

DIFFERENT APPROACHES TO HANDLING CIRCOVIRUS

John Harding
Department of Large Animal Clinical Sciences
Western College of Veterinary Medicine
University of Saskatchewan
52 Campus Drive, Saskatoon, Saskatchewan S7N 5B4
E-mail: john.harding@usask.ca

INTRODUCTION

Since its discovery and characterization in western Canada in 1995, the significance and dissemination of post-weaning multisystemic wasting syndrome (PMWS) has grown and it has become a serious disease affecting the global swine industry. More recently, there is a heightened interest in PMWS due to the explosive outbreaks in eastern Canada, particularly in Quebec starting in late 2004.

PMWS is caused by Porcine Circovirus type 2 (PCV2), a small single stranded DNA virus. It is the only circovirus known to cause disease in mammals, but other circoviruses cause numerous diseases in birds. By comparison, porcine circovirus type 1 (PCV1) does not cause disease in pigs, and is genetically and antigenically distinct from PCV2. In addition to PMWS of swine, PCV2 contributes to porcine respiratory disease complex (PRDC) and proliferative and necrotizing pneumonia (PNP). It has also been associated with several other conditions including humpy-back swine, porcine dermatitis and nephropathy syndrome (PDNS), congenital tremors (CT-AII), pre-natal myocarditis and reproductive failure. It is important to note that PCV2's involvement in these latter conditions has not been proven.

POST-WEANING MULTISYSTEMIC WASTING SYNDROME (PMWS)

There are several classic clinical signs of PMWS that form the basis of a preliminary clinical diagnosis including enlarged lymph nodes, wasting, dyspnea, diarrhea, pallor, and jaundice (Harding, et al., 1998a & 1998b; Cottrell, et al., 1999; Harms, 1999). While all of these signs will not be noted in a single pig, affected farms will present with the majority, if not all, over a period of time. Confirmation of PMWS requires the presence of clinical signs, hallmark histological lesions and the identification of PCV2 within lesions (Sorden, 2000).

The clinical signs of PMWS are traditionally restricted to the post-weaned aged groups, particularly the late nursery and early grower stages, between 7 and 15 weeks of age (Harding, et al., 1998b). Ironically, the 2004/05 eastern Canadian outbreak appears to affect older hogs, likely due to the dynamics of co-infections and PCV2 viral load specific to the affected farms. Between 1995 and 2005, PMWS in North America most commonly caused low grade and sporadic death loss. On rare occasions particularly in western Canada, severe epidemics resulting in three to four fold increases in post-weaning mortality rates occurred. Persistently high mortality has been noted commonly in some European countries over the

last decade. Interestingly, it is likely that the same is happening in Canada after an 8-year period of quiescence. The reasons for these sudden explosive outbreaks are unknown but current theories include the mutation of PCV2 into a more virulent strain(s), the presence of indigenous or exotic infective cofactors (Agent X), or changes in farm management that “trigger” the onset of disease.

PCV2 STRAINS

The 2004-05 eastern Canadian outbreak is similar in many ways to the PMWS outbreaks in many European Union countries, except that it coincides with the frequent isolation of an apparently novel PCV2 strain, identified as PCV2-321, based on restriction fragment length polymorphism (RFLP) analysis (Carman, et al., 2005). This novel PCV2 strain is over 99% homologous to virulent French and Dutch PCV2 isolates recovered from PMWS cases (Gagnon, unpublished). By comparison, the PCV2 strains recovered in eastern Canada prior to 2005 are only 95-96% homologous to the same French and Dutch PCV2 isolates. Recent Orf2 sequencing of PCV2 strains recovered from diagnostic cases in France, UK, China and Canada in 2004-05 suggests this novel PCV2-321 belongs to a unique cluster of isolates, that is genetically distinct from PCV2 isolates recovered between 1997-99 from western Canada and USA (Hamel, unpublished). Based on the sudden appearance of this new RFLP pattern and the severity of clinical disease and mortality, it is proposed that the 2004-05 PMWS outbreaks in eastern Canada was caused by the dissemination of this novel PCV2 “321” strain which is of increased virulence and possibly imported from France via semen. However, the virulence of this novel PCV2-321 strain has not yet been proven experimentally or by field studies. Furthermore, case-control studies evaluating the molecular characterization of PCV2 strains in France and the Netherlands failed to identify any single mutation or variant strain that was correlated with clinical disease or increased virulence (Boisseson, et al., 2004; Grierson, et al., 2004).

CO-FACTORS AND VIRAL LOAD

While PCV2 infection is clearly a necessity (Allan & Ellis, 2000; Ellis, et al., 2000) and is the only virus consistently recovered from PWMS cases, other co-factors are required for inducing PMWS. These co-factors may include other diseases such as PRRS (Harms, et al., 2001; Pallares, et al., 2002; Allan et al., 2000); mycoplasma, swine influenza (Harms, et al., 2002) and parvovirus (Ellis, et al., 2000; Krakowka, et al., 2000); immune stimulation or vaccination (Krakowka, et al., 2001; Opriessnig, et al., 2003; Kyriakis, et al., 2002); or the absence of good production practices (Rose, et al., 2003; Wallgren, et al, 2005). However, virtually all commercially raised pigs are subclinically infected with low levels of PCV2 (Larochelle, et al., 2003; Harding, 2000) yet most remain healthy and do not develop PMWS. By contrast, very high levels of PCV2 are consistently recovered from various tissues and organs of pigs with PMWS (Brunborg, et al., 2004). In fact, the amount of PCV2 in tissues and serum of PMWS pigs (a.k.a. viral load) is correlated with the severity of clinical signs and associated histological lesions in experimentally (Krakowka, et al., 2005; Ladekjaer-Mikkelsen, et al., 2002; Krakowka, et al., 2001) and naturally (Brunborg, et al., 2004;

Segales, et al., 2005) infected pigs. While the viral load in healthy subclinically infected pigs is typically less than 10^6 (per mL serum or 500 ng tissue), the viral load in clinical PMWS pigs generally exceeds 10^7 (Brunborg, et al., 2004). Thus, amplifying viral load is critical for the development of PMWS. In pigs as in all species, antigen presenting cells (APCs) play a fundamental role in the early immune response, by presenting foreign antigens such as viral particles to the effector cells of the immune system. In healthy subclinically infected pigs, PCV2 is contained within APCs in a quiescent state that does not impact APC function or result in cytotoxic effects (McCullough, et al., 2003). Although the immunologic details are unclear, this appears to be a fundamental mechanism of PCV2 induced disease and results in the persistent low level PCV2 infection of lymphoid tissue in healthy pigs. Moreover, co-factors may induce PMWS by attracting PCV2-infected APCs to sites of immune stimulation or infection, where PCV2 is amplified beyond the critical biological “threshold”. This amplification leads to the development of the hallmark lesions of PMWS (granulomatous inflammation, lymphoid depletion) through a number of immune mechanisms, and ultimately to the dissemination of PCV2 to distant systemic sites. Thus, I propose the key to controlling and preventing PMWS in any farm regardless of PMWS status, location, strain or co-factors involved is to reduce and maintain PCV2 viral load below this biologically critical “threshold”.

PCV2 VACCINES

At the time of writing, there are no licensed vaccines in the North American market, although several pharmaceutical companies have products in their pipeline. CFIA has recently granted Merial Canada an import permit for their vaccine (February 2006). Public domain research documenting the efficacy of these experimental vaccines is limited, but the experimental and field research available is promising (Charreyre, et al., 2005; Meng, 2005). The products under development are targeted at both the breeding herd, to enhance the passive immunity of piglets, and feeding herd, to initiate active immunity post-weaning. Both killed and attenuated live vaccines are in the pipe. The use of autogenous vaccines has been suggested, however it is unlikely that autogenous PCV2 vaccines would be effective, and more importantly may not be safe, because PCV2 is difficult to grow in tissue culture, and is very resistant to inactivation.

CONCLUSIONS

Our understanding of the factors impacting the emergence and severity of PMWS on affected farms is not complete; however it is clear that severe disease is associated with high amounts of PCV2 in tissues. Thus, the amplification or upregulation of PCV2 in tissues is a prerequisite to disease expression. Our understanding of the epidemiology and potential triggering factors is improving, particularly the role of adjuvants, vaccines and co-infections. Until vaccines are widely available in Canada, the key to controlling severe PMWS is to implement good production practices and eliminate coinfections to prevent the amplification of viral load. While the emergence of a new PCV2 strain has received considerable attention

in eastern Canada, the industry should be cognizant that the superior virulence of these strains has not been proven.

LITERATURE CITED

- Allan, G.M., and J.A. Ellis. 2000. Porcine circoviruses: a review. *J. Vet. Diagn. Invest.* 12:3-14.
- Allan, G.M., F. McNeilly, J.A. Ellis, S. Krakowka, B. Meehan, I. McNair, I. Walker, S. Kennedy. 2000. Experimental infection of colostrum deprived piglets with porcine circovirus type 2 (PCV-2) and porcine respiratory and reproductive syndrome virus (PRRSV) potentiates PCV-2 replication. *Arch. Virol.* 145:2421-2429.
- Boisseson, C., V. de Beven, L. Bigarre, R. Thiery, N. Rose, E. Eveno, F. Madec, A. Jestin. 2004. Molecular characterization of Porcine circovirus type 2 isolates from post-weaning multisystemic wasting syndrome-affected and non-affected pigs. *J. Gen. Virol.* 85(2): 293-304.
- Brunborg, I.M., T. Moldal, C.M. Jonassen. 2004. Quantification of porcine circovirus type 2 from serum/plasma and tissue samples of healthy pigs and pigs with postweaning multisystemic wasting syndrome using TaqMan-based real-time PCR. *J. Virol. Meth.* 122:171-178.
- Carman, S., B. McEwen, J. Delay, H. Vai, J. Fairles, T. van Dreumel. 2005. Porcine circovirus type 2 - associated disease continues to increase. *AHL Newsletter Dec., 2005*; www.ahl.uoguelph.ca/newsletters/.
- Charreyre, C., S. Beseme, A. Brun, M. Boblot, F. Joisel, B. Lapostolle, P. Sierra, A. Vaganay. 2005. Vaccination strategies for the control of porcine circoviral diseases in pigs. *Proc. International Conf. Animal Circoviruses and Associated Diseases, Belfast, Ireland.* Sept. 11-13; p26-30.
- Cottrell, T., B. Friendship, C. Dewey, G. Josephson, G. Allan, F. McNeilly, I. Walker. 1999. Epidemiology of post-weaning multisystemic wasting syndrome in Am Ontario. *Proc. Am. Assoc. Swine Pract.* 389-390.
- Ellis, J.A., A. Bratanich, E.G. Clark, G. Allan, B. Meehan, D.M. Haines, J. Harding, K.H. West, S. Krakowka, C. Konoby, L. Lassard, K. Martin, F. McNeilly. 2000. Coinfection by porcine circoviruses and porcine parvovirus in pigs with naturally acquired postweaning multisystemic wasting syndrome. *J. Vet. Diagn. Invest.* 12:21-27.
- Grierson, S.S., D.P. King, G.J. Wellenberg, M. Banks. 2004. Genome sequence analysis of 10 Dutch porcine circovirus type 2 (PCV-2) isolates from a PMWS case-control study. *Res. Vet. Sci.* 77(3):265-268.
- Harding, J.C.S. Sept. 2000. Understanding the epidemiology of porcine circovirus. Saskatchewan Agriculture Development Fund, Project 97000099, 14 pgs.
- Harding, J.C.S., and E.G. Clark. 1998a. Recognizing and diagnosing post-weaning multisystemic wasting syndrome (PMWS). *Swine Health and Prod.* 5, 201-203.
- Harding, J.C.S., E.G. Clark, J.H. Strokappe, P.I. Willson, J.A. Ellis. 1998b. Post-weaning multisystemic wasting syndrome: epidemiology and clinical presentation. *Swine Health and Prod.* 6, 249-254.

- Harms, P.A., P.G. Halbur, S.D. Sorden. 2002. Three cases of porcine respiratory disease complex associated with porcine circovirus type 2 infection. *J. Swine Health Prod.* 10(1): 27-30.
- Harms, P.A., S. Sorden, P. Halbur, S.R. Bolin, K.M. Lager, I. Morozov, P.S. Paul. 2001. Experimental reproduction of severe disease in CD/CD pigs concurrently infected with PRRSV and type 2 porcine circovirus and porcine reproductive and respiratory syndrome virus. *Vet. Pathol.* 38:528-539.
- Harms, P.A. 1999. Hepatopathy associated with spontaneous type 2 porcine circovirus infection in caesarian derived/colostrum deprived pigs. *Proc. Amer. Assoc. Vet. Lab. Diagn.*, 4.
- Krakowka, S., J.A. Ellis, F. McNeilly, C. Waldner, G. Allan. 2005. Features of porcine circovirus-2 disease: correlation between lesions, amount and distribution of virus and clinical outcome. *J. Vet. Diagn. Invest.* 17:213-222.
- Krakowka, S., J.A. Ellis, B. Meehan, S. Ringler, M.D. Rings, G. Allan. 2001. Activation of the immune system is the pivotal event in the production of wasting disease in pigs infected with porcine circovirus-2 (PCV-2). *Vet. Pathol.* 38:31-42.
- Krakowka, S., J.A. Ellis, B. Meehan, S. Kennedy, F. McNeilly, G. Allan. 2000. Viral wasting syndrome of swine: experimental reproduction of postweaning multisystemic wasting syndrome in gnotobiotic swine by coinfection with porcine circovirus 2 and porcine parvovirus. *Vet. Pathol.* 37:254-263.
- Kyriakis, S.C., K. Saoulidis, S. Lekkas, C.H.C. Miliotis, P.A. Papoutis, S. Kennedy. 2000. The effects of immunomodulation on the clinical and pathological expression of postweaning multisystemic wasting syndrome. *J. Comp. Pathol.* 126:38-46.
- Ladekjær-Mikkelsen, A.S., J. Nielsen, T. Stadejek, T. Storgaard, S. Krakowka, J.A. Ellis, F. McNeilly, G. Allan, A. Bøtner. 2002. Reproduction of postweaning multisystemic wasting syndrome (PMWS) in immunostimulated and non-immunostimulated 3-week-old piglets experimentally infected with porcine circovirus type 2 (PCV2). *Vet. Microbiol.* 89:97-114.
- Larochelle, R., R. Magar, S. D'Allaire. 2003. Comparative serologic and virologic study of commercial herds with and without postweaning multisystemic wasting syndrome. *Can. J. Vet. Res.* 67:114-120.
- McCullough, K.C., I.E. Vincent, A. Summerfield, J. Neilsen, S. Krakowka, J.A. Ellis, H.J. Nauwynck, C. Charreyre, F. McNeilly, G.M. Allan. 2003. The immunology of PCV2 infections and PMWS. *White Book Four, Merial SAS*, p25-34.
- Meng, X.J. 2005. Molecular biology of porcine circoviruses: identification of antigenic epitopes and genetic determinants for virulence. *Proc. International Conf. Animal Circoviruses and Associated Diseases, Belfast, Ireland.* Sept. 11-13, p17-19.
- Opriessnig, T., S. Yu, J.M. Gallup, R.B. Evans, M. Fenaux, F. Pallares, E.L. Thacker, C.W. Brockus, M.R. Ackermann, P. Thomas, X.J. Meng, P.G. Halbur. 2003. Effect of vaccination with selective bacterins on conventional pigs infected with type 2 porcine circovirus. *Vet. Pathol.* 40:521-529.
- Pallares, F.J., P.G. Halbur, T. Opriessnig, S.D. Sorden, D. Villar, B.H. Janke, M.J. Yaeger, D.J. Larson, K.J. Schwartz, K.J. Yoon, L.J. Hoffman. 2002. Porcine circovirus type 2 (PCV-2) coinfections in U.S. field cases of postweaning multisystemic wasting syndrome (PMWS). *J. Vet. Diagn. Invest.* 14(6):515-519.

- Rose, N., G. Larour, G. Le Diguierher, E. Eveno, J.P. Jolly, P. Blanchard, A. Oger, M. Le Dimna, A. Jestin, F. Madec. 2003. Risk factors for porcine multisystemic wasting syndrome (PMWS) in 149 French farrow-to-finish herds. *Prev. Vet. Med.* 61:209-225.
- Segales, J., M. Calsamiglia, A. Olvera, M. Sibila, L. Badiella, M. Domingo. 2005. Quantification of porcine circovirus type 2 (PCV-2) DNA in serum and tonsillar, nasal, tracheo-bronchial, urinary and faecal swabs of pigs with and without porcine multisystemic wasting syndrome (PMWS). *Vet. Microbiol.* 111:223-229.
- Sorden, S.D. 2000. Update on porcine circovirus and postweaning multisystemic wasting syndrome (PMWS). *Swine Health Prod.* 8(3):133-136.
- Wallgren, P., F. Hasslung, G. Bergstrom, A. Linder, K. Belak, C. Hard af Segerstad, M. Stampe, B. Molander, T.B. Kallay, E. Norregard, C.J. Ehlorsson, M. Tornquist, C. Fossum, G.M. Allan, J.A. Robertsson. 2005. Postweaning multisystemic wasting syndrome (PMWS). The first year with the disease in Sweden. *Vet. Quart.* 26(4):170-187.