Porcine Circovirus: A Review

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Introduction

Post weaning multisystemic wasting syndrome (PMWS) has been described as a "new and unique disease of swine."[1,2,3] PMWS is characterized as a disease of 5 to 12 week old pigs.[4] The disease causes wasting and dyspnea with affected pigs also presenting with lymph node enlargement and icterus.[1,2,4]

The disease first appeared in 1991, in Saskatchewan, Canada. In 1994, the disease reoccurred in Saskatchewan and Alberta. Since then, PMWS has been diagnosed in other provinces of Canada, and the states of Iowa, Illinois, Indiana and California. France and Spain have also reported a similar disease presentation.[4]

The etiological agent associated with PMWS is porcine circovirus (PCV) from the family Circoviridae. The virus was first described as a contaminant of the pig kidney cell line (PK-15) by Ilise Tischer in 1973.[5,6,7] Other animal viruses in the same family that have been described include: psittacine beak and feather disease virus, chicken anemia virus, and pigeon circovirus. At the present time, no relationship between these viruses and porcine circovirus have been identified through DNA sequence homologies or common antigenic determinants.[6]

PMWS is a new and possible economically important disease of swine that may be mistaken for other diseases such as porcine reproductive and respiratory syndrome (PRRS) or post weaning anorexia and starvation.[8] This paper will review the epidemiology and pathogenesis of porcine circovirus, describe the pathology associated with clinical disease, and discuss diagnostic and prevention/management procedures.

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Description

PCV is a small isometric virus 17nm in diameter with monopartite circular ssDNA genome.[6,9] The genomic size is 1.76kb and the virus has a buoyant density of 1.37 gm/ml in CsCl.[6,9] From the completed sequence, PCV exhibits positive sense and a circular genome with an absence of intergenic regions and six overlapping open reading frames.[6,9] The virus shows no hemagglutinating activity, has been shown to be resistant to a pH of 3, and remains active after exposure to chloroform.[10] PCV has the ability to infect various types of cell cultures with the exception of avian cell lines. However, complete replication occurs only in porcine-derived and vero (fibroblastic cell line derived from African Green Monkey) cell cultures.[10] The physiochemical properties of PCV have been shown to be more closely related to plant circoviruses and geminiviruses than animal circoviruses.[9, 11] This relationship may help in understanding PCV's replication processes.

Epidemiology

PCV is proposed to be the cause of PMWS because of the epidemic spread through naive herds. Lesions seen in pigs with PMWS have been shown to be associated with PCV.[3] PCV typically occurs in high health status pigs 5 to 12 weeks of age. The syndrome is characterized by low morbidity (8-10% in post-weaning pigs[1,3,4]) and high care fatality rate. Post-weaning mortality where PMWS has been accused has been found to be greater than 50%.[1,4] This high rate in some situations suggests that there are perhaps other factors of the production system that may influence the severity of the disease such as continuous flow, crowding, commingling pigs, and mixing pigs of different ages.

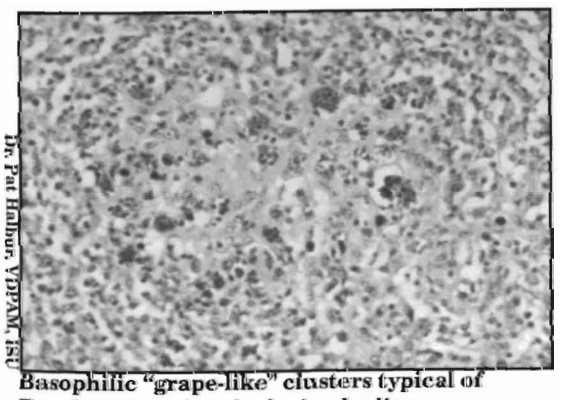
PMWS is a chronic insidious and occasionally protracted disease in most infected herds. The syndrome appears to have a long

incubation period, evident by the transfer to downstream nurseries via segregated early weaned pigs at 10-14 days of age. The disease tends to be spread out amongst the population of pigs in a pen or barn and does not appear to infect pigs in pockets or groups.[1]

Clinical signs of PMWS include weight loss, tachypnea, dyspnea, jaundice, and enlarged lymph nodes. Other associated clinical signs are diarrhea, a productive cough and central nervous system signs.[1] Congenital tremors have been described in piglets.[7]

Currently 25-30 herds in Saskatchewan and Alberta, as well as several herds in Iowa, have been diagnosed with PMWS.[1] Based on antibody detection surveys, the prevalence of PCV in Europe is 95%.[12] Antibodies to PCV have been identified in Germany, England, Ireland, and Canada, but no associated disease syndrome was described before 1996.[4] In 1994, PCV was implicated as the cause of congenital tremors in piglets.[7] It has been found that an average of 53% of United States swine herd have been exposed to PCV.[13]

In studies reported by Tischer in 1986, 60% of slaughter pigs in the United States had PCV antibody titers equal to or greater than pigs experimentally infected with PCV.[6] From this information, the conclusion was made that infection appears to occur in the grower/finisher stage. In another study, piglet litters were traced from birth to slaughter. Data revealed PCV maternal antibody disappears 8-9 weeks after birth. Within the study group, PCV antibodies reappeared 13-15 weeks after birth.[6] Likewise, this study also suggested PCV infection occurred at the grower/finisher phase. At the present time, only one serotype of PCV has been identified.[6]



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Basophilic "grape-like" clusters typical of Porcine circovirus inclusion bodies.

Pathogenesis

The pathogenesis of PCV is not well understood. Experimental studies have discovered some information on the processes associated with PCV, but much of the work is still ongoing. In this section, experimental research and field studies are presented to help compare findings and stimulate ideas on how PCV moves through a swine herd.

In an early study reported by Tischer, six PCV seronegative nine-months-old minipigs were infected intranasally with PCV.[6,14] Intranasal inoculations were done because this appears to be the most logical route of infection. One unexposed minipig was placed within the infected group of six to detect shedding of the virus. Nasal swabs and fecal samples were collected and cultured. PCV was isolated from nasal swabs on days 3 through 6 from four of the infected animals and the control pig placed within the group. Three pigs were shown to shed virus through their feces on days 13 and 14 post-infection.[6,14] No clinical signs were evident in any of the pigs and no post-mortem lesions were observed. Isolation of the virus was unsuccessful from multiple tissue samples.[6,14] Antibodies were detected by IFA one week post-infection. Titers increased for 2 to 5 weeks then declined. Antibodies were detected out to 39-47 weeks. Since it has been found that the multiplication of PCV in cell cultures depends on the cellular DNA synthesizing enzymes, fast replicating tissues like nasal and intestinal mucosa are needed for virus replication in the animal.[14] Whether PCV can cause disease in pigs is still questionable.

A field study done on a 500 sow total confinement farrow-to-finish farm found the infectivity level of the virus to be low. Pockets of infection were found within the crated gestation barn with positive and negative animals side by side. The study concluded that distribution of infection would follow airflow when the virus is spread by aerosol route. Since negative and positive sows were found adjacent to each other, infectivity in

healthy sows is low.[5] The study also found that seronegative sows became positive in the farrowing barn. No explanation was given but the stress of farrowing may have allowed sows to be more susceptible to the greater concentration of virus that may have replicated in the placenta or fetus.[15] Overall, no production differences were found in PCV positive versus PCV negative sows.

This same study incriminated PCV as a cause of congenital tremors. One seronegative sow seroconverted in the farrowing room and also had pigs demonstrating signs of congenital tremors. One piglet from the litter had PCV antibody titers and primary pig kidney cell cultures were positive for PCV based on IFA. Virus isolation was successful from the pig kidney cell cultures and confirmed to be PCV by electron microscopy and immunoelectron microscopy.[7] This isolate was then used to infect four seronegative pregnant sows. The sows never demonstrated clinical disease, but subsequent litters had evidence of congenital tremors. PCV was then isolated from these piglets.[7]

Extensive studies have been done on the replication process of chicken anemia virus (CAV) and psittacine beak and feather disease virus (PBFDV). Both are thought to be immunosuppressive although they are believed to have different pathogenesis. CAV is thought to cause damage to thymic T-cells and precursor cells in the bone marrow. PBFDV is found to replicate in macrophages, the thymus and bursa Fabricus.[16]

PCV appears to be more closely related to PBFDV in its infective process. Experimentally, PCV has been found to infect and replicate in cultures of pig monocyte/macrophages cells without having any affect on cultures of pig T or B cells.[16, 17] There was no cytopathic effect described on cell cultures, but there may be other pathological processes occurring.

McNeilly looked at the effects of PCV on alveolar macrophage function. PCV had no effects on expression of Fc and complement receptors and the ability of the cell to phagocytose and kill was not impaired.[9, 17] However, MHC class II expression decreased at one point in the study. This could be caused by virus mediated membrane turnover associated with virus infection of the cell and movement of the virus to replication areas in the cytoplasm.[17] Lastly, PCV infected alveolar macrophages had a decreased ability to reconstitute the proliferation response of monocyte/macrophage depleted porcine peripheral blood mononuclear cells.[17] This led to the assumption that infected alveolar macrophages have a lessened ability to act on B and T cells and stimulate proliferation.

In a study reported by Allan, colostrum deprived pigs were inoculated with PCV. These animals never developed clinical signs and gross lesions were not observed at necropsy. However, PCV antigen was found in many tissues taken from pigs in the study group. In previous studies, tissue samples were found to be negative in infected pigs at six weeks post-inoculation, ruling out the possibility of a persistent or latent infection. Yet, possibilities of persistency may exist.[18]

The same study examined pig fetuses and stillborn piglets submitted from commercial breeding herds. Although no PCV antigen was detected in the 160 fetuses examined, PCV virus was isolated from a spleen sample and two pooled serum samples collected from two separate stillborn piglet cases. Speculation is that transplacental infection with PCV before immunocompetence could result in persistent infection. This may cause a different distribution of antigen and virus compared to piglets infected at late gestation or 1 day of age.[18]

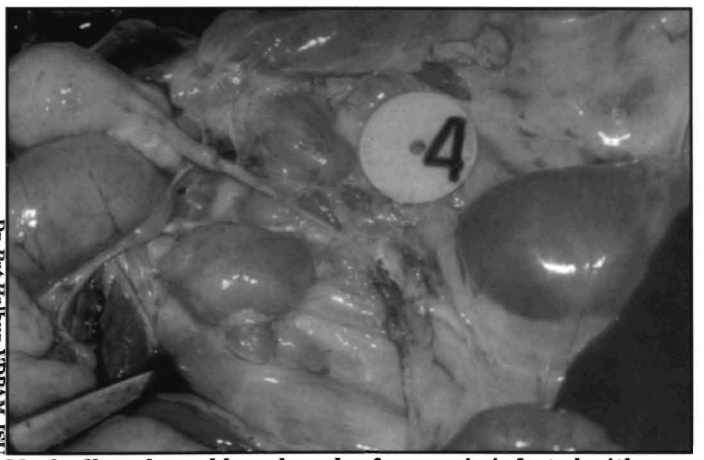
Necropsy Findings

To make a diagnosis of PMWS, a postmortem examination is required. Although lesions may be evident and striking in case of severe disease, variability does exist from pig to pig. For this reason it is important to necropsy as many pigs as economically possible to form a good understanding of how PMWS pigs present.

The majority of PMWS pigs are in poor body condition with muscle atrophy or evidence of muscle wasting. The skin is mild to moderately pale and pigs tend to have a long hair coat. Icterus can be seen in 20% of the pigs suspected of PMWS. Significant lymph node enlargement is observed. Both visceral and peripheral lymph nodes become

three to four times normal size. On cut surface, the lymph nodes tend to have a homogenous white appearance.[2,8]

The lung pathology of PMWS pigs can be quite variable. The lungs tend to consist of a diffusely noncollapsing, interstitial type appearance. The lungs are generally heavy and firm to rubbery when palpated. The lung surface generally has a gray to tan mottled appearance, with some cases showing dark red to brown lobules of alveolar hemorrhage. The lungs may also appear to have gray to red cranial ventral atelectasis or consolidation.[2,8]



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Markedly enlarged lymph nodes from a pig infected with Porcine circovirus.

Variability is also seen in the kidneys of PMWS pigs. The kidneys of 50% of the pigs will be spotted with variably sized white foci scattered throughout both the cortical and medullary regions. The kidneys will have a waxy appearance due to edema of the peripelvic area of the kidney and the kidney itself. The kidneys may be enlarged up to five times the normal size in some cases. In other pigs, the kidneys may be normal in size with varying degrees of the lesions described.[2,8]

The spleen may be mildly enlarged and have a meaty consistency. In 50% of the cases, the liver will be normal. On other occasions, the liver may be small with prominent interlobular connective tissue. The liver may have a mild yellow to orange mottled appearance.[2,8]

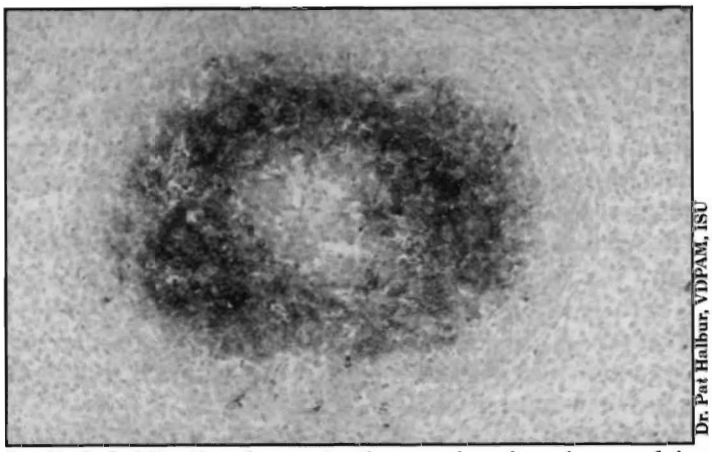
The gastrointestinal tract presents with varying degrees of lesions. The fundic mucosa of the stomach may appear mottled with patchy-opaque white areas. The cecum and proximal spiral colon can be hyperemic with areas of petechiation. The wall of the cecum may be thickened due to edema. The small intestines will generally have little ingesta present.[2,8]

Histopathology

In order to have success at diagnosing PMWS, tissues for histopathology should be submitted.

In all cases of PMWS, lung lesions tend to persist. In early stages of disease a lymphocytichistiocytic patchy to diffuse interstitial pneumonia is present. Chronically infected pigs have more of a granulomatous interstitial pneumonia with giant cells and multinucleated syncitial cells predominate with fewer numbers of lymphoblastic cells. Varying degrees of partial to complete airway epithelial sloughing may be seen along with replacement of the mucosa and submucosa by fibroplasia and lymphohistiocytic cell infiltrates throughout all lung lobes. Airways of all sizes at end-stage disease show bronchiolitis obliterans and regeneration of airway epithelium is common. Neutrophils are only seen in airway lumens. Eosinophils are present in PMWS cases but plasma cells are rarely seen.[2,8]

Pigs with PMWS tend to have lesions involving Peyer's patches of the ileum, lymph nodes, spleen and tonsils. Early loss of B cell follicles is seen in these lymphoid organs and tissues. The T cell area is likely to be expanded by large histiocytic and multinucleated syncitial cells. In B cell dependent areas, basophilic intracytoplasmic inclusion bodies that are predominately in mononuclear cells can be found in early to mid-stage disease. Lymph nodes will have multifocal areas of coagulative necrosis amd necrotic cells will contain intranuclear eosinophilic inclusions. A vasculitis/arteritis of the pericapsular lymph node vessels may also be seen.[2,8]



***In situ* hybridization demonstrating porcine circovirus nucleic acid (darker staining area) in lymphoid tissue.**

The kidneys may have cellular infiltration of lymphocytes, eosinophils and histiocytes. Mainly, these infiltrates will be located in the peripelvic connective tissue. As with lymph node vessels, a vasculitis may be seen with vessels of the kidney. In most areas of the cortex, cortical tubular atrophy can be found with some areas showing hyperplasia. Connective tissue between tubules will be edematous and fibroblastic proliferation will be evident.[2,8]

The portal zones of the liver in PMWS pigs tend to be infiltrated with lymphocytic-histiocytic cells. The hepatocytes generally have a swollen cytoplasm that is vacuolated. The nuclei of the hepatocytes may also appear swollen and enlarged. In icteric pigs, most hepatocytes are destroyed that results in a congested liver.[2,8]

Occasionally, the pancreas will show interstitial lymphocytic-histiocytic infiltration. The zymogen granules are not apparent and pancreatic ducts tend to be surrounded by inflammatory cells. Epithelial sloughing of the pancreatic ducts can also be seen with some indications of regeneration.[2,8] The gastrointestinal tract may be infiltrated by lymphocytic-histiocytic cells. The glandular and crypt epithelium will show areas of sloughing and regeneration. Dilation of the submucosa of the gastrointestinal organs may be edematous.[28]

Differential Diagnosis / Diagnostics

In order to make a sound diagnosis of PMWS, complete diagnostic testing should be performed along with the postmortem examination. The reason is simple. Postweaning wasting can be caused by a variety of factors and/or a combination of those factors. Without complete knowledge of what is happening in a herd, treatment and management strategies may be faulted. Possible causes other than PMWS for postweaning wasting in pigs include:

1. Porcine reproductive and respiratory syndrome virus
2. Hemagglutinating encephalomyocarditis virus
3. Swine influenza
4. Porcine proliferative enteropathy
5. *Mycoplasma hyopneumoniae*
6. *Haemophilus parasuis*
7. Postweaning colibacillosis
8. Cryptosporidiosis
9. Postweaning anorexia and starvation

Diagnostic tests used to confirm lesions associated with PCV are immunoperoxidase staining and electron microscopy.[2,4,8] There are reports of a diagnostic polymerase chain reaction test being used for PCV detection.[8,9] Serology has limited value until an etiologic agent is identified. Because Koch's postulates have not yet been fulfilled, any diagnostic testing results for PCV must be evaluated carefully.

Management

Without complete understanding of PMWS, treatment of infected animals is difficult, if not impossible. A good solid management program is needed to control the disease

within the herd. Recommendations include all in/all-out pig flow, good sanitation, and creation of a sick pen for infected pigs. Feed grade and injectable antibiotics may be used to control secondary pathogens but have proven unsuccessful against PMWS.[1] The long term control scheme depends on the herd developing solid immunity and a reduction in viral spread and challenge.[1,3]

Conclusion

A tremendous amount of information is still needed to associate PCV with PMWS. Without a doubt, PCV is present in tissues from pigs suffering from a wasting syndrome, but the relationship of the virus to PMWS has not been identified. Some people believe that PCV and PMWS may not be related because of the ubiquitous presence of the virus in the swine population.[17,19] However, there could be a synergism between PCV and other pathogens that allow the virus to exploit its destructiveness. With the devastating PRRSV sweeping across the land, this idea cannot be left out. In fact, CAV has been shown to demonstrate increased invasiveness when dual infection with other avian viruses occurs.[18]

For the time being, PMWS should be thought of as a immunosuppressive dysfunction caused by the possible combination of PCV and other swine pathogens. The mechanism of action is unknown so it is imperative to concentrate on the information currently known. When a PMWS pig presents, gross pathology, histopathology, serology, and other diagnostic testing should be used to rule in or out the presence of other infectious agents. PCV should also be looked for through immunohistochemistry, electron microscopy, and PCR to help make decisions on the relatedness of this virus. At the present time, we need to control the effects of other swine pathogens on pigs through good management, pig flow, and vaccination programs.◆

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