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Porcine circovirus type 2: A critical appraisal of its role in PMWS

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Introduction

Porcine circovirus (PCV) was first discovered in 1974 as a noncytopathic contaminant of the PK-15 porcine kidney continuous cell line[1] and was later characterized as a small icosahedral DNA virus with a circular genome.[2] The complete genome was subsequently sequenced[3] and PCV infection of PK-15 cells was characterized electronmicroscopically.[4] The PK-15 cell line is widely used throughout the world. It is not known for how long or the number of clones of PK-15 cells that were/are infected with PCV. Likewise, it is not known to what degree PCV mutated during passage in PK-15 cells. Later work demonstrated two genetic types of PCV: PCV1 and PCV2.[5] The PCV from PK-15 cells is the type strain of PCV1. The worldwide prevalence and distribution of PCV1 in pigs is unknown. Recently in a limited study in Germany, PCV1 was demonstrated by PCR in 5% of sampled pigs.[6] Inoculation studies with PK-15 PCV1 in young pigs have not demonstrated clinical disease or lesions.[6–9] The significance of PCV1 as a porcine pathogen remains unclear.

Recently a second type of porcine circovirus, PCV2, has been described in association with a porcine multisystemic wasting syndrome (PMWS). Numerous studies have implicated PCV2 as the central pathogen in PMWS (see below). PCV2 has also been associated with congenital tremors,[10,11] lesions in aborted, stillborn or weakborn pigs[12,13] and porcine dermatitis and nephropathy syndrome (PDNS)[14–16]; however, the role of PCV2 in these other (not PMWS) syndromes is unclear.

PMWS: The disease

Porcine circovirus was first associated in weaned pigs with disease and unique microscopic lesions by Daft and others.[17] Later, the epidemiological and pathologic features of a PCV-associated disease syndrome in weaned pigs in Western Canada was more completely described as porcine postweaning multisystemic wasting syndrome (PMWS).[18,19] Subsequently, PMWS was described worldwide.[20–25] PCV isolates from cases of PMWS are antigenically and genetically distinct from PCV1 and are known as PCV2.[5,22,23,26] PCV2 isolates are relatively alike and share at least 95% nucleotide homology, whereas they share less than 80% homology with the PK-15 PCV1 virus.

PMWS most often affects 8- to 18-week-old weaned pigs. Morbidity and mortality are variable and may be as high as 50%, but are typically less than 10%. Pigs with PMWS most often exhibit progressive weight loss and dyspnea, but may also be icteric, have diarrhea, or exhibit pallor.[27] Gross lesions in pigs with PMWS consist of generalized lymphadenopathy in combination with one or a multiple of the following:

- cutaneous pallor or icterus,
- interstitial pneumonia,
- mottled atrophic livers,
- grossly enlarged, edematous kidneys with a semitranslucent appearance,
- an enlarged, meaty, noncongested spleen,
- fluid-filled, thin-walled sections of lower intestine, with occasional edema of the wall of the cecum, and
- gastric ulcers and gastric wall edema.[18,27]

The most distinctive microscopic lesions of PMWS are lymphoid cell depletion and granulomatous inflammation in lymphoid organs with inconsistently occurring clusters of basophilic intracytoplasmic inclusion bodies in macrophages and multinucleate giant cells.[18,22,24,25,28] Other lesions include interstitial lympho-histiocytic pneumonia, lympho-histiocytic periportal hepatitis with hepatocellular necrosis, lympho-histiocytic peripelvic and interstitial nephritis with nonsuppurative perivasculitis, lympho-histiocytic infiltration of gastric, cecal, and colonic mucosa and marked edema of the submucosa, and lympho-histiocytic interstitial pancreatitis. Cells containing PCV2 antigen or nucleic acid are highly associated with lesions and the number of virus infected cells correlates positively to the severity of lesions. The primary cells infected by PCV2 are macrophages and other antigen presenting cells; less often infected are B and T lymphocytes, hepatocytes, respiratory and intestinal epithelial cells, pancreatic acinar cells, and endothelial cells.[18,22,25,29]

Epidemiology

PMWS typically affects 4–30% of pigs with 70–80% mortality.[30] Affected pigs are infected with PCV2 and usually one or more other pathogens including PRRS virus, porcine parvovirus, pseudorabies virus, *Mycoplasma hyopneumoniae*, and/or a variety of other bacteria.[30,31] PCV2 is readily transmitted from inoculated to sentinel pigs, can be detected in tissues of inoculated pigs for at least 125 days, and is shed in nasal secretions, feces, urine, semen, and blood.[32] A French on-farm study reported that pigs with PMWS originated from few litters, suggesting a litter affect.[33] Other studies have shown that castrated male pigs were more likely to develop PMWS than females.[34] On PCV2-infected farms, serological testing demonstrates a typical viral infection pattern with colostral antibodies declining through suckling and nursery stages and active seroconversion indicating viral infection during the early grower period.[34] PMWS mortality typically coincides with seroconversion to PCV2.

Subclinical infection with PCV2 appears to be common. Serological surveys have demonstrated PCV2 in high prevalence in pigs worldwide on farms with and without PMWS. Serological surveys have also demonstrated PCV2 in high prevalence in pig populations in Canada and Ireland prior to the appearance of PMWS.[35,36] Likewise, PCV2 has been demonstrated commonly in pigs in Belgium where PMWS has not been observed.[37]

Presence or absence of PMWS on PCV2-infected farms is not likely due to differences in virulence between PCV2 strains. Phylogenetic analysis of the entire genomic sequence of PCV2 isolates from around the world has demonstrated ≥95% nucleotide homology among all strains.[6,38] Analysis of strains originating from herds with and without PMWS demonstrated no consistent differences.[38]

Pigs with PMWS have higher concentrations of PCV2 in serum compared to subclinically infected pigs on the same farm.[39]

Inoculation studies

Inoculation studies with PCV2 in pigs have partially clarified the role of PCV2 in PMWS. In most studies where PCV2 alone is inoculated into germ-free, gnotobiotic, cesarean-derived-colostrum-deprived (CDCD) or conventional pigs, typical microscopic lesions and few to moderate gross lesions of PMWS are induced while clinical disease is mild or absent.[9,40–43] PMWS has been completely reproduced with typical wasting disease, gross lesions and microscopic lesions by inoculation of CDCD pigs with only PCV2 [32,44] and by dual inoculation of pigs in several other studies with both PCV2 and porcine parvovirus (PPV).[9,40,41,45] In one of the PCV2-only models[32], PCV2 virus was detected in tissues of inoculated pigs up to 125 days post-inoculation indicating persistent viral infection and was transmitted to sentinel pigs placed in contact with PCV2-inoculated pigs beginning 42 days PI. In the combined PCV2 and PPV model of PMWS, much PCV2 and little PPV was detected in lesions that were typical of PMWS. The primary role of PPV seemed to be enhancement of PCV2 replication. Large numbers of PCV2-infected cells in dually inoculated pigs relative to few in pigs without disease that were inoculated with only PCV2 suggested that enhanced PCV2 replication produced a threshold level of PCV2 needed to cause PMWS. The mechanism of PPV-enhancement of PCV2 replication is unknown, but the authors suggested PPV-induced immunosuppression or activation of macrophages as possibilities. The immediate cause of death in inoculated pigs appeared to be liver failure.[9] Other studies demonstrated that concurrent inoculation of PRRSV with PCV2 also resulted in enhanced PCV2 replication and induction of PMWS.[46,47] A novel study in germ-free pigs confirmed PCV2 as the sole essential infectious cause of PMWS, fulfilling Koch's postulates.[48] Fatal PMWS was reproduced by the concurrent inoculation of PCV2 and the administration of a non-infectious antigen, keyhole limpet haemocyanin that was emulsified in Freund's incomplete adjuvant (KLH-ICFA). Fatal PMWS and enhanced replication of PCV2 was observed only in pigs receiving both PCV2 and KLH-ICFA and not in controls that received either PCV2 or KLH-ICFA alone. It was speculated that the combination of adjuvant and antigen resulted in immunostimulation that, in turn, enhanced replication of PCV2. An identical study conducted in conventional pigs resulted in PMWS in both pigs that received only PCV2 and in those receiving PCV2 and KLH-ICFA.[49] There was not enhancement of disease associated with KLH-ICFA induced "immunostimulation."

PCV2: Cause or curiosity?

A consistent finding in field studies of PMWS is the presence of PCV2 in affected pigs within unique lesions in proportion to lesion severity. Likewise, clinically ill pigs have higher concentrations of PCV2 in their serum than do subclinically infected pigs on the same farm. These findings strongly suggest that PCV2 plays a central role in PMWS. Inoculation studies further implicate PCV2. In all models of PMWS, PCV2 is demonstrated in typical lesions in proportion to lesion severity. In models employing dual inoculation with a second infectious agent, only PCV2 is consistently demonstrated in lesions. Finally, PMWS has been reproduced in a model employing PCV2 as the only infectious agent. Taken together, these findings suggest that PCV2 is the essential infectious cause of PMWS.

However, PMWS seems to be a multifactoral disease and presence of PCV2 alone does not guarantee disease. PCV2 replicates predominantly in monocyte-derived cells and PCV2 can only replicate in dividing cells.[50] A common denominator in all experimental models of PMWS is that

replication of PCV2 is enhanced and concentrations of PCV2 are higher in animals that develop PMWS. It is likely that viral cofactors such as PRRS virus or porcine parvovirus or non-viral cofactors such as KLH-ICFA contribute to the development of PMWS, at least in part, by increasing the population of dividing monocyte-derived cells. This, in turn, would increase the number of host cells for PCV2 replication, resulting in enhanced PCV2 replication and development of PMWS. It may be most reasonable to view PCV2 as a secondary pathogen that is reliant on infectious and non-infectious cofactors to enhance PCV2 replication up to a threshold required for disease development.

Even so, the pathogenesis of PMWS is likely even more complicated since there are many observations that are yet not explained by our current understanding. For example, the combined prevalence of PRRSV, porcine parvovirus or other co-factors and PCV2 is higher in most parts of the world than is the prevalence of PMWS. It is also unclear why PCV2 was prevalent as early as 1973 in Northern Ireland[36] or 1985 in Canada[35] and yet PMWS did not emerge as a problem until the mid-1990s. Some researchers have proposed that enhanced PCV2 replication may require more than just dividing host cells.[48] Host cells might also need to be activated by certain cytokines that in turn would require specific conditions for their secretion. There may also be host-specific factors that render certain animals or litters more or less susceptible to PMWS. Clearly, there is much that remains unknown.

Conclusion

PCV2 is the essential infectious cause of PMWS, but is not likely a primary pathogen in the conventional sense. PCV2 is best viewed as a ubiquitous secondary pathogen that can cause disease given adequate co-factors and susceptible hosts. The problem is, we do not yet recognize all possible co-factors nor do we understand the determinants of host susceptibility.

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