Terminology

Porcine Circovirus Diseases (PCVD) and Porcine Circovirus Associated Disease (PCVAD) are synonymous names for a disease syndrome caused by porcine circovirus type 2 (PCV2) and originally described in the mid-1990’s as Postweaning Multisystemic Wasting Syndrome (PMWS).\(^1\), \(^2\) While the acronyms PCVD and PMWS have been widely used over the last decade, the American Association of Swine Veterinarians in 2006 endorsed “PCVAD”. Their desire to avoid using “PMWS” was due to the potentially negative connotations the word “wasting” may have on consumer purchasing habits, and because PCV2-affected pigs do not always demonstrate weight loss.

Historical Perspective of PCVD

Postweaning multisystemic wasting syndrome (PMWS) was first described by Harding\(^1\) and Clark\(^2\) at the Western Canadian Association of Swine Practitioners’ (WCASP) conference in 1996, and later at the American Association of Swine Veterinarians’ (AASV) meeting in Quebec City in 1997. These conference presentations described a novel, devastating disease in a select number of biosecure high health western Canadian herds affecting nursery and grower pigs characterized by wasting, respiratory disease, enteritis, enlarged lymph nodes, pallor and jaundice. These first herds were located in Alberta and Saskatchewan, two provinces in the Canadian prairies, and included the widely publicized Saskatchewan 600 sow farrow to finish farm that experienced a 16-20 month epizootic.\(^3\) The fact that this 600 sow farm was a closed, high health status farm, confirmed negative for porcine reproductive and respiratory syndrome (PRRS) virus, and virtually all other swine respiratory and enteric pathogens, strongly suggested the epizootic was associated with a new pathogen. Moreover the frequent observation of liver and kidney lesions in early PMWS cases was paramount to our recognition in 1995 of a novel syndrome, and more specifically that this was not a manifestation of PRRS.

PCV2 was later isolated from lesions of affected pigs\(^4\) and the first experimental reproduction of PMWS clinical signs and lesions was completed using PCV2 and porcine parvovirus (PPV) coinfection in gnotobiotic pigs.\(^5\) In 2001 Krakowka\(^6\) experimentally reproduced disease in immunostimulated gnotobiotics using PCV2 alone leading to the hypothesis that PCV2 is the necessary but insufficient cause of PMWS. Since the mid-1990’s, PCVD has been diagnosed in virtually all pig rearing areas of the globe with the notable exception of Australia.

Stain variation and virulence

The circular genome of PCV2 consists of single stranded DNA of 1768 nucleic acids,\(^7\) and 4 major open reading frames (ORF). ORF 1 and 2 code for proteins essential for replication and
the capsid respectively.\textsuperscript{7} ORF 3 appears to code for protein(s) involved in apoptosis.\textsuperscript{8} The function of ORF 4 and 2 other minor ORFs is not known. PCV2 isolates from diseased and non-diseased pigs are genetically similar, and all greater than 90\% homologous. However, the simultaneous identification of a distinct porcine circovirus genomic cluster (PCV2b; genogroup 1; RFLP 321)\textsuperscript{9-11} with the devastating 2005-2006 epizootic outbreaks in North America led to speculation that PCV2b is of higher virulence than is PCV2a (genogroup 2; RFLP 422). Except for one recent study in which a genotype 1 PCV2 isolate was reported to be highly pathogenic,\textsuperscript{12} there is no clear evidence that isolates from genotype 1 are more pathogenic or virulent than those from genotype 2.\textsuperscript{13-18} While PCV2b provided a possible explanation for the eastern Canadian outbreak, concurrent infections with new strains of PRRSv and SIV, which were also circulating in the eastern Canadian pig population, cannot be ruled out as contributors to the PCVD outbreak. Our research team has recently completed experiments indicating that the severity and incidence of clinical signs and lesions in PCV2b-infected gnotobiotes, with and without KLH/ICFA immunostimulation, were indistinguishable from those of gnotobiotes inoculated with PCV2a using the same experimental model and reported in the past decade.\textsuperscript{19} This data suggests that infection of swine with PCV2b alone is not the single causal event responsible for the North American PCVD epizootic.

Case Definitions

Sorden was the first in North America to formally propose a case definition for PMWS\textsuperscript{20} which provided pathologists and field veterinarians critical diagnostic guidelines for individual animals. Faced with a mounting number of herd epizootics in 2005/06, the AASV developed a PCVAD case definition\textsuperscript{21} for affected herds in an attempt to distinguish farms with epizootic disease (PCVAD positive), from those experiencing only sporadic losses (PCVAD negative). Fulfillment of this latter case definition requires the presence of characteristic lesions associated with PCV2 antigen or DNA in affected pigs, as well as significant levels of clinical disease affecting the population. The AASV’s case definition is not specific to PMWS; it is more encompassing and recognizes other clinical manifestations of PCV2 including respiratory, reproductive or enteric disease (Figure 1). However, this case definition fails to objectively set out intervention levels or upper-level mortality or prevalence targets that are indicative of PCVD. Although a global agreement on such targets does not exist, Segales proposed that elevations in herd mortality more than 1.66 standard deviations above a historic mean for the herd, or exceeding a national or regional standard by more than 50\% should be used.\textsuperscript{22}

Pathogenesis and clinical expression of PCV2

\textit{Multisystemic immunosuppressive disease with wasting, enteric and/or respiratory involvement:}

Known also as PMWS, multisystemic disease implies the involvement of multiple organ systems and requires by definition, systemic lymphoid depletion in advanced cases. There are several classic clinical signs and gross lesions of PMWS that should form the basis of a preliminary clinical diagnosis including: enlarged lymph nodes, wasting, dyspnea, diarrhea, pallor, nephropathy, thymic atrophy and jaundice.\textsuperscript{3,23,24} While all of these signs or lesions will not be noted in a single pig, the majority if not all of these signs will be noted over a period of time on affected farms.
The clinical signs of PMWS were historically restricted to the post-weaned aged groups, particularly the late nursery and early grower stages, between 7 and 15 weeks of age. More recently however, some of the 2005/06 North American outbreaks affected older hogs, likely due to farm-related production factors altering PCV2 epidemiology, or the type and infection dynamics of other pathogens on the affected farms.

Passively acquired PCV2 antibody appears to be protective in young piglets, and may persist for 6-8 weeks in the progeny of unvaccinated sows (Harding, unpublished). While piglets are clearly exposed to PCV2 in their dam’s milk and feces at a very young age, the timing of initial infection is not fully understood. Following infection PCV2 is most commonly associated with monocytic cells and less frequently in endothelial, epithelial cells or lymphocytes. In vitro analysis has demonstrated that PCV2 accumulates in monocytic cells, particularly the plasmacytoid dendritic cell (DC), for prolonged periods of time, but the virus does not replicate substantially within these cells. PCV2 both infects and replicates in endothelial cells and gut epithelium, particularly when these cells are stimulated as would occur during inflammation. This possibly explains why high levels of PCV2 can be found in DC, and why clinical disease is associated with immune stimulation and/or coinfections with other pathogens such as PRRS, *Mycoplasma hyopneumoniae*, *Mycoplasma hyorhinis*, swine influenza virus, porcine parvovirus, swine hepatitis E virus, and torque teno virus. Moreover, through its modulation of the innate immune response, specifically the impairment of interferon alpha and tumour necrosis factor alpha production in some plasmacytoid DC, PCV2 infection eventually induces immunosuppression and may render the host more susceptible to secondary infections.

**Reproductive failure and prenatal myocarditis:**

Porcine embryos and fetuses are susceptible to PCV2 infections, but their susceptibility is dependent on their developmental stage. Embryos appear to be resistant to PCV2 infection as long as the zona pellucida is intact, but are susceptible to infection after hatching. PCV2 replicates in fetuses experimentally infected in mid- and late-gestation, but replication is age dependent being significantly higher in young (57 day) versus older (75 and 92 day) fetuses. Moreover, fetuses inoculated at 57 days of gestation demonstrated myocarditis resulting in congestion and heart failure, whereas fetuses inoculated at gestation day 75 and 92 were free of pathological lesions but developed PCV2 antibodies within 21 days of inoculation.

Although transplacental infection has been demonstrated experimentally and PCV2-induced reproductive failure has been reported in commercial herds, it is not a consistent finding in PCVD outbreaks. Affected farms experience elevated abortions, stillbirth and fetal mummification rates, prenatal myocarditis, and variable amounts of PCV2 antigen present in fetal tissues and sera. It must be noted that PCV2-associated reproductive failure is most commonly reported in start-up herds. While other causes of infective and non-infective reproductive failure including PRRS are no doubt significant, the relevance of PCV2 as a cause of reproductive failure in commercial farms needs to be clarified. However, several studies have demonstrated the presence of PCV2 in semen of experimentally and naturally infected boars. In one study involving a commercial AI stud, boars less than 52 weeks of age, or less than 26 weeks post-entry were 2.6 and 3.0 times more likely to shed PCV2 in their semen than older...
boars. While it is clear that PCV2 can be shed intermittently in semen, the duration and frequency of seminal shedding, and concentration of PCV2 in semen is poorly understood. However, PCV2 appears to be more concentrated in the seminal fluid and nonsperm fractions rather than adherent to the spermatozoa suggesting that PCV2 enters semen via migrating immune cells or in seminal plasma.

**Diagnosis**

Although the classical gross and histopathological lesions associated with PMWS were described over a decade ago, the definitive diagnosis of PCVD in individual pigs is not always simple. In individual cases, the classical clinical signs (wasting, diarrhea, dyspnea, lymphomegaly, jaundice), histopathological lesions (granulomatous inflammation, lymphoid depletion) and PCV2 antigen associated with the lesions must be evident. However, the presence of simultaneous infections often makes diagnosis difficult. In these cases, diagnostic results may yield multiple viral or bacterial pathogens in addition to PCV2, and the significance of each must be ascertained.

Another complicating factor in the diagnosis of PCVD is that PCV2 may be present, albeit at low levels, in the tissues of subclinically infected but seemingly healthy pigs. Thus, antibody testing in serum is not an effective tool to determine if a farm or pig is affected by PCVD. It only shows that PCV2 is present in a herd or that a pig was exposed. However, quantifying the levels of PCV2 in tissues may be useful in ascertaining the significance of PCV2 infection. In research settings, PCV2 levels may be semi-quantified in any lymphoid or non-lymphoid tissue using immunohistochemistry (IHC) or *in situ* hybridization (ISH). In commercial laboratories, semi-quantitative IHC or ISH is rarely performed, but quantitative polymerase chain reaction (qPCR) is becoming routine. However, ring tests performed in North America, indicate that the detection limits and the capability of qPCR assays to accurately quantify PCV2 DNA levels varies widely. Given this, the establishment of a “cut-off” level that could be used by all laboratories globally to distinguish PCVD from non-PCVD tissues is impractical. Cut off levels must be established and validated independently at each lab using their own PCR assay and standards. At our laboratory, PCV2 DNA levels above logarithm (base 10) $10^7$ to $10^8$ per gram in tissues, and above $10^4$ to $10^5$ per milliliter in serum are indicative of PCVD. Moreover, PCV2 DNA levels are most highly correlated (.75 < r < .84) with PCV2 IHC staining intensity scores in Peyer’s Patches, spleen, lung and lymph nodes (Figure 2). Variable levels of PCV2 DNA are noted in some tissues of unvaccinated pigs in the absence of PCV2 IHC staining.

**PCVD Control & PCV2 Vaccines**

Good production practices might help to reduce the impact of PCVD and coinfections. Thus, the MADEC 20 principles were at least partially effective in controlling PMWS in many European herds prior to the use of PCV2 vaccine. However, the concensus view is that the use of PCV2 vaccines, led to the rapid decline in the number of PCV2 herd epizootics in North America in 2007. At the time of writing, there are four vaccines licensed (full or restricted) in Canada, and 3 licensed in the USA. Peer-reviewed research demonstrating the efficacy of these vaccines is expanding and clearly demonstrates that PCV2 vaccines, particularly if administered to piglets, are very efficacious reducing viremia, viral load, mortality, lesions and improving
growth rates. PCV2 vaccination around weaning (about 3 weeks of age) is the routine protocol for piglet vaccination and protects until slaughter. Because PCV2 exposure typically coincides with the waning of passive immunity between 6 and 8 weeks of age, it is not necessary to determine the time course of infection through serology or PCR prior to implementing an optimal vaccination program. However, if unacceptable levels of mortality continue after a PCV2 vaccination program is implemented, additional diagnostics to identify significant coinfections should be undertaken.

Serology (testing for antibodies) is not a tool for testing efficacy of PCV2 vaccines. One-dose vaccines do not always induce seroconversion, but nevertheless are very successful in stimulating protective immunity. To judge the efficacy of a vaccine in the field, reduction of mortality, reduced number of culls and improved performance are the most relevant parameters. Based on these the economical benefit of piglet vaccination can be estimated, and is reported to be as high as 9.85 USD in a non-complicated case of PCVD. Importantly, the piglet vaccines are efficacious in the presence of maternal antibodies and when coinfections including PRRS and Mycoplasma hyorhinis exist. Moreover, there appears to be cross-protection between the two PCV2 genogroups. Thus, the North American and the global swine industries are very fortunate, in that effective PCV2 vaccines are now widely available, and enhance the profitability of pork production systems.


19. Harding JCS, Ellis JA, Krakowka S. Dual heterologous porcine circovirus type 2 (PCV2) genogroup a/b infection, not PCV2b alone, is associated with enhanced disease severity in germ-free pigs. *submitted for publication.*


Figure 1. Porcine circovirus associated disease (PCVAD) case definition

**PCVD (PCV diseases)**

**PCVAD (PCV-associated disease)**

- PMWS* (Multisystemic disease with weight loss)
- Respiratory disease
- High Mortality
- Prenatal myocarditis
- Enteric disease
- Reproductive failure
- PDNS?

* PMWS is a component of PCVD/PCVAD
Figure 2. Box and whisker plots demonstrating the correlation between tissue porcine circovirus type 2 (PCV2) DNA concentrations and the intensity of PCV2 immunoperoxidase staining in tissue.\(^{53}\)

Y-axis: Viral load \(\log_{10}\) DNA concentration copies per gram (tissues); X-axis: PCV2 (immunohistochemistry) staining intensity score (0–3) in the tissue characterized. Abbreviations: LN = lymph node; Spl = spleen, PP = Peyer’s patches, Lng = lung; Staining intensity scores: 0 = absent, 1 = mild, 2 = moderate, 3 = severe.