Sponsors

University of Minnesota
College of Veterinary Medicine

College of Food, Agricultural and Natural Resource Sciences

Extension Service

Swine Center

Thank you to **IDEXX Laboratories** for their financial support to reproduce the conference proceeding book.

Production Leader
Steven Claas

Production Assistant
Steven Claas
Janice Storebo
Sarah Summerbell

Layout and CD-ROM
David Brown
Tina Smith

Logo Design
Ruth Cronje, and Jan Swanson;
based on the original design by Dr. Robert Dunlop

PCV2 isolate variation: Its role in pathogenicity and disease control

C. Charreyre, DVM, MSc, F. Joisel
Merial SAS, Lyon, France

Introduction

After 10 very eventful years of PCV2 research across the world, we can now make a difference and minimize the importance of PCVD by vaccinating against PCV2. The very good results obtained with vaccination have already demonstrated that vaccinal protection occur against all the existing various PCV2 isolates.

In this presentation we will attempt to describe why isolate variation today has a very limited effect on PCV2 pathogenicity and PCVD control.

Variations at the genetic level

In appears obvious that a molecular biological approach would be the most precise of tools to evaluate and map changes of viruses. The exact nature of one isolate should be more precisely represented by its genomic sequence. PCV2 virus is small (1700 bases) and has a DNA genome; therefore it is easier to sequence with fewer errors than with other larger and/or RNA viruses. One should take into account the often-low fidelity of PCR tests used in diagnostic, but nevertheless there has been now hundreds of isolates sequenced and of course some degree of variations has been observed. These small variations allowed graphing various phylogenetic trees for PCV2 isolates although all "branches" regroup within more than 93% homology at the nucleotide level (Ellis *et al*., 2006; Olvera *et al*., 2007). However PCV2 isolates are now distributed into two main genotypes: the "older" genotype 2 (2A or 422-like pattern) and the "newer" genotype 1 (2B or 321-like pattern) (Segales *et al*., 2007).

Of course there is the previous example of the Parvovirus (PPV), also a small single-stranded DNA virus, for which a change in a few bases resulted in a major change in biological behavior. The virus changed tropism and switched from cats to dogs, a very major jump. One can observe that the very magnitude of this change (change of host species) could be in fact indicative of the stable balance of the PPV (or PCV2) population within its regular host. Once a virus is well adapted to its host, it has most likely already tried out in vain most strategies to adapt even better. Everything else considered, such a simple life form as PCV2, once genetically balanced within its host will stay in balance and small changes should not matter tremendously. Nevertheless continuous sequencing and comparison are mandatory to monitor genetic changes as they inevitably occur. This is also critical to maintain accuracy of the PCR tests used for diagnostic.

Generally, sequencing is a much better tool than Restriction Fragment Length Polymorphism (RFLP). RFLP tests are based on the ability of bacterial enzymes to cut DNA between specific sequences. Incubation of PCV2 DNA with a pre-defined set of enzymes will result in DNA fragments of different sizes that can be visualized on gels. However, RFLP should not be used without frequent sequencing checks, since some biologically irrelevant changes can be detected by RFLP, or conversely a significant change will not be seen, depending on the enzymes used. As an example, the RFLP pattern 321 can be obtained as well with "old PCV2 isolates" of the genotype 2 or with "new PCV2 isolates" of the genotype 1. Therefore, RFLP patterns alone should not be used to classify PCV2 isolates beyond current diagnostic purposes.

Finally it seems quite logical to use cloned viruses experimentally to sort out between variations at the genetic level and at least one study suggests that cloned PCV2 isolates can differ in virulence in an SPF pig model (Opriessnig *et al*., 2006). One can notice however that cloned viruses are never present in nature. They are useful models but cannot represent what is happening with a normal viral population.

In fact available data to date indicate that there are no changes in PCV2 sequences that can explain the emergence of PCVD or be clearly associated with aggravated disease.

Variations at the protein level

Serological tests are used to describe serotypes within close virus isolates. This allows separating them at the antigenic level with the help of biological reagents, monoclonal or polyclonal antibodies. The virus neutralization test (VNT) is the classical gold standard among such tests since it is directed towards the very ability of the virus to infect. It is most useful and significant to acquire such knowledge, since it clearly represents a more complete and biologically related view on isolate variations than mere sequencing.

To date however little information exists on the serological classification of PCV2 isolates. Using a reproducible VNT with a transformed-lymphocyte cell line and specific rabbit anti-sera, researchers from Belfast were nevertheless able to

study antigenic relationships between 6 PCV2 isolates of different geographical origins within genotype 2 (Duffy *et al*., 2007). Based on at least a 10-fold difference in neutralization titres, 2 isolates could be considered serogically distinct from each other and moderately distinct from the 4 other very close isolates. Although all sera neutralized all isolates fairly strongly, this is a first indication that fine epitopic variations could have some biological significance, even within one genotype. Similar studies using both genotypes 1 and 2 showed a greater variability (G. Allan, personal communication).

Monoclonal antibodies have also been used to demonstrate antigenic differences among PCV2 isolates (Lefebvre *et al*., 2007).

Another study looked at the *in vitro* interaction of PCV2 virus from genotypes 2 (SG1 based on sequencing of Swedish isolates) and 1 (SG3), with porcine pulmonary macrophages (Stevenson *et al*., 2007). The accumulation of PCV2 in the cytoplasm of monocytic cells is a major feature of PMWS and the hallmark of its pathogenic effect (Gilpin *et al*., 2003). Specific monoclonal against PCV2 ORF2 displayed the same affinity to both genotypes grown in PK15 cells, and the same antigenic mass was detected in those cells using a PCV2 antigen capture ELISA. However, genotype 1 was detected with a significantly higher rate and in a higher numbers of cells in primary macrophage cultures from 2 pigs. There was some degree of variation as well between macrophage reactivity between the 2 pigs.

These results suggest that genotype 1 viruses may have a higher tropism for pulmonary macrophages and/or have a greater capacity to persist in these cells than viruses from genotype 2.

Nevertheless again, all sera neutralized all isolates, all isolates grew in PK15 cells to similar levels and all viruses accumulated in macrophages. Therefore these results characterized serotypes and some difference in tropism *in vitro*, but they did not distinguish pathotypes among PCV2 isolates.

Variations at the clinical level

It has been a constant observation that the outcome of PCV2 infection is highly variable in the pig. It has even been a constant source of confusion and frustration for the researchers trying to establish challenge models.

PCV2 infection is characterized by a constellation of clinical signs including progressive weight loss, jaundice and respiratory disease with variable intensity and mortality rates (Allan *et al*., 1999; Ellis *et al*., 1999; Kennedy *et al*., 1999; Krakowka *et al*., 2002).

Experimental acute clinical cases with more than 50% incidence were obtained only with pigs younger than 3 to 4 weeks of age and mostly in animals that were co-stimulated or co-infected with another pathogenic agent. Close to 100 % incidence is obtained only in 3 day old gnotobiotic co-stimulated piglets. In older pigs PCV2 infection appear to induce less severe clinical consequences. The oldest pigs experimentally affected with the occasional acute clinical case were 6 weeks old, conventional and co-infected with *Mycoplasma hyopneumoniae* (Opriessnig *et al*., 2004).

With the different challenge models available, the different PCV2 isolates showed similar results: all of them are able to cause disease to similar degrees.

A Swedish isolate obtained from lymph nodes of a healthy pig collected in 1993 and which did not produce disease for 10 years in Sweden was used in parallel with a reference Canadian isolate to infect 3-day-old snatch- farrowed colostrum-deprived piglets co-infected with PPV. (Hasslung *et al*., 2005). Both the Swedish isolate from 1993 and the Canadian isolate were of genotype 2, now suspected of a lower pathogenicity. However, despite very different histories, both isolates produced to similar extend specific clinical signs and lesions consistent with PMWS.

More recently, a vaccination challenge study allowed to compare directly the pathogenicity of PCV2 isolates of different genotypes and all genotypes gave similar results experimentally (Segales, J. *et al*., 2007).

It is now generally acknowledged that PCV2 is the necessary but usually not sufficient cause of PMWS and PCVD. PCVD are multi-factorial diseases. The great variability observed in clinical signs is not due to variations between PCV2 isolates, but overwhelmingly to other factors besides the virus itself. To this day, all genotypes/serotypes tested are able to cause PCVD and no pathotype have been described.

Variations at the epidemiological levels

Logically an even greater variability in PCVD expression is observed at the herd level, geographically and over time. Indeed such observations between Canada and Europe induced the very debate on isolate variations and on the possibility of more pathogenic mutants. Faced with a clinical disaster of enormous proportions, it was difficult to take into account the natural variability of viral populations and easy to conclude that a "new" variant was present. However logical in appearance, this hypothesis could not be confirmed.

In fact, PCV2 populations will evolve and change as a matter of course. If the changes are as prevalent in healthy pigs and farms as in diseased animals it will be difficult to relate viral change with increased virulence of infection. By definition however disease investigation will focus on diseased animals seldom addressing the general population including healthy animals.

It has been shown that PCV2 genotypes recovered from 26 PMWS affected and non-affected farms in Northern Ireland and Ireland from 1997 to 2006 naturally evolved from a dominant genotype 2 to a dominant genotype 1.

The switch happened between June 2003 and January 2004 and the recovery of genotype 1 in non-diseased herds during the disease epidemic argues against the possibility that genotype 1 was more virulent (McMenamy *et al*., 2007).

In a multifactorial disease as PCVD, changes of co-factors might also be extremely important, even more important than changes in PCV2 itself.

The very final argument against the existence of pathotypes in the PCV2 viral population at this time is of course the great efficacy of the various vaccines. All vaccines have been produced with genotype 2 and all vaccines are described as miraculously efficacious in a large geographic range where genotype 1 is now predominant.

Vaccination of the sow herds with CIRCOVAC® induced a marked improvement of the sanitary and economical status of the farms with a decrease in PCV2-linked and global mortalities, a decrease of the use of medications especially antibiotics and a significant increase of daily gains at all stages of the pig life.

To more precisely evaluate the nature and predictability of the efficacy of CIRCOVAC®, a linear regression model was used to compare the results obtained before and during vaccination in Germany where the largest body of data exist for the longest time (233 farms, 67,000 sows and more than 2 millions pigs since 2005). For each farm where data were available, the decrease of mortality figures after vaccination was plotted versus the initial mortality rate before vaccination.

Regression lines were calculated using Excel® 2003, and equations validated using Systat® 5.0 in **Figures 1** and **2**.

These graphs answer to the question: "What reduction of mortality over a normal baseline value may be expected after vaccination?"

Strong positive linear correlations were found between the reductions of mortality figures and mortality before vaccination. The higher the mortality before vaccination, the larger the improvement after vaccination, as the farms returned to normal baseline mortality figures in weaned pigs and in fattening units. These findings showed that pigs born from vaccinated sows were protected against the deleterious impact of PCV2 during their whole lifespan.

Identical results were obtained in France and in Canada.

Conclusion

Available data today allow distribution of PCV2 isolates into 2 genotypes, the "older" genotype 2 and the "newer" genotype 1. This switch naturally occurred all over the world in 2003-2004. Studies at the antigenic level support this classification and distinguish further between a few PCV2 serotypes. It is however acknowledged that PCVD are multi-factorial diseases. The great variability observed in clinical signs and epidemiologically is not due to genetic or antigenic variations between PCV2 isolates, but overwhelmingly to other factors besides the virus itself.

In particular, there are no changes in PCV2 sequences today that can explain the emergence of PCVD or be clearly associated with aggravated disease.

The final argument against the existence of pathotypes in the PCV2 viral population at this time is the great efficacy of the various vaccines. Vaccination of the sow herds with

Figure 1: Reduction of mortality in weaned piglets

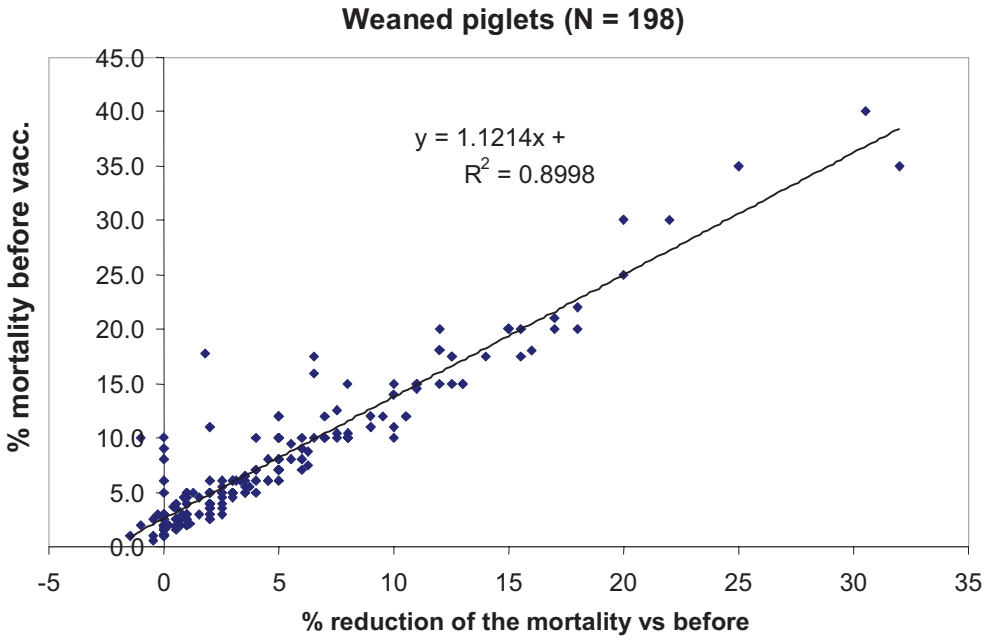
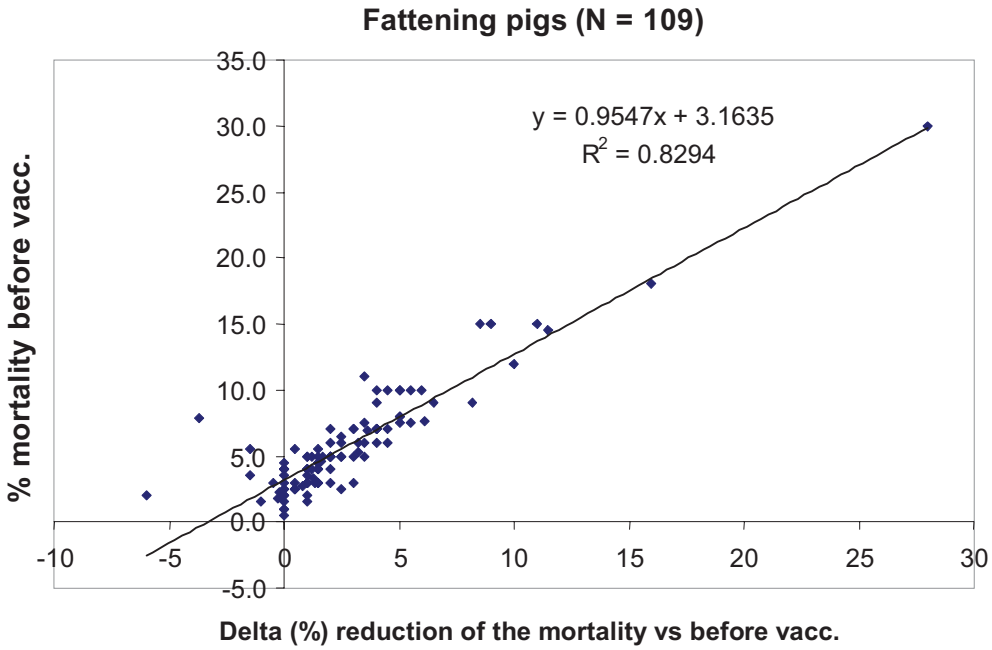


Figure 2: Reduction of mortality in fatteners



CIRCOVAC® (PCV2 genotype 2) resulted in very significant decreases in PCV2-linked and global mortalities while PCV2 genotype 1 is now predominant.

This vaccination allows a return to normal or even improved baseline mortality figures in the farms and a marked improvement of their sanitary and economical status.

Although we must remain cautious and continue close monitoring of PCV2 viral populations, isolate variation today has a very limited effect on PCV2 pathogenicity and PCVD control.

References

Allan G. *et al*., 1999, Experimental reproduction of wasting disease and death by co infection of piglets with porcine circovirus and porcine parvovirus. *J. Comp Pathol*. 121: 1-11

Duffy C. *et al*., 2007, The serological characterization of PCV2 isolates using an established virus neutralization test for PCV2 antibodies, 5th International Symposium on Emerging and Re-emerging Diseases, Krakow, Poland, 52.

Ellis J. *et al*., 1999, Reproduction of lesions of PMWS in gnotobiotic piglets. *J Vet Diag Inves*t 11: 3-14

Ellis, J. *et al*., 2006, Porcine Circovirus Disease in Canada... a circle within a wheel, in "PCVD Coming Full Circle" Merial White Book July 2006, 23- 34

Gilpin *et al*., 2003, In vitro studies on the infection and replication of PCV2 in cells of the immune system, *Vet. Immunol. Immunopathol*., 94:149-161

Hasslung F. *et al*., 2005, Experimental reproduction of PMWS in pigs in Sweden and Denmark with a Swedish isolate of PCV2, *Veterinary Microbiology* 106: 49-60

Kennedy S., *et al*., 1999, Reproduction of lesions of PMWS by infection of conventional pigs with PCV2 alone or in combination with porcine parvovirus. *J Comp Path* 122: 9-24

Krakowka S. *et al*., 2002, Immunologic features of porcine circovirus type 2 infection. *Virol Immunol* 15: 567-582

Lefebvre D. *et al*., 2007, Monoclonal antibodies demonstrate antigenic differences between PCV2 strains, 5th International Symposium on Emerging and Re-emerging Diseases, Krakow, Poland, 47

McMenamy M. *et al*., 2007, The temporal distribution of PCV2 genogroups recovered from PMSW-affected and non-affected farms in Ireland and Northern Ireland, 5th International Symposium on Emerging and Re-emerging Diseases, Krakow, Poland, 85

Olvera A. *et al*., 2007, Molecular evolution of PCV2 genomes: phylogeny and clonality, *Virology* 357: 175-185

Opriessnig T. *et al*., 2004, Experimental reproduction of PMWS in pigs by dual infection with Mycoplasma hyopneumoniae and porcine circovirus type 2, *Vet. Pathol.* 41(6): 624-40

Opriessnig, T. *et al*., 2006, Genetic and experimental comparison of PCV2 isolates from cases with and without PCV2-associated lesions provides evidence for differences in virulence, *Journal of General Virology*, 87: 2923-2932

Segales, J. *et al*., 2007, PCV2: strains, pathogenicity and vaccines, in Intervet Satellite Proceedings and Symposium Abstracts July 2007, 5-11

Stevenson L. *et al*., 2007, Biological comparison of PCV2 isolates, 5th International Symposium on Emerging and Re-emerging Diseases, Krakow, Poland, 51