

WHAT DO WE KNOW ON EPIDEMIOLOGY, CONTROL AND PREVENTION OF PORCINE CIRCOVIRUS DISEASES?

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Introduction

PCV2 is a ubiquitous virus in domestic pig and wild boar, both in countries where porcine circovirus diseases (PCVDs) have or have not been described (Segalés et al., 2005a). PCV2 was associated with postweaning multisystemic wasting syndrome (PMWS) by mid 90s, and since then it has also been linked to a number of diseases and conditions such as reproductive failure, porcine dermatitis and nephropathy syndrome (PDNS), porcine respiratory disease complex (PRDC) and proliferative necrotizing pneumonia. The acronym PCVD has been used to group all these conditions. However, a scientific demonstration of PCV2 causality in those pathologies has only been established for PMWS and reproductive failure. On the other hand, PMWS is, by far, the most economically significant PCVD; it has been estimated that PCVD costs (direct and indirect losses) around 600 million Euros per year to the European Union (Armstrong and Bishop, 2004). Therefore, the objective of this review is to summarise what is currently known (or unknown) on epidemiology, prevention and control of PMWS, with special emphasis on the most practical and challenging issues.

How to diagnose PMWS on a herd basis?

The internationally accepted PMWS case definition based on three criteria, including clinical signs, characteristic lymphoid lesions, and presence of PCV2 within lesions (Sorden, 2000), is based on the result of individual animals. However, it is known that farms with very good performance scores can have individual animals fulfilling the three abovementioned criteria (Jorsal et al., 2006). For this reason, scientists from the European consortium on PCVD research (European Project No. 513928 from the VI Framework Programme, Priority 5. Food Quality and Safety) discussed and agreed on a PMWS herd case definition. Of course, this definition might be debatable, but at least gives practical clues on how to determine the relative importance of PMWS on farm basis. The agreed definition, which can be found at the web site of the project (www.pcvd.org) includes:

Criterion 1. Clinical appearance on herd level. The occurrence of PMWS is characterized by a significant excessive increase in postweaning mortality and wasting compared to the historical level in the herd. When historical mortality is available in the herd, increase in mortality may be recognized based on statistical tests. If no records of the mortality in the herd do exist, the increase in mortality should exceed the national or regional level by 50%.

Criterion 2. Pathological & histopathological diagnosis of PMWS. Necropsy should be performed on at least five pigs per herd (this value considers the possibility of detecting at least one affected pig with PMWS when this disease is the cause of problems in 50% of the total diseased animals with 95% confidence, independently of the number of diseased pigs). A herd is considered positive for PMWS when the pathological and histopathological findings mentioned above, indicative for PMWS, are all present at the same time in at least one of the autopsied pigs.

As it can be seen, the presented PMWS herd case definition tries to establish objective (statistical) criteria to assess what can be considered or not as a disease concern for the affected farm. The given herd case definition is especially applicable to outbreaks of disease, but much more difficult to apply on enzootic scenarios.

How is the PCV2 infection dynamics in a pig population?

Under field conditions, colostral antibodies typically decline during lactation and nursery and follow an active seroconversion by late nursery and/or fattening (Rodríguez-Arrijoja et al., 2002; Rose et al., 2002; Blanchard et al., 2003a; Larochelle et al., 2003). This seroconversion usually occurs around 7 to 12 weeks of age, and antibodies may last at least until 28 weeks of age (Rodríguez-Arrijoja et al., 2002). PMWS is not usually observed in pigs youn-

ger than 4 weeks of age (Segalés and Domingo, 2002), which may be associated with protective maternal immunity against the development of PMWS, based on field and experimental studies (McKeown et al., 2005; Ostanello et al., 2005; Calsamiglia et al., 2007; Rose et al., 2007). Although PCV2 serum-profiles observed in PMWS and non-PMWS affected farms could be fairly similar, case-control studies have shown that an earlier infection with PCV2 might be a risk factor for PMWS expression (Rose et al., 2003; López-Soria et al., 2005).

How PCV2 is transmitted?

PCV2 can be detected by PCR in nasal cavities, tonsillar and bronchial secretions, faeces and urine (Segalés et al., 2005b) of both naturally PMWS and non-PMWS affected pigs; the latter ones shed a lower amount of PCV2 compared to sick animals. These results are further sustained by experimental infections with PCV2; the virus has been isolated or detected by PCR from nasal, rectal, urinary, salivary, ocular and tonsillar swab specimens (Krakowka et al., 2000; Bolin et al., 2001; Shibata et al., 2003). Therefore, this virus can be potentially shed by all routes of excretion. However, the oro-nasal route is considered the most likely and frequent route of PCV2 infection and transmission. This idea is supported by most experimental studies on PCV2 infection, which have mainly used the intranasal route (Allan et al., 1999; Balasch et al., 1999; Ellis et al., 1999; Krakowka et al., 2000, 2001; Rovira et al., 2002). A practical demonstration of horizontal transmission of PCV2 is the infection of susceptible pigs commingled with already infected pigs. Infection by direct contact with pigs previously inoculated with PCV2 has resulted in transmission of virus to contact control pigs (Albina et al., 2001; Bolin et al., 2001). Taking into account the latter result and the fact that in a given farm almost all pigs (if not all) have seroconverted by 6 months of age (Rose et al., 2003; López-Soria et al., 2005), the horizontal transmission of PCV2 between pigs must be very efficient.

Is PMWS transmissible?

Recent studies conducted in Denmark (Kristensen et al., 2006) and New Zealand (Jaros et al., 2006) were able to transmit PMWS to healthy pigs after mingling them with pigs from PMWS affected herds. In the Danish study, over a period of 46 days, 14 commingled healthy pigs developed PMWS: 10 corresponded to pigs that were placed by direct contact with sick pigs (same pen), 3 by close indirect contact (neighbouring pen) and 1 by indirect contact (across the aisle). The study of New Zealand, of a 56 day-period duration, also demonstrated disease development of healthy pigs in contact or indirect exposure to PMWS affected pigs; moreover, only those healthy pigs that were commingled at four weeks of age developed the disease, but not those animals exposed when they were 12 or more weeks of age. Therefore, age of PCV2 exposure (probably related to remaining PCV2 passive immunity) seem to play a major role on PMWS development.

Is PCV2 transmitted by semen?

It is known that PCV2 can be present in semen (Hamel et al., 2000; Larochelle et al., 2000; Le Tallec et al., 2001). Viral DNA has been detected in semen at least until day 47 post-inoculation, the latest day so far tested in an experimental study (Larochelle et al., 2000). On the other hand, PCV2 DNA detection in semen of naturally infected pigs has also been achieved (Hamel et al., 2000; Le Tallec et al., 2001). These studies could not conclude if infectious PCV2 was present in the semen since no virus isolation or swine bioassay was performed. Therefore, artificial insemination and natural mating have to be considered potential ways to disseminate PCV2 infection in the reproductive stock. It is not known at present if this situation is related to PMWS transmission or not.

Is PCV2 vertically transmitted?

Experimental studies have demonstrated a deleterious effect to foetuses (San-

chez et al., 2001, 2003, 2004; Johnson et al., 2002; Pensaert et al., 2004; Yoon et al., 2004) when PCV2 is directly inoculated into them and to PCV2 infected zona pellucida free embryos (Mateusen et al., 2004; Nauwynck et al., 2007). Moreover, evidence of vertical transmission (defined as transmission from one generation to the next by infection of the embryo or foetus in utero) exists (West et al., 1999; O'Connor et al., 2001), although it is not really known how this transmission takes place and if it is a frequent event or not. Potential natural routes of infection have been assessed in a very limited number of studies. On one hand, transplacental transmission of PCV2 has been demonstrated once following experimental intranasal infection of sows (Park et al., 2005), indicating that vertical transmission of PCV2 is feasible; however, in this study, authors did not give any information on the status of the semen they used to inseminate the sows. Opposite results were obtained when inoculating pregnant SPF sows via the intramuscular and tracheal routes; although acute symptoms were observed in sows, no PCV2 was detected in tissue samples from delivered piglets at birth (Cariolet et al., 2001a). On the other hand, another experiment dealt with intrauterine PCV2 infection with artificial insemination; the four inoculated SPF PCV2-seronegative sows got infected, one of them aborted and three out of four sows delivered an abnormally high number of stillborns and/or mummified foetuses (Cariolet et al., 2001b). Most of the delivered foetuses or neonatal pigs of those two studies contained PCV2 in their tissues. A more recent study on SPF PCV2 seropositive sows confirmed that the use of PCV2 infected semen can lead to severe reproductive disorders (mummifications) even in PCV2 seropositive sows, owing to the infection of foetuses during gestation (Rose et al., 2007). However, the frequency of these reproductive alterations under field conditions is apparently variable, since it has been reported as very rare in Europe (Maldonado et al., 2005; Pensaert et al., 2004), but data from Korea showed PCV2 infection in about 13% of aborted fetuses and stillborn piglets (Kim et al., 2004). The significance of embryo effects and return-to-estrus due to PCV2 infection (Nauwynck et al., 2007) under natural conditions has not been assessed yet.

Is vertically transmitted PCV2 infection linked to subsequent PMWS development?

Although deleterious effects of PCV2 on foetuses and zona pellucida free embryos have been described (see above), it is unknown if, under natural conditions, 1) viable delivered PCV2 congenitally infected piglets do exist, 2) the proportion of them if this is the case, and 3) the probability they have (if any) to develop PMWS subsequently at postweaning ages. The answer on the first point is probably yes, since it has been achieved experimentally (Sanchez et al., 2003; Park et al., 2005), but not contrasted data exist nowadays to answer the other two issues. Very recent data, however, indicates that piglets early infected during pregnancy and deprived of passive immunity are at a higher risk of suffering from PMWS subsequently (Rose et al., 2007).

Is semen playing a role in PMWS transmission?

Different epidemiological studies have been conducted throughout the world, but all of them, performed in the United Kingdom, Denmark, France, Sweden and New Zealand, ruled out semen as a major cause of PMWS diffusion and transmission (Desrosiers, 2007).

Which is the minimal PCV2 dose needed to infect a pig or to cause PMWS?

The minimal dose required to infect a pig with PCV2 has not been determined yet. Based on experimental trials, a dose of 102 TCID₅₀/pig (Ellis et al., 1999) or higher is enough to cause infection in inoculated pigs. On the other hand, it seems that the dosage of inoculum is not the key factor in the development of PMWS or PCV2 subclinical infection: PMWS has been reproduced with 2x10³ TCID₅₀/pig (Harms et al., 2001) intranasally inoculated, whereas a viral inoculum of 4,3x10⁶ TCID₅₀/pig in gnotobiotic pigs (Krawowka et al., 2001) or inoculation of a tissue homogenate containing PCV2 (106.8 TCID₅₀/pig) in conventional, PCV2 seronegative pigs (Balasch et al., 1999) have failed in disease reproduction. This could potentially be due to the existence of pathogenicity differences among PCV2 isolates; however, the multifactorial nature of PMWS prevents us to establish a given minimal dose of PCV2 to cause disease.

Does PCV2 cause a persistent infection in the pig?

PCV2 DNA has been demonstrated in serum from pigs up to 22 weeks of age under field conditions (Rodríguez-Arrijoja et al., 2002). Pigs with long-lasting PCV2 infection can occur in both PMWS and non-PMWS affected herds (Larochelle et al., 2003; Sibila et al., 2004). However, these studies did not assess whether pigs were continuously or intermittently viremic. Field data are further supported by experimental studies, since PCV2 has been

detected in blood and tissues of a high proportion of experimentally inoculated pigs at termination (days 21-71 post inoculation) of the experiments (Allan et al., 1999; Balasch et al., 1999; Krawowka et al., 2000; Magar et al., 2000; Pogranichny et al., 2000; Harms et al., 2001; Rovira et al., 2002; Resendes et al., 2004), and in one experiment, PCV2 nucleic acid was detected in tissues of a single pig sacrificed at 125 days PI (Bolin et al., 2001). Therefore, PCV2 is able to cause a persistent infection in a proportion of pigs, but the mechanism by which PCV2 persists in the pig is unknown.

Is PCV2 able to infect other species than pigs?

PCV2 infection and PMWS have been described both in domestic swine and wild boar (Segalés et al., 2005a). In regards other non-suidae species, serological surveys in cattle, goats, sheep, horses, dogs, cats, mice and humans have shown no evidence of infection (Allan et al., 2000; Ellis et al., 2001; Rodríguez-Arrijoja et al., 2003), and further serological data on a theoretically "high risk" populations, such as veterinarians, have also yielded negative results (Ellis et al., 2000). However, the first serological study in which serum samples from humans, cattle and mice were tested gave positive results (Tischer et al., 1995). Nowadays it is believed that those initial serological reactants probably corresponded to false positive results.

How many triggering factors do we know for PMWS occurrence?

PMWS is defined as a multifactorial disease which involves infection of pigs with PCV2 and the influence of infectious and non-infectious factors or triggers for the clinical disease occurrence.

Infectious risk factors or triggers: Viral (porcine reproductive and respiratory syndrome virus [PRRSV] and porcine parvovirus [PPV]) and bacterial (Mycoplasma hyopneumoniae) co-infections with PCV2 have been used to experimentally reproduce PMWS (Allan et al., 1999; Krawowka et al., 2000; Harms et al., 2001; Rovira et al., 2002; Opriessnig et al., 2004b). These experimental results have been further supported by epidemiological data (Rose et al., 2003; Vigre et al., 2006) and a wide spectrum of infectious agents have been observed concomitant with PCV2 infection in PMWS-affected farms (Rodríguez-Arrijoja et al., 1999; Pallares et al., 2002; Pogranichny et al., 2002; Segalés et al., 2002; Segalés and Domingo, 2002; Ellis et al., 2004). The immune status to PCV2 must also be considered as a risk factor for PMWS development, since experimental infections of PCV2 seropositive pigs do not yield clinical disease or caused lower severity of histological lymphoid lesions (McKeown et al., 2005; Ostanello et al., 2005). Moreover, PCV2 infection or low serological titers to PCV2 in sows at farrowing had a significant effect on the overall mortality of its offspring due to PMWS (Calsamiglia et al., 2007).

Non-infectious risk factors or triggers: Several conditions are believed to worsen the clinical outcome in PMWS affected farms. 1) Some of these "worsening" or risk factors have been assumed as a consequence of empirical farm work, such as management. Initial studies in France on PMWS affected farms (Madec et al., 2000) detected significant management deviations and the implementation of what is today known as the Madec's 20-point plan (a list of management measures to lower the impact of the disease) significantly decreased the percentage of mortality in severely affected farms. 2) Several case-control studies to assess risk factors have been conducted in Denmark (Enøe et al., 2006), France (Rose et al., 2003), Spain (López-Soria et al., 2005) and the United Kingdom. Some factors that were linked to lower risk of PMWS included biosecurity measures, quarantine of purchased pigs, changes of boots/clothes, long empty periods in weaning and farrowing facilities, regular treatment against external parasites and housing sows in collective pens during pregnancy all decreased the odds of PMWS. 3) The experimentally induced PMWS in gnotobiotic pigs following PCV2 infection and stimulation of the immune system (Krawowka et al., 2001) has also been supported by a number of on-farm studies, where PCV2 infection and the use of certain commercially available pig vaccines (mineral oil adjuvanted vaccines) or immunomodulators have acted as apparent triggering factors for PMWS (Allan et al., 2001; Kyriakis et al., 2002; Grasland et al., 2005). Therefore, although some controversial results have been generated on this topic on conventional pigs, it should be at least considered that immune activation may be a potential triggering factor of PMWS under certain non-determined circumstances. 4) Field observations from farmers and veterinarians have suggested that certain genetic lines of pigs, specifically in relation to boar lines, are more or less susceptible to PMWS. This observation has been supported by recent experimental studies where Landrace pigs were experimentally shown to be more susceptible to development of PMWS lesions than Duroc and Large White pigs (Opriessnig et al., 2006c). Other studies have shown contradictory results with the use of Pie-

train boar line; while the use of this genetic line did not seem to have any effect on the offspring in one study (Rose et al., 2005), another study showed lower general postweaning and PMWS associated mortalities (López-Soria et al., 2004). Further work is nowadays ongoing to determine the role of genetics in susceptibility/resistance to PMWS.

Do we know how to control PMWS?

Since PMWS is a multifactorial disease, consequently, effective control measures, without the control of PCV2 infection, have focused on the understanding of the co-factors and triggers involved on individual farms and the control or eradication of these triggers. The most studied co-factors and triggers in relation to disease progression or protection have been outlined above. Following PMWS control recommendations are based on scientifically and technically oriented research.

Management: Significant positive results have been obtained when the so-called "Madec's 20 point plan" measures; best improvement was achieved when the rate of compliance with the recommended measures was high (Guilmoto and Wessel-Robert 2000).

Concurrent infections: Control of concurrent viral and bacterial infections in the postweaning area should decrease the incidence of PMWS. From a practical point of view, attempts to control PMWS with PPV vaccination on finishing sites in the USA with confirmed PPV circulation have been repeatedly successful (Halbur, 2001). However, this positive effect of PPV vaccination in reducing the clinical incidence of PMWS has not been experimentally proven (Opriessnig et al., 2004a). To date, no published results are available on the control of PRRSV or Mycoplasma hyopneumoniae infection (by vaccination or other systems) to mitigate effects of PMWS.

Immunomodulation: From a practical point of view, removal of vaccines from sanitary programs may be inappropriate, since the risk of eliminating effective vaccines may be greater than the risk of inducing PMWS in a low percentage of pigs in a given pig population. Therefore, based on the available results, producers with PMWS affected herds should consider determining the approximate timing of PCV2 infection, with the objective of re-scheduling the timing of vaccination as a potential plan to minimize the disease (Opriessnig et al., 2006a).

Nutrition: Partial control of epizootic PMWS was achieved in some farms in the United Kingdom by changes in the diet of affected pigs (Donadeu et al., 2003). On the other hand, a single study has shown that conjugated linoleic acid ameliorates PCV2 experimental infection (Bassaganya-Riera et al., 2003). Although some preliminary field and experimental data suggest that certain nutritional factors might favor a decrease in PMWS outcome, there is not enough scientific information to establish the real effect of nutrition on this disease.

Serum-therapy: Subcutaneous injection of PCV2 hyperimmune sera from commercial slaughterhouse age pigs in suckling or nursery pigs has been reported as successful in reducing mortality in PMWS affected farms (Ferreira et al., 2001; Waddilove and Marco, 2002). However, success of this procedure has been variable, and the use of "serum-therapy" in some farms did not result in any significant effect. A more controlled use of PCV2 hyperimmune sera in case-control (natural or experimental) studies did not show a uniform or general significant effect of this practice on controlling PMWS or decreasing the severity of PMWS lesions (Hassing et al., 2006; Opriessnig et al., 2006b). The mechanism of action of serum-therapy has not been elucidated as yet. Moreover, serum-therapy should be used in a strictly controlled way to prevent from the spreading of other potentially present highly infectious diseases.

Do we know how to prevent PMWS?

The newest information in regards PMWS/PCV2 infection comes from the advent of PCV2 vaccines and its field application, both from Europe and North-America. Previous scientific studies already showed that different vaccine prototypes, including those based on inactivated PCV2 isolates (Pogranichniy et al., 2004), a chimeric PCV1/PCV2 virus (Fenaux et al., 2003), and PCV2 DNA and subunit vaccines (Blanchard et al., 2003b), were able to decrease lesional severity in lymphoid tissues, and to shorten PCV2 shedding and viremia length. However, those prototypes were never tested under field conditions and its real efficacy was really unknown.

In 2004, an inactivated, adjuvanted PCV2 vaccine for use in sows and gilts was commercially available and in use under special license in some European countries (France and Germany). The same vaccine was available in

Denmark, some farms in the United Kingdom and Canada since 2006. Nowadays, more than a half million of sows has been vaccinated in Europe and a similar amount in Canada, obtaining significant reductions in mortality in the postweaning area. In 2006, three PCV2 vaccines for use in piglets came to the market in North-America (Canada and/or USA). One of them was based in the inactivated chimeric virus cited above while the other two were based on the PCV2 ORF2 product (capsid protein) expressed in a baculovirus system. Preliminary reports have also shown a significant improvement of postweaning mortality, being in some cases extremely positive. Certainly, PCV2 vaccines seem to be one of the major weapons that can be used to control or prevent PMWS in those severely affected farms. Surprisingly, the overall mortality levels achieved after PCV2 vaccination has dropped, sometimes, to values even lower than those previous to the PMWS outbreak in some farms. These results may suggest that those vaccines, at least in some cases, may have been also able to counteract certain unknown or subclinical effects caused by or associated to PCV2 infection.

Discussion

Known and unknown factors on PMWS epidemiology, control and prevention have recently been reviewed (Segalés and Torremorell, 2006). The present paper tried to highlight some of the most significant issues related to those topics. During the last ten years we have addressed many investigations on PCV2 infection, but PMWS epidemiology is still poorly understood. The key epidemiological question that still remains to be answered regarding PMWS is the sudden appearance of PMWS cases despite the widespread of PCV2 infection. While PMWS appeared as a worldwide epidemic, PCV2 has been present in pigs for many decades. It is also important to note that PCV2 causality on PMWS has been a matter of debates for many years both in Europe and North-America. Is the epizootic form of PMWS the result of the introduction of a new PCV2 strain? The introduction of a new, yet not identified, infectious agent ("agent X")? Due to a still unknown non-infectious factor? Or just due to a combination of infectious and non-infectious factors together with PCV2? Most of these questions are still unanswered. On the other hand, the apparent clear efficacy of PCV2 vaccines in controlling PMWS further supports the central role of this virus in PMWS causality. However, vaccine situation does not change the status of PMWS as a multifactorial disease; therefore, other concomitant factors may have a worsening effect in PCV2 infected piglets and the improvement of management, control of concurrent diseases, immunostimulation, etc. must still be considered when designing a plan to prevent PMWS in a given farm.

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