Postweaning multisystemic wasting syndrome (PMWS) – a threat to Swedish pig production?

Caroline Fossum, Frida Hasslung, Eva Wattrang, Per Wallgren

Background

The disease complex postweaning multisystemic wasting syndrome (PMWS) was first diagnosed in Sweden in December 2003. The factor(s) that elicit the disease are not fully specified but it is generally accepted that porcine circovirus type 2 (PCV2) is a necessary agent for development of PMWS and demonstration of PCV2 is one criteria that has to be fulfilled at diagnosis of PMWS. At the section for Veterinary Immunology, SLU and the division for Ruminant and Pig Diseases, National Veterinary Institute (SVA), research on PCV2/PMWS was initiated in 2001 via collaboration with Prof. Gordon Allan and co-workers at Queen's University in Belfast, UK, and reagents and methods for diagnosis of PMWS had been transferred to pathologists at SVA in Uppsala. The research was then financed via a grant from FORMAS and the division of Vet Immunology also became one of 16 partners collaborating in the EU-consortium "Control of porcine circovirus diseases (PCVDs): Towards improved food quality and safety" established in Dec. 2004.

The PCV2/PMWS-research carried out within the frame of the SLF-grant has focused on the epidemiology of PMWS in Sweden, with special reference to identification of PCV2 genogroups and their possible role in the spread of PMWS. Furthermore, the research has aimed to identify risk factors for development of PMWS at herd level and the protective role of antibodies to PCV2 in individual pigs.

Material and Methods

Reagents and methods for the diagnosis of PMWS, detection of PCV2 and quantification of antibody levels to the virus have to a great extent been provided from our collaborators at Queen's University in Belfast. One work package within the EU research project is dedicated to harmonisation of technologies with ring-trials to ensure the quality and similarity of test results obtained in the various European laboratories. Subcommittees on Epidemiology, Pathology, and Immunology are established within the EU consortium to make certain that similar criteria are used for definition and diagnosis PCV2-related diseases. Accordingly, standard operating procedures (SOPs) are established and a list of defined reagents as well as a case definition for PMWS is available at the EU consortium home page (www.pcvd.org).

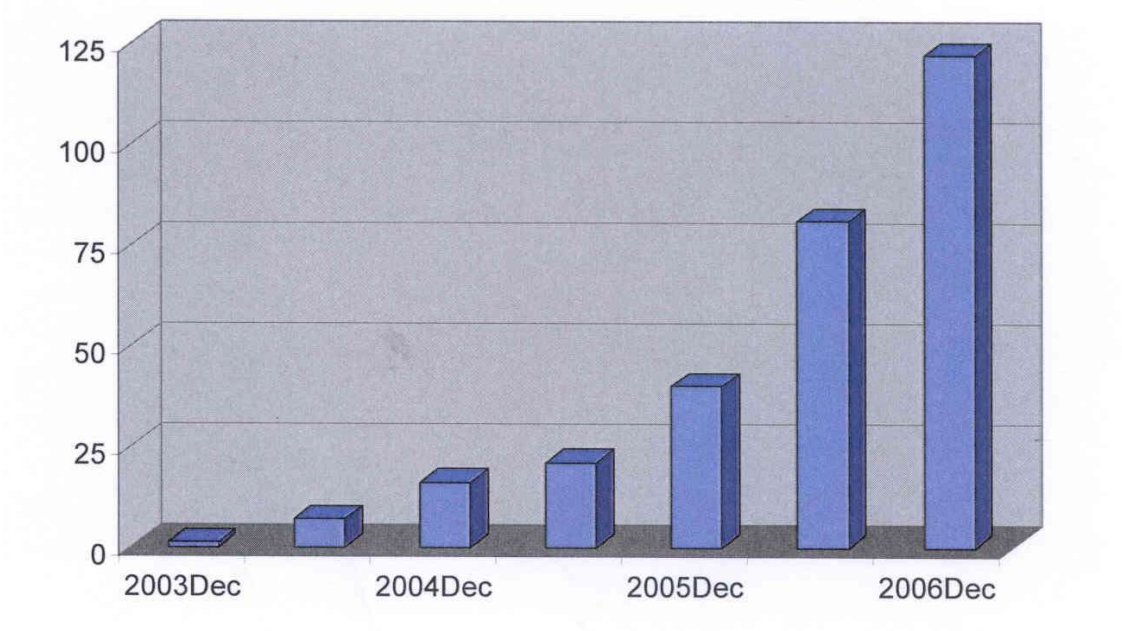
Results

The spread of PMWS in Sweden

The spread of PMWS in Sweden has been carefully monitored since the first confirmation in 2003. During the first three years all herds where the disease was suspected or diagnosed were registered and various forms of "contacts" between diseased and healthy herds have been determined. These studies are documented in two publications: PMWS – the first year with the disease in Sweden (Wallgren et al., 2004) and PMWS in Sweden: From an exotic to an endemic disease (Wallgren et al., 2007). In summary, PMWS has spread slowly but consistently from the southern part of Sweden and is at present reaching Mälardalen. Compared to other European country the morbidity due to PMWS is low. Over the three-year period, the overall percentage of runts, culled and dead pigs in affected herds was 7.4% ± 3.2% at the time point when the herds were diagnosed for PMWS. In Sweden, PMWS is more frequently diagnosed in sow pool satellites and integrated herds than in other production systems. Furthermore, PMWS is more frequently diagnosed in herds producing breeding stock

than in piglet producing herds. An under-diagnosing of PMWS in fattening herds is likely, probably partly due to the fact that these herds empty themselves between batches. Nevertheless, the results obtained link PMWS to larger herds and to herds with intensive rearing systems.

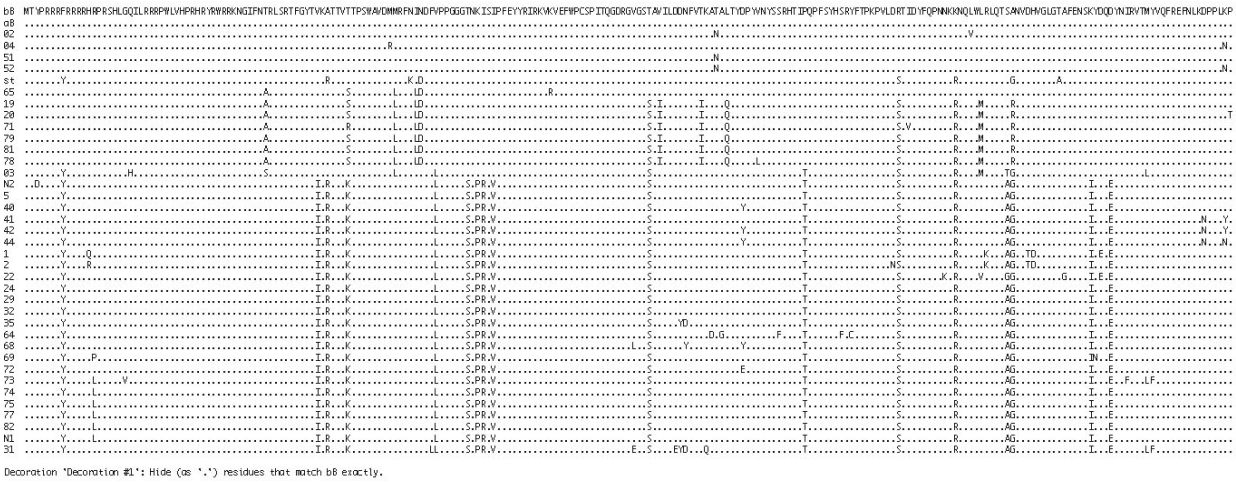
An effect of intensity in rearing on the development if PMWS is also suggested from studies in sow pool satellites. In this production system, PMWS has been diagnosed more frequently in herds with intensified systems (1 to 8 weeks between farrowing) than in satellites performing the initial production cycle of 16 weeks. It is notable that satellites with 16 weeks between consecutive batches of farrowing empty themselves completely between every batch. In general, there are no clear links between affected herds but it is notable that whenever PMWS has been diagnosed on a herd level, management errors or short to non-existing empty time between consecutive batches on the farms has constantly been noted. The accumulated increase in Swedish farms affected by PMWS is summarized in the figure below.



Identification of various genogroups of PCV2

During the epidemiological studies of PMWS, PCV2 was isolated from healthy and diseased pigs in farms affected or not by PMWS. The sequence of the viral genome was determined after PCR-amplificaiton of the whole or parts of the genome and alignments revealed clear differences at both the nucleotide and amino acid levels between various PCV2 isolates. Although the overall identity was high (>90%), distinct genogroups of the virus could be discerned, and interestingly one certain genogroup of PCV2 was only found in healthy animals on non-PMWS farms whereas another genogroup predominated in pigs from farms affected by PMWS. These results were first presented at the "International Conference on Animal Circoviruses and Associated Diseases" (Timmusk et al., 2005) and were then regarded as controversial because previous surveys in e.g. France had not been able to establish any relationship between PCV2 sequence and pathogenicity. The Swedish studies have since then been extended, including samples from Norway, that is free from PMWS, and Estonia, where the disease recently was diagnosed (Timmusk et al., manuscript). Taken

together, the alignments of the ORF2 gene (see below) that codes for the structural capsid protein of PCV2, show that the differences between genogroups are focused to certain regions within this part of the genome. Interestingly studies by others have indicated that these regions are those that compose B-cell epitopes, i.e., viral parts that are targets for the antibody-mediated immunity.



It seems that the Swedish studies encouraged to similar surveys in other countries and a recent publication from Denmark confirm that PCV2 at present can be divided into at least three genogroups (Dupont et al. 2007. Genomic analyses of PCV2 isolates from Danish archives and a current PMWS case-control study support a shift in genotype. Vet Microbiol. In press). However, it is not known if the existence of various genogroups of PCV2 is related to the development of PMWS or not.

No difference in pathogenicity between PCV2 genogroups in naïve pigs

The only reproducible experimental model for induction of PMWS uses naïve pigs obtained by snatch farrowing or caesarean sections. The experimental model has been established in Sweden and used to evaluate the pathogenicity of PCV2 in Sweish and Danish pigs (Hasslung et al., 2005). Furthermore, the pathogenicity of two of the Swedish genogroups of PCV2 was tested and compared to that of the reference isolate (PCV2 1010 Stoon) during experimental infection carried out in Belfast. As reported at IPVS (Allan and McNeilly, 2006) the mortality was similar (50%) in all three experimental groups. Thus, all Swedish isolates of PCV2 tested today are potentially pathogenic under certain experimental conditions.

The role of antibody-mediated immunity to PCV2 in protection against PMWS

In should be noted that the success of the experimental model for reproduction of PMWS is dependent on that the animals are free from antibodies to PCV2 which indicate that the antibody-mediated immunity is pivotal for protection against PMWS. To be protective however, the antibodies need to neutralise the virus and thus not all serological test are suitable for estimating the biological role of antibodies. The various genogroups of PCV2 differ at amino acid positions that can affect the three-dimensional structure of the virus capsid and consequently, conformational alterations at these position are likely to reduce the binding affinity of antibodies generated against different genogroups of PCV2. At present, polyclonal antisera from pigs naturally or experimentally infected with the various genogroups of PCV2 are scrutinized for their “cross-reactivity” in a number of serological tests measuring binding and neutralising activity.

No clear effects of co-infection of weaning pigs with PCV2 and E. coli on the development of PMWS

The epidemiological studies in Sweden have, as in other countries, pointed out that in addition to PCV2, other viral and/or bacterial infections present at the farm are likely to influence the likelihood for development of PMWS. In Sweden, many herds that have been deemed for PMWS also have experienced problems with enteric diseases (Wallgren et al., 2007) and therefore attempts have been made to induce PMWS by co-infection of weaning pigs with PCV2 and *E. coli* according to an experimental model previously established for reproducing post-weaning diarrhoea at the National Veterinary Institute, Uppsala. As previously reported in detail (first year report of the present project, Oct 2005) some of the dual infected pigs displayed mild clinical symptoms of PMWS and PCV2 DNA was also demonstrated by Q-PCR in blood lymphocytes obtained from the animals approximately three weeks after infection. These results, in combination with results from *in vitro* studies on effects of lipopolysaccaride (LPS) from *E. coli* on poPBMC (C Wolff, 2005) encouraged further studies using an experimental co-infection with *E. coli* and PCV2.

During the spring 2006 four experimental groups with ten pigs in each were established. The pigs were offspring to sow L X Y raised at the SPF-herd "Serogrisen, Hagalund, Ransta and transported the day of weaning (at 28 days of age) to animal experimental facilities. Before allocation into experimental groups the serum antibody titres to PCV2 were determined and the pigs were genotyped regarding MUC4 in order to determine their expression of F4ab/F4ac receptors for *E. coli* fimbriae. Four experimental groups were established, one uninfected control group, a group with pigs only exposed to *E.coli* (day 4: O149; day 11: O147 and; day 15: O141). All pigs in the other two experimental groups also received the similar exposure to *E.coli* but in addition they were infected intranasally on three consecutive days (day 5, 6, and 7) with PCV2 1247 pool 9 representing genogroup 1, or PCV2 1452 pool 3, representing genogroup 3. Both these isolates have when used in the experimental model with SFCD pigs and given to pigs co infected with PPV caused PMWS. In contrast, no clinical evidence of PMWS was registered in any of the experimental pigs dually infected with *E. coli* and PCV2. Neither was any evidence of PMWS registered at autopsy performed on experimental day 37. The only measurable effect of the dual infections with *E. coli* and PCV2 were an enlargement of the inguinal lymph nodes registered from experimental day 25 and onwards. Taken together, none of the two experimental infection studies using *E. coli* in combination with PCV2 demonstrated that co-infection with *E.coli* and PCV2 is sufficient to induce PMWS in seropositive weaning pigs.

Discussion

The studies of PCV2 and PMWS performed within the present research project have provided a unique documentation of the spread of PMWS in a country during the early phase of the disease outbreak. This close monitoring of herds allowed us to detect that at least three genogroups of PCV2 exist in Sweden. It could also be clarified that one of the genogroups was more prominent in samples collected from pigs at PMWS affected herds whereas one genogroup of PCV2 exclusively was found in pigs from healthy herds. The existence of PCV2 genogroups that differ in pathogenicity is still a matter of debate but it is becoming more and more evident that a shift in genotype of PCV2 has occurred with time and that this alterations in Europe coincided with the emergence of PMWS.

Experimental infection studies using Swedish PCV2 isolates have clearly shown that they do

not differ in their capacity to induce PMWS during certain conditions, e.g. in young pigs devoid of any antibody-mediated immunity to PCV2. Thus, future studies will focus on the protective role of cross-immunity to various genogroups of PCV2.

Publications

2004

Wallgren P, Hasslung F, Bergström G, Linder A, Belak K, Hård af Segerstad C, Stampe M, Molander B, Björnberg Kallay T, Nörregård E, Ehlorsson CJ, Törnquist M, Fossum C, Allan GM, Robertsson JA. (2004) Postweaning multisystemic wasting syndrome (PMWS) - the first year with the disease in Sweden.Vet Q. Dec;26(4):170-87.

2005

Timmusk, S., Wallgren, P., Belák, K., Berg, M., Fossum, C. Genetic analysis of PCV2 capsid protein sequence reveals two main groups of Swedish isolates. Int. Conference on Animal Circoviruses and Associated Diseaes, Belfast, N. Ireland 11-13 Sept 2005.

Wolff, C. Co-infection of weaned piglets with PCV2 and *E. coli*. Degree project 2005: no. 2006:17 ISSN 1652-8697, Veterinary Medicine Programme, Swedish Univrsity of Agricultural Sciences, Uppsala.

Hasslung F, Wallgren P, Ladekjaer-Hansen AS, Bøtner A, Nielsen J, Wattrang E, Allan GM, McNeilly F, Ellis J, Timmusk S, Belák K, Segall T, Melin L, Berg M, Fossum C. Experimental reproduction of postweaning multisystemic wasting syndrome (PMWS) in pigs in Sweden and Denmark with a Swedish isolate of porcine circovirus type 2. Vet Microbiol. (2005) 106:49-60.

2006

Bergström, G & Wallgren, P. The incidence of thin-to-wasting pigs with respect to race in a progeny test station affectedby PMWS. Proc 19th IPVS Congress, Copenhagen, Denmark 2006 Vol 1 p 169.

Wallgren, P. & Ehlorsson, CJ. Diverging signs of PMWS in satellites belonging to a sow pool affected by PMWS. Proc 19th IPVS Congress, Copenhagen, Denmark 2006 Vol 1 p 171.

Wallgren, P. & Robertsson JÅ. PMWS in Sweden – the first two years with the disease. Proc 19th IPVS Congress, Copenhagen, Denmark 2006 Vol 2 p 89.

Blomqvist, G., Bergström, G., Persson, M., Fuxler, L., Fossum, C. & Wallgren, P. The prevalence of PCV2 antibodies in pigs allocated to a progeny test station affected by PMWS at weaning. Proc 19th IPVS Congress, Copenhagen, Denmark 2006 Vol 2 p 104.

2007

Wallgren, P., Belák, K., Ehlorsson, C-J., Bergstöm, G., Lindberg, M., Fossum, C., Allan, G.M. and Robertsson, J.Å. (2007) Postweaning Multisystemic Wasting Syndrome in Sweden: From an exotic to an endemic disease!. Vet Q. Dec. Vol. 29. In press.

Allan GM, McNeilly F, McMenamy M, McNair I, Krakowka SG, Timmusk S, Walls D, Donnelly M, Minahin D, Ellis J, Wallgren P, Fossum C. (2007) Temporal distribution of porcine circovirus 2 genogroups recovered from postweaning multisystemic wasting syndrome affected and nonaffected farms in ireland and Northern Ireland. J Vet Diagn Invest. 19:668-73.

Timmusk, S., Wallgren, P., Brunborg, I.M., Hasslung Wikström, F., Allan, G.M., Meehan, B.,

McMenamy, M., McNeillym F., Fuxler, L., Belák, K., Podersoo, D., Saar, T., Berg, M., Fossum, C. Phylogenetic analysis of porcine circovirus type 2 (PCV2) pre- and post- epizootic potweaning multisystemic wasting syndrome (PMWS). Submitted for publication.

Wallgren, P., Hasslung Wikström, F., Blomqvist, G., Belak, K., Tenk, M., Fuxler, L., Wolff, C., McNeilly, F., Krakowka, S., Allan, G.M. & Fossum, C. Experimental co-infection of 4-week-old colostrum fed pigs with PCV2 and *E. coli* (manuscript)

Dissemination of data

Hasslung, F. Interaktion mellan PCV2 och immunsystemet – varför blir grisar sjuka? Allmänna veterinärmötet, Uppsala, 12 Nov 2004.

Hasslung, F., Wallgren, P., Fossum, C. PMWS (Postweaning multisystemic wasting syndrome) hos gris – ny sjukdom i Sverige. Jordbrukskonferensen, Uppsala, 23-24 Nov 2004.

Wallgren, P. (2005) Nyheter rörande PCV2. Svenska Djurhälsovårdens Fortbildnings-konferens 15-16 mars 2005. Muntligt föredrag.

Hasslung, F. (2006) Nyheter rörande PCV2. Svenska Djurhälsovårdens Fortbildnings-konferens 22-23 mars 2006. Muntligt föredrag.

Fossum C. Early interactions of PCV2 with the porcine immune system. Oral presentation: 19th IPVS Congress, Copenhagen, Denmark 2006.

Wallgren, P (2006) PMWS – en ny grissjukdom i Sverige. Svavet Nr 2, sid 6-7.

Wallgren, P. and K. Belák (2006) Allvarlig grissjukdom här för att stanna. Svavet Nr 3, sid 9-10.

Wallgren, P. and C.J. Ehlorsson (2006) Flera faktorer bakom PMWS i suggpooler. Svavet Nr 3, sid 11-13.

In addition, research projects and results are presented in the homepage for the EU-project on PCVD (www.pcvd.org) with special refeence to pig producers and breeders, politicians and comsumers via COPA-COEGA (Committee of Agricultural Organisation). A forum or discussion is established at <http://www.pcvd.org/>www.pcvd.org) and at thePigSite (<http://www.thepigsite.com/>www.thepigsite.com). A “PCVD newsletter” can be downloaded from <http://www.pcvd.org/news.php>www.pcvd.org/news.php