present within the gastrointestinal tract. The liver in this pig was diffusely pale and slightly yellow and orange. Semifluid intestinal contents were present in the majority of the pigs.

The pulmonary lesions were characterized microscopically by moderate thickening of the alveolar septa due to inflammatory cell infiltration by mononuclear cells (primarily macrophages and lymphocytes) and occasional type II pneumocyte hyperplasia. Proteinaceous and karyorrhectic debris was sometimes present within alveolar spaces (Fig. 1).

Most lymphoid tissues, including visceral and peripheral lymph nodes, spleen, and Peyer's patches of the ileum and tonsils, had variable degrees of lymphoid depletion, scattered lymphocytolysis, and histiocytosis within germinal centres (Fig. 2). T-cell–dependent areas of lymphoid follicles were moderately to markedly expanded, primarily by the presence of histiocytic cells. Large, multiple, basophilic, or amphophilic ''botryoid'' intracytoplasmic inclusions were often seen within the cytoplasm of histiocytic cells within cell-depleted germinal centers. Microscopic lesions and intracytoplasmic inclusions were present throughout the lymphoid

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Table 1. Main postmortem findings and serological and virological results in six affected pigs.*

		PRRS virus			Porcine circovirus			
		IFAT	FAT		PCR		TEM	
Pig no.	Main postmortem findings	Serum	Tissue	Results	Tissue	Results	Tissue	Results
1	Bronchointerstitial pneumonia	ND	lung	positive	lung intestine	positive positive	intestine	positive
2	Bronchointerstitial pneumonia	negative	lung	negative	lung	positive	lymph node	positive
3	Bronchointerstitial pneumonia, enterocolitis	ND	lung	negative	lung	positive	ND	ND
4	Icterus, hepatitis, enterocolitis, gastric ulcer	ND	ND	ND	ND	ND	ND	ND
5	Bronchointerstitial pneumonia, enteritis	positive	lymph node	negative	lymph node	positive	ND	ND
6	Bronchointerstitial pneumonia, generalized lymphoadenomegaly, enterocolitis	positive	lymph node	negative	lymph node	positive	ND	ND

* PRRS = porcine reproductive and respiratory syndrome; IFAT = indirect fluorescent antibody test; FAT = fluorescent antibody test; PCR = polymerase chain reaction; TEM = transmission electron microscopy; ND = not determined.

tissues of the body but were consistently found within Peyer's patches of the ileum (Figs. 2, 3) and associated mesenteric lymph nodes.

Ultrastructural examination of affected macrophages revealed variably sized, membrane-bounded, electron-dense cytoplasmic inclusions containing paracrystalline arrays of nonenveloped virions scattered throughout the cytoplasm (Fig. 3). Viral particles, 14–17 nm in size, were interpreted as morphologically compatible with circovirus. Pig no. 6 had scattered small foci of necrosis and prominent clusters of multinucleated syncytial cells within B-cell–dependent areas of the spleen, lymph nodes, and mucosa-associated lymphoid tissue (Fig. 4). Gram's, acid-fast, and Gomori's methenamine silver stains (GMS) nitrate failed to reveal bacterial or fungal agents in the tissues of this pig.

Hepatic lesions were detected only in pig no. 4 and were characterized by moderate inflammatory cell infiltration of portal areas, moderate hepatocellular vacuolation and swelling, sinusoidal collapse, and scattered widespread single cell necrosis (Fig. 5). The inflammatory cell infiltrate consisted primarily of lymphocytes, plasma cells, and macrophages with occasional neutrophils and eosinophils. Moderate hepatocellular anisokaryosis and karyomegaly were also seen. Some lobules had moderate to prominent hepatocellular loss with preservation of the sinusoids and connective tissue meshwork.

Porcine circovirus nucleic acid was detected by PCR in the

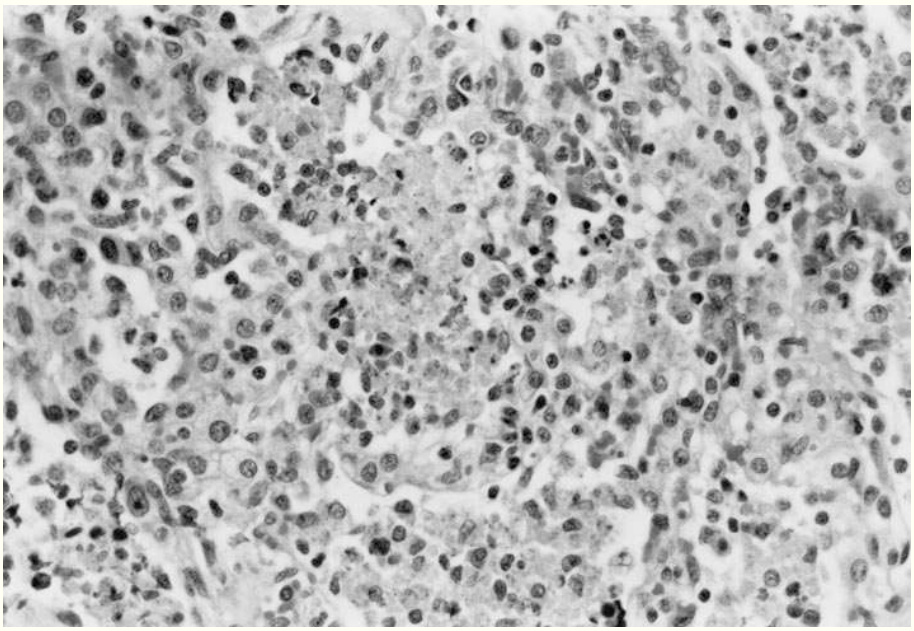

Figure 1. Bronchointerstitial pneumonia characterized by diffuse thickening of alveolar septa and occasional karyorrhectic debris within alveoli. Lung, pig no. 1. HE.

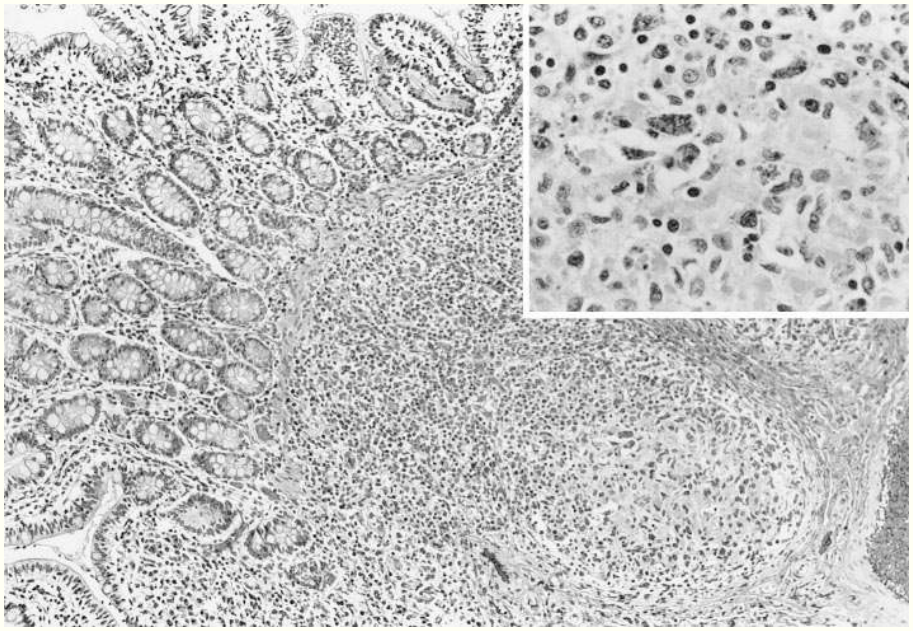

Figure 2. Moderate B-cell depletion within a Peyer's patch. Ileum, pig no. 5. HE. **Inset**, higher magnification of the cell-depleted Peyer's patch reveals numerous intracytoplasmic inclusions within phagocytic cells. HE.

lungs of pig nos. 1, 2, and 3, in the Peyer's patches of the small intestine of pig no. 1, and in the enlarged internal lymph nodes of pig nos. 5 and 6. Viral antigens of PRRSV were detected by FAT in the lung of pig no. 1. Pig nos. 5 and 6 were positive for PRRSV antibodies, with IFAT titers of 1:256 and 1:128, respectively. In these two pigs, viral antigens of PRRSV were not detected within enlarged lymph nodes.

Porcine circovirus was first detected as a noncytopathic contaminant of a continuous porcine kidney (PK/15) cell line.[14] Even though seroepidemiological studies have demonstrated that PCV is highly prevalent among swine populations worldwide,[5,10] only recently has PCV been linked with a specific pathological condition. Interestingly, in spite of the consistent association of PCV with lesions in pigs with clinical signs and postmortem changes compatible with those of PMWS, Koch's postulates, until recently, were unfulfilled. Ellis et al.[7] demonstrated that lesions of PMWS can be experimentally reproduced in gnotobiotic piglets with filterable

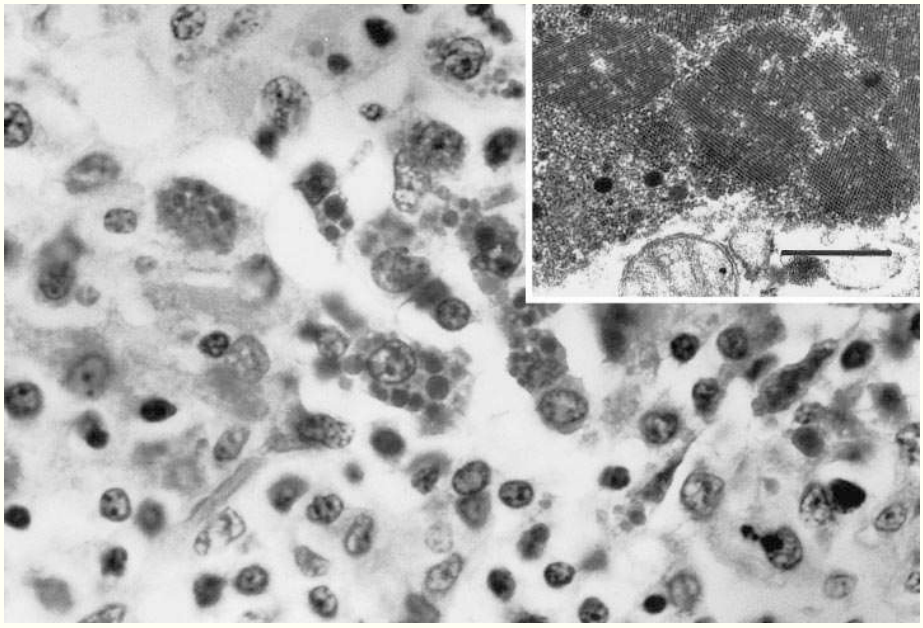

Figure 3. Multiple amphophilic intracytoplasmic inclusions within histiocytic cells of a germinal center. Lymph node, pig no. 2. HE. **Inset**, transmission electron micrograph of a cytoplasmic inclusion containing paracristalline arrays of virus particles. Lymph node, pig no. 2. Bar = 400 nm.

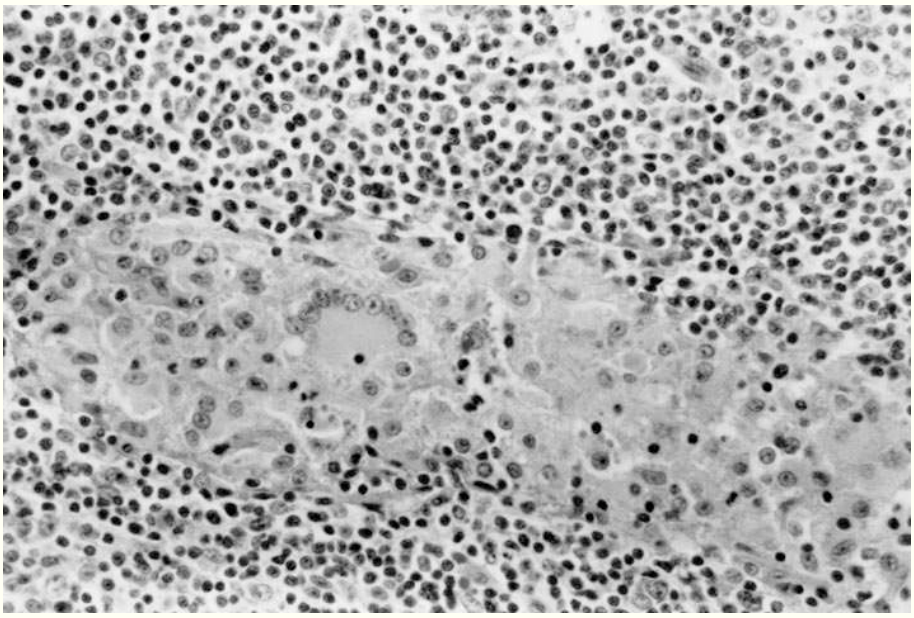

Figure 4. Cluster of histiocytes and multinucleated syncytial cells within the lamina propria. Small intestine, pig no. 6. HE.

material from field cases of PMWS. Interestingly, PCV and porcine parvovirus (PPV) were detected in the tissues of the experimental piglets that developed the lesions characteristic of PMWS. The presence of PPV in the inoculated pigs was unexpected, and studies are currently under way in order to dilucidate the role of coinfection with these two viruses in field cases of PMWS.

Clinical signs and gross and microscopic lesions in the field cases reported here are compatible with those described in PMWS.[2,4,11] In agreement with previously published reports, the majority of the pigs in this study had patchy to diffuse interstitial or bronchointerstitial pneumonia. Two of the pigs (nos. 5 and 6) had antibodies to PRRSV, and in pig no. 1, PRRSV antigens were detected by FAT within the consolidated pulmonary parenchyma. The common occurrence of PRRSV infection in pigs with PMWS has been previously reported.[3,6] Furthermore, epidemiological data suggest that PMWS acquires more importance in PRRSV-positive herds because the increased incidence of secondary bacterial and other opportunistic infections within these herds increases the morbidity and mortality associated with PMWS.[3] Concomitant *Pneumocystis carinii* infection is not uncommon in piglets with PRRSV-in-

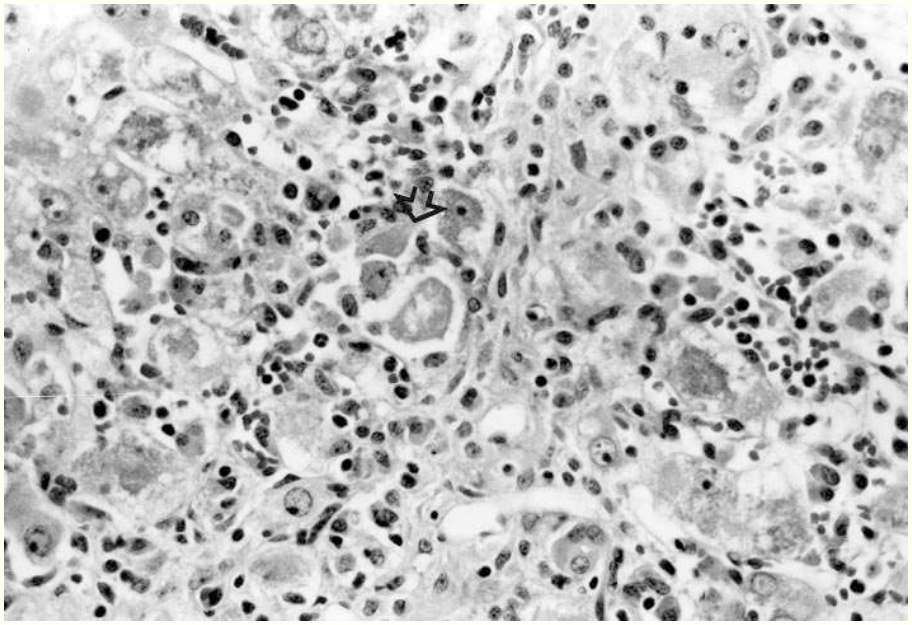

Figure 5. Hepatocellular loss and mononuclear inflammatory cell infiltration of a periportal space. Note degeneration and necrosis of hepatocytes (arrow). Pig no. 4. HE.

duced interstitial pneumonia, and *P. carinii* organisms have been found within the alveoli of approximately 5% of the pigs with PMWS.[2] In the present study, no *P. carinii* organisms were detected in hematoxylin and eosin– or GMS-stained sections of lung. No attempt to determine the presence of PPV was done in the cases presented here.

It is interesting to note that some of the microscopic lesions described in PMWS overlap those of PRRS, which makes the differential diagnosis of these two conditions particularly challenging. For example, necrosis, hypertrophy, and hyperplasia of the monocytic cell population of lymph nodes, some of the hallmarks of PMWS, are also present in PRRS.[13] In addition, widespread lymphoid depletion within follicular centers in the spleen, thymus, tonsils, and mesenteric lymph nodes, is also reported in PRRS.[12] In the study reported here, lesions in pig no. 6 were considered unusual because, in addition to histiocytic proliferation, clusters of large multinucleated cells were a prominent feature within enlarged lymph nodes, spleen, and mucosa-associated lymphoid tissue including tonsils and Peyer's patches. Similar cells, referred to as polykaryocytes, have been described within lymph nodes of pigs experimentally infected with PRRSV.[13] Multinucleated cells have also been reported in pigs with PMWS,[2] so the polykaryocytes present in pig no. 6 may have been a manifestation of PMWS rather than PRRS. Even though this pig was serologically positive for PRRS, no PRRSV antigens were detected in affected lymph nodes by FAT. Furthermore, PCV nucleic acid was detected by PCR within enlarged lymph nodes, and special stains (GMS, periodic acid–Schiff, acid fast) failed to reveal bacteria or fungi within the lesions.

Detailed studies are under way in several countries with the hope of clarifying the pathogenic potential of PCV in pigs affected with this recently described wasting syndrome. It is generally accepted that PCV is involved in the pathogenesis of PMWS; the magnitude of this involvement and the cellular mechanisms responsible for lesion development remain to be elucidated.

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