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**A META-ANALYSIS ON EXPERIMENTAL INFECTIONS WITH PORCINE
CIRCOVIRUS TYPE 2 (PCV2)**

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Short title: A meta-analysis on PCV2 experimental infections

Abstract

A meta-analysis was performed with the aim to identify factors with a relevant influence on the expression of clinical postweaning multisystemic wasting syndrome (PMWS) under experimental conditions. Data from 44 studies were included in the analysis. Several variables were studied: number of pigs in the experiment, intake of colostrum, serological status against porcine circovirus type 2 (PCV2), strain of PCV2 used for inoculation, the route and dose of inoculation, use of potential triggering factors (such as co-infections, vaccinations, or immunomodulator products). Multiple correspondence analysis and log-linear regression methods were used to establish the relationships between the studied variables and the number of PCV2 infected pigs that developed PMWS. Based on the results of the meta-analysis, the most successful animal experiment aimed to develop PMWS should include: 1) colostrum-deprived pigs, 2) age of inoculation below three weeks, 3) high doses of PCV2 inoculum, 4) PCV2 strain from genotype 1, and 5) co-infection with another swine pathogen as a triggering factor.

Keywords: experimental infections / meta-analysis / porcine circovirus type 2 / postweaning multisystemic wasting syndrome

1. Introduction

Porcine circovirus type 2 (PCV2) has been recognized as the essential infectious agent of postweaning multisystemic wasting syndrome (PMWS) (Allan et al., 1999; Bolin et al., 2001). This syndrome is a multifactorial disease that mainly affects nursery and fattening pigs of almost all types of farms (Madec et al., 2000; Rose et al., 2003; López-Soria et al., 2005). PMWS characteristic clinical signs are severe weight loss (wasting), pallor of skin, respiratory distress, diarrhoea and, occasionally, jaundice (Harding et al., 1997). The morbidity and mortality rates due to PMWS are highly variable from farm to farm, ranging from 4 to 30%, depending on the type of farm, husbandry and management practices, co-infections with other swine pathogens, or host genetics (Segalés et al., 2005).

Although PCV2 infection has been shown to be necessary for the full expression of PMWS, it is known that the virus is present in both healthy and diseased pigs and herds (Rose et al., 2003; Sibila et al., 2004). However, it has been traditionally difficult to reproduce the severe clinical expression of the disease observed under field conditions through experimental infections with PCV2 alone (Allan et al., 1999, Albina et al., 2001, Resendes et al., 2004). Therefore, it has been suggested that other infectious or non-infectious factors must be concomitantly influencing the development of PMWS in the field.

Experimental models are essential for researchers to study the pathogenesis of infectious diseases, host immunological responses, as well as to evaluate the efficacy of vaccines against an etiological agent. Up to date, however, it has been difficult to establish a universal effective model for the reproduction of PMWS in swine. Few studies have been able to reproduce PMWS by inoculating PCV2 alone (Bolin et al., 2001, Okuda et al., 2003, Lager et al., 2007). The apparently most successful model, understanding it as the achievement of clinical disease, implies the infection with PCV2 together with other swine pathogens, such as porcine parvovirus (PPV) (Allan et al., 1999, 2003; Krakowka et al., 2000; Kim et al., 2003;

Opriessnig et al., 2004a; Hasslung et al., 2005), porcine reproductive and respiratory syndrome virus (PRRSV) (Allan et al., 2000b; Harms et al., 2001; Rovira et al., 2002; Opriessnig et al., 2006c) or *Mycoplasma hyopneumoniae* (Opriessnig et al., 2004b), or the use of non-infectious immunomodulators like the keyhole limpet hemocyanin in incomplete Freund's adjuvant (KLH-ICFA) (Krakowka et al., 2001, 2002; Ladekjaer-Mikkelsen et al., 2002; Grasland et al., 2005). However, a larger number of unsuccessful (lack of clinical PMWS reproduction) experiments have been reported (Allan et al., 2000a,b; Loizel et al., 2005; Ostanello et al., 2005; Opriessnig et al., 2006a,c,d; Fernandes et al., 2007; Fort et al., 2008).

Reasons for the inconsistent results achieved by independent studies are not yet known, but there are several factors that may explain the different outcomes of experimental infections with PCV2: origin of pigs, age of the animals at inoculation, immunological status, genetic predisposition, PCV2 strain, type of inoculum, infectious dose, and route of administration. In the present work we have performed a meta-analysis aimed at identifying the most relevant factors that must be accounted for the successful reproduction of PMWS under experimental conditions.

2. Materials and methods

2.1. Data source

The studies included in the present meta-analysis were obtained from peer reviewed papers available at the public database PubMed (<http://www.ncbi.nlm.nih.gov/sites/entrez>) as well as from not yet published results presented in scientific international congresses and conferences which provided a detailed description of the methodology used and results obtained (LeCann et al., 1998; Stockhofe-Zurwieden et al., 2003; Krakowka et al., 2006). The

articles retrieved dealt with infections in non-adult pigs for experimental reproduction of PMWS. Inoculations in boars and/or pregnant sows were excluded from the analysis since they were beyond the scope of this study. Only information regarding PCV2-inoculated pigs with or without concomitant treatments was considered. Therefore, control pigs which were mock inoculated, immunostimulated or infected with other swine viruses alone, or contact-exposed to PCV2 were not included in the analysis. An experimental unit was defined as a group of pigs that had received an identical treatment within the same study. Several variables were annotated from each experimental unit:

- 1) Research group: 12 categories (RG1 to RG12).
- 2) Sample size: number of inoculated pigs in each experimental unit (three categories used for multiple correspondence analysis: $N \leq 6$, $7 < N < 10$, or $N \geq 11$).
- 3) Age of pigs at inoculation: 4 categories (1-7 days, 1-3 weeks, 4-5 weeks, or > 5 weeks).
- 4) Colostrum deprivation: 2 categories (CD_YES or CD_NO).
- 5) Seronegative for PCV2 before inoculation: 2 categories (SN_YES or SN_NO). This category is different from the previous one, since PCV2 seronegativity was achieved either by colostrum deprivation or by colostrum antibody waning.
- 6) Route of inoculation: 2 categories (intranasal or other routes; latter one included intraperitoneal, intratracheal, intramuscular, intralymphoid, or intrahepatic inoculation routes).
- 7) PCV2 strain: 22 different strains.
- 8) PCV2 genotype: 2 categories (genotypes 1 or 2, Grau-Roma et al., 2008).
- 9) Dose of inoculum: 4 categories ($< 10^4$, 10^4 - 10^5 , 10^5 - 10^6 , or $> 10^6$ TCID₅₀ or equivalent per pig).

10) Type of experiment: 4 categories (PCV2 alone (PCV2), PCV2 plus immunostimulation and/or vaccination (ISV), PCV2 plus co-infection (CI), or PCV2 plus immunostimulation and/or vaccination plus coinfection (ISV-CI).

11) Success: a response variable was defined to classify experimental units on the basis of the percentage of PMWS-affected pigs. This variable allowed defining 4 categories (0%, 0-25%, 25-50%, and >50% of PMWS-affected pigs).

2.2. Statistical analyses

2.2.1. Univariate analysis

Main descriptive statistics were calculated for quantitative (mean, median, standard deviation, maximum, and minimum) and for qualitative (frequency tables) variables.

2.2.2. Bivariate analysis

Contingency tables were used to explore the relationships between each independent variable and the percentage of pigs affected (4 categories of success).

2.2.3. Multivariate Analysis

Multiple Correspondence Analysis (MCA) techniques combined with classification methods were used to analyse the pattern of relationships of the related variables. MCA is a descriptive, exploratory technique designed to analyse multi-way contingency tables with cases as rows and categories of variables as columns (Greenacre 2007). The main objective of this technique is to summarize a group of categorical variables, whose associations are jointly considered, thus reducing initial variability. This analysis shows a group of subjects with similar profiles regarding the chosen characteristics to describe them (categorical variables).

Classification methods were used to complement the results of the MCA making the interpretation of the results easier. The advantage of classification methods is that they incorporate a new element (the classes) easier to interpret than factorial axes. The classification algorithm used was the mixed classification method (Lebart et al., 2000), a combination of the algorithm of aggregation around gravity centres and the hierarchical classification method. Nominal active variables used for MCA and direct hierarchical classification were: age at inoculation, colostrum deprivation, PCV2 serological status, route of inoculation, PCV2 genotype, dose of PCV2 inoculum, and type of experiment. Other variables were considered as illustrative variables which were not used in the analysis but were helpful for interpretation of results. These illustrative variables were: research group, sample size, PCV2 strain, severity of PMWS, and the percentage of affected pigs (variable success). The SPAD software (*Système Portable pour l'Analyse des Données, Centre International de Statistiques et d'Informatique Appliquées* (C.I.S.I.A.), Saint Mandé, France) was used for classification and multiple correspondence analyses.

2.2.4. Poisson regression models

A Poisson regression model (Cameron and Trivedi, 1998) was established using the count of affected pigs following PCV2 inoculation as a response variable. The presence of over-dispersion of the response variable was taken into account in the model. The sample size used in the analysed experiments was highly heterogeneous among them and was, therefore, considered as an offset term of the model. Due to the fact that experimental units coming from a certain experiment are correlated, dependencies between non-independent observations were also considered in the model. Firstly, a Poisson regression model was established separately for each covariate to identify those that had a significant effect on the number of affected pigs following PCV2 inoculation. Secondly, a global Poisson regression

model was fitted including all covariates simultaneously. This strategy allowed us to account for possible interactions between variables non-detectable under the bivariate model approach. Then, after a selection procedure, only those variables that remained significant were kept in the final global model.

The GLIMMIX procedure of the SAS software (SAS Institute Inc., Cary, NC, USA) was used for fitting the Poisson regression models. The offset parameter was introduced into the model statement and the random statement was used for taking into account the possible non-independence among observations and the presence of over-dispersion. The significance level was set to 0.05.

3. Results

3.1. Descriptive statistics

A total of 100 experimental units, derived from 44 independent studies, were included in the analyses (Table 1). Sixty percent of the experimental units were performed by only two Research groups (RG1 and RG2), which accounted for 31% and 29% of them, respectively. The remaining 40% of experimental units were done by 10 different Research Institutes.

Sample size was highly variable between experimental units, ranging from 3 to 58 pigs, with a mean sample size of 10.02 inoculated pigs per experimental unit. The age of pigs at inoculation day was also variable ranging from one day to more than five weeks old.

With regards to the source of the pigs used, animals were fed with colostrum in 61% of the experimental units, among which 65.6% (40 out of 61) and 34.4% (21 out of 61) were seronegative and seropositive against PCV2 at the time of challenge, respectively. As expected, all colostrum-deprived pigs (in 39 out of 100 experimental units, 39%) were seronegative against PCV2 at the moment of inoculation.

The effect of genetics could not be considered because in most studies the breed of the pigs used was not specified.

As much as 22 different PCV2 strains have been tested, although the ISU-40895 and the Stoon-1010 ones have been the most extensively used, comprising more than 50% of the experimental units (28 and 27 experimental units, respectively). Given the high level of imbalance on PCV2 strain data we considered the genotype of the virus (Olvera et al., 2007; Grau-Roma et al., 2008). Two strains from 3 different works were not classified in any phylogenetic group since their genomic sequence was not available (Balasch et al., 1999; Rovira et al., 2002; Ostanello et al., 2005). Almost 80% of the remaining strains corresponded to genotype 2.

The most frequent route of inoculation was the intra- or oro-nasal route (87%), which used variable doses of inoculum ranging from 10^2 TCID₅₀/pig to 10^6 TCID₅₀/pig. In 57% of the experimental units, PCV2 was inoculated alone; the remaining most frequent designs used PCV2 and co-infection with PPV (12%), immunostimulation with KLH-ICFA (8%), co-infection with PRRSV (7%), and co-infection with *M. hyopneumoniae* (6%).

Only 38% of the experimental infections resulted in reproduction of PMWS, with a mean percentage of affected pigs of 15.1%.

3.2. Bivariate analysis

Table 2 summarizes the distribution of the number and percentage of experimental units within each variable category classified by the percentage of PMWS-affected pigs (four categories). More than 80% of the experimental units that reproduced overt signs of PMWS in more than 50% of the PCV2-infected pigs, inoculated the virus at ages comprised between the first day and 3 weeks of life (9 out of 11 experimental units). Conversely, none of the

experimental units inoculating pigs older than 5 weeks of age could reproduce disease in such a high percentage of pigs.

More than 50% (21 out of 39) of experimental units using colostrum-deprived pigs could reproduce PMWS in at least one pig. Of them, 23.1% yielded more than 50% of affected pigs. Conversely, in colostrum-fed models, only 27.9% (17 out of 61) succeeded in the reproduction of disease in at least one pig and only 3.3% of the cases with more than 50% of affected pigs. Similarly, models using PCV2-seronegative pigs were able to reproduce PMWS in approximately 45% of the cases, with 13% (10 out of 77) of them yielding more than 50% of affected pigs, whereas inoculation of PCV2-seropositive pigs only led to 13% of successful cases (4.3% with more than 50% of affected pigs).

With regards to the PCV2 genotype, a higher percentage of successful experiments (50%, 10 out of 20) were annotated in experiments using a PCV2 inoculum from genotype 1 compared to those experiments using strains from genotype 2 (36%, 27 out of 75), despite the lower number of experiments using PCV2 strains belonging to genotype 1. Although similar percentages of affected pigs were achieved with inoculums from both genotypes, they were slightly higher for those experiments using inoculums from genotype 1. While 30% (6 out of 20) of the experimental units using an inoculum from genotype 1 achieved more than 25% of affected pigs, only 18.7% (14 out of 75) of experimental units using a PCV2 inoculum from genotype 2 reached this percentage of affected pigs.

If dose of inoculum is taken into account, it can be observed that the higher the dose of PCV2, the higher the percentage of PMWS-affected animals. However, several experiments have been proven to be successful with low doses of PCV2 (Allan et al., 1999; Harms et al., 2001).

Finally, some experiments inoculating PCV2 alone reproduced PMWS (26.3% of total PCV2-alone experimental units), but the vast majority of successful experiments were those

in which co-infection or immunostimulation-vaccination models were used (75% and 41.7% of successful experiments recorded within each model, respectively). Interestingly, co-infections produced higher percentages of PMWS-affected pigs (31.2% of experimental units with more than 50% of affected pigs) than the use of immunomodulators or vaccines/adjuvants (16.7%).

3.3. Multivariate Analysis

Figure 1 shows the result of the MCA in a two dimensional plane, according to the two first axes of the MCA (32.9% of inertia, Table 3). It can be seen that the percentage of PMWS affected pigs is positively correlated to the first axis (horizontal) and negatively correlated to the second axis (vertical). The nominal active modalities that appear to be more associated to the percentage of affected pigs are the ones that fall into the right and bottom square of Figure 1 (i.e.: 1-7 days, CD_YES, SN_YES, 10E5-10E6 TCID50, >10E6 TCID50, CI, and ISV). This figure, however, should be interpreted with caution due to the fact that only information on the first two axes is used and a small proportion of variability of data is explained by them. For classification and statistical description of classes the first seven axes of the MCA analysis, which jointly explained 76.3% of total data variability, were used (Table 3). Results of the mixed classification method are shown in Table 4. Experimental units were assigned, based on the similarity of their experimental design (i.e., similar modality characteristics), in four different classes (namely I, II, III, and IV). Two main classes, II and IV, accounted for 30% and 42% of the total number of experimental units, respectively. These two classification groups appeared to discriminate experimental units by their level of success on reproducing PMWS.

Experimental units included in class II were characterized by the use of piglets fed with colostrum, inoculated at an older age than 5 weeks and that received low to moderate

doses of PCV2 inoculum (10^4 - 10^5 TCID₅₀/pig). Seventy percent (21 out of 30) of the experimental units associated to class II did not reproduce PMWS disease (all inoculated piglets remained healthy throughout the experiment). Amongst the remaining 30% (9 out of 30) of experiments within this class, the average percentage of affected pigs was 2.5%. Thus, class II mostly grouped non-successful experimental units.

Conversely, class IV was mainly associated to successful experiments. The most representative modalities of this class were deprivation of colostrum, inoculation during the first week of life, and inoculation of high doses of PCV2 ($>10^6$ TCID₅₀/pig). Remarkably, more than 80% of all the reported experimental units with rates of success above 50% of affected pigs were grouped in this class, representing 21.4% of all the experimental units associated to class IV (9 out of 42). Finally, the average percentage of affected pigs of all experimental units belonging to class IV was considered high (23.8%).

The route of inoculation and the PCV2 genotype did not appear to be relevant variables in any class because identical modalities (genotype 2 and intranasal route of inoculation) were present in both class II and class IV groups.

No remarkable association with the rate of success on reproducing PMWS was found for the remaining classes I and III.

3.4. Poisson regression models

The response variable of the models (affected number of piglets) was assumed Poisson distributed. However, the response variable was overdispersed with respect to the Poisson distribution (deviance/df = 2.38). To model this situation we allowed the variance function to have a multiplicative overdispersion factor Φ : $\text{Var}(y) = \Phi \mu$. This is the NB1 model described by Cameron and Trivedi (1998). The standard errors of the parameters fitted by the Poisson regression model were corrected by using the estimated value of Φ .

In the individual model, variables with a significant effect on the number of affected pigs were, in decreasing order of significance: intake of colostrum ($F=13.7$, $P=0.0005$), type of experiment ($F=4.5$, $P=0.007$), age at inoculation ($F=3.1$, $P=0.0357$), and serological status of pigs before infection ($F=4.0$, $P=0.0495$). PCV2 genotype, route of inoculation, and dose of inoculum used for infection of pigs did not have a significant individual effect. The estimated differences of least squares means (β) and the ratio of the expected number of affected pigs ($\exp(\beta)$) are displayed in Table 5. As it is shown, the ratio of expected number of PMWS affected pigs was about four times higher ($\exp(\beta)=4.3$, $P=0.0005$) for colostrum-deprived pigs compared to colostrum-fed pigs. Similarly, seronegative piglets were 3.5 times ($P=0.0495$) more prone to develop PMWS after infection with PCV2 than seropositive piglets. Furthermore, the mean number of affected pigs was significantly higher in piglets inoculated within the first 3 weeks of age compared to those inoculated at older ages than 5 weeks. With regards to the type of experiment, co-infections with other swine viruses significantly resulted in higher expected mean number of affected pigs compared to experiments inoculating with PCV2 alone ($\exp(\beta)=3.6$, $P=0.0006$). Co-infections tended to produce two times more affected pigs than experiments using other immunostimulant factors ($\exp(\beta)=2.2$, $P=0.0791$).

In the global model, the only variables that remained significant were colostrum intake status of pigs ($F=28.8$, $P<0.0001$) and genotype of PCV2 strain ($F=12.0$, $P=0.0008$). The effect of colostrum deprivation was even more marked in the global analysis than in the individual analysis, with a mean expected number of affected pigs almost six-fold higher in colostrum-deprived pigs compared to colostrum-fed pigs ($\exp(\beta)=5.8$, $P<0.0001$, Table 5). The PCV2 genotype effect, that was not significant in the individual analysis, now had a significant effect when the intake of colostrum was jointly considered in the model. As shown in Table 5 the ratio of expected number of affected pigs was more than three-fold higher for PCV2 genotype 1 compared to genotype 2 ($\exp(\beta)=3.5$, $P=0.008$). The interaction effect

between colostrum deprivation and PCV2 genotype was included in the model but did not reach the statistical threshold for significance (data not shown).

4. Discussion

Meta-analysis is a powerful statistical tool that has become widely used for the validation of previous research on a common topic and has been applied to multiple areas of knowledge including epidemiology (Greenland, 1987; Blettner et al., 1999). To our knowledge, this is the first meta-analysis done to evaluate the performance of experimental infections with an infectious agent in pigs. Two statistical approaches were used, including a multiple correspondence analysis (MCA) to summarize the relationships between the studied variables, and log-linear regression models to determine the effect of significant variables on the number of PMWS-affected pigs. Both methodologies used are complementary. MCA allows the characterization of groups of papers sharing similar characteristics and regression techniques model the frequency of success. By their combination a better understanding of data structure is achieved, thus facilitating the interpretation of results (Panagiotakos and Pitsavos, 2004).

The MCA analysis indicated that the main common characteristics shared by successful experiments (i.e., experiments that reproduce PMWS clinical signs in at least one PCV2-inoculated animal) are the inoculation of colostrum-deprived pigs during the first week of age with high doses of PCV2 inoculum. These results were mostly confirmed by regression models. In this sense, the intake of colostrum and the age at inoculation had a significant individual effect on the expected number of pigs that develop PMWS after infection with PCV2. In addition, the type of experiment (co-infections with PCV2 and other viruses, or the use of immunomodulators or vaccine/adjuvants) also had a significant individual effect.

Passively acquired antibodies through colostrum ingestion confer protection against viral infections to newborn piglets (Ward et al., 1996; Parreno et al., 1999; Suradhat and Damrongwatanapokin, 2003). The presence of antibodies to PCV2 has been shown to reduce the peak viremia levels (McKeown et al., 2005) and to protect pigs from development of severe PCV2-associated lesions (Allan et al., 2002; Opriessnig et al., 2006b). This situation would explain why naturally PMWS-affected pigs are usually aged between 5 to 12 weeks (Segalés et al., 2005), once maternal derived antibodies waned; however, a single case of diseased pigs at earlier ages has been reported (Hirai et al., 2001). The higher potential of success obtained for inoculations at younger ages could be due to the fact that colostrum-deprived pigs are used to assure that no maternal immunity was acquired by piglets before infection. In contrast, inoculations at older ages usually imply that piglets have been fed with colostrum. This latter fact means that, in most cases, piglets have acquired maternal antibodies against PCV2 and certain levels of maternal immunity (maybe not detectable by current laboratorial techniques) that may remain at the time of inoculation at older ages. In this sense, only one experiment has been reported in which caesarean-derived, colostrum-deprived (CD-CD) piglets were inoculated at 8 weeks of age; however, none of the infected piglets developed PMWS (Progranichnyy et al., 2000).

The dose of inoculum was not a significant variable in the regression models, although it has been suggested that the severity of the disease is dose-dependent and, for most of the cases, higher doses of inocula led to reproduction of PMWS experimentally (Albina et al., 2001). However, the administration of relatively high amounts of inocula does not always guarantee the development of PMWS (Resendes et al., 2004; Yu et al., 2007; Fernandes et al., 2007). Conversely, Allan et al. (1999) could reproduce PMWS by administering only $10^{2.4}$ TCID₅₀ to each infected piglet.

The use of co-infections (Allan et al., 1999; 2003; Krakowka et al., 2000; Harms et al., 2001; Rovira et al., 2001; Kim et al., 2003; Stockhofe-Zurwieden et al., 2003, Opriessnig et al., 2004a; 2004b; Hasslung et al., 2005), immunomodulators (Krakowka et al., 2001, 2002; Nielsen et al., 2003; Grasland et al., 2005; Loizel et al., 2005), or vaccine adjuvants (Krakowka et al., 2006; 2007; Hoogland et al., 2006) have been proven effective to reproduce PMWS in several experiments. However, many authors have reported that stimulation of the immune system does not have a critical effect on intensifying the disease (Ladekjær-Mikkelsen et al., 2002; Resendes et al., 2004; Loizel et al., 2005; Fernandes et al., 2007). The underlying mechanism by which activation of the host immune system interacts with PCV2 and empowers its replication still remains unsolved. However, results of the meta-analysis study indicate that these schemes have a higher potential for reproducing PMWS than the inoculation of PCV2 alone.

An interesting finding was that the genotype of the PCV2 strain used for inoculation was significant when the intake of colostrum was jointly considered in the global analysis. In contrast, PCV2 genotype did not have an individual significant effect, probably due to the low number of experiments using PCV2 strains from genotype 1. Therefore, it is very likely that no enough statistical power was available to detect such differences. However, different PCV2 genotypes seem to differ in pathogenicity, as it has been suggested by both *in vitro* (Meerts et al., 2005) and *in vivo* experiments (Hasslung et al., 2005; Opriessnig et al., 2006; Lager et al., 2007). Therefore, further studies should be performed to elucidate if certain PCV2 strains have a higher potential of developing PMWS under experimental conditions.

The main limitation of this meta-analysis stems from the inherent limitations of the publications included in the analysis. Our conclusions could be biased due to the fact that negative results obtained from experiments with identical experimental design to those with positive results are finally not published. Another reason for bias in our study is the fact that

most of the experiments included in this meta-analysis were reported by few research groups, which tend to use a determined strategy (successful or not) and their relative weight could have had an important impact on the results.

Finally, several factors could not be studied because they were not explicitly mentioned in a substantial number of publications (i.e., genetics of the pigs and number of passages of the virus inoculum). Genetic susceptibility to suffer from PMWS has been pointed out both under field (Madec et al., 2000; López-Soria et al., 2005; McIntosh et al., 2006; Zhou et al., 2006) and experimental (Opriessnig et al., 2006b) conditions. On the other hand, it has been demonstrated that, after serial cell passages, the original PCV2 virus experiences adaptive genetic changes that can lead to virulence attenuation *in vivo* (Fenaux et al., 2004). The number of PCV2 passages in cell culture for the corresponding inocula was available in a very few number of publications.

In summary, this meta-analysis contributes to a better understanding of the factors that have a major influence on the successful development of PMWS under experimental conditions. Specifically, the highest likelihood to achieve PMWS in an experiment includes pigs younger than 3 weeks of age, colostrum-deprived, inoculated with high doses ($>10^5$ TCID₅₀/pig) of PCV2 from genotype 1 and, preferably, in co-infection with another porcine pathogen.

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Tables

Table 1. List of references included in the meta-analysis. The research group (RG) and the number of experimental units (N) of each study are specified.

Research Group	References	N	Research Group	References	N
RG1	Fenaux et al 2002	3	RG2	Krakowka et al 2002	4
RG1	Fenaux et al 2003	1	RG2	Krakowka et al 2005	5
RG1	Fenaux et al 2004	4	RG3	Balasch et al 1999	1
RG1	Lager et al 2007	2	RG3	Fernandes et al 2008	2
RG1	McKeown et al 2005	2	RG3	Fort et al 2008	4
RG1	Opriessnig et al 2004a	3	RG3	Resendes et al 2004	2
RG1	Opriessnig et al 2004b	2	RG3	Roca et al 2004	3
RG1	Opriessnig et al 2006a	2	RG3	Rovira et al 2001	2
RG1	Opriessnig et al 2006b	6	RG4	Albina et al 2001	2
RG1	Opriessnig et al 2006c	3	RG4	Grasland et al 2005	2
RG1	Opriessnig et al 2006d	2	RG4	Le Cann et al 1998	2
RG1	Yu et al 2007	1	RG4	Loizel et al 2005	2
RG2	Allan et al 1995	1	RG5	Bolin et al 2001	1
RG2	Allan et al 1999	2	RG5	Harms et al 2001	2
RG2	Allan et al 2000a	2	RG5	Progranichnyy et al 2000	1
RG2	Allan et al 2000b	2	RG6	Ladekjaer-Mikkelsen et al 2002	2
RG2	Allan et al 2003	2	RG7	Stockhofe-Zurwieden et al 2003	5
RG2	Allan et al 2007	2	RG8	Ostanello et al 2005	2
RG2	Ellis et al 1999	1	RG9	Magar et al 2000	1
RG2	Hasslung et al 2005	2	RG10	Wang et al 2007	2
RG2	Krakowka et al 2000	3	RG11	Okuda et al 2003	1
RG2	Krakowka et al 2001	3	RG12	Kim et al 2003	1

Table 2. Distribution of the number of experimental units (and row percentage) within each variable category classified by the percentage of PMWS-affected pigs.

^a PCV2: PCV2 alone, ISV: PCV2 + immunostimulator or vaccination, CI: co-infection, ISV-CI: combination of categories ISV and CI.

Variables	Percentage of PMWS-affected pigs				Total (N=100)
	0%	< 25 %	25-50 %	> 50 %	
Age at inoculation					
1-7 days	16 (53.3)	6 (20.0)	2 (6.67)	6 (20.0)	30
1-3 wk	7 (46.7)	3 (20.0)	2 (13.3)	3 (20.0)	15
4-5 wk	15 (62.5)	4 (16.67)	3 (12.5)	2 (8.33)	24
> 5 wk	24 (77.4)	5 (16.1)	2 (6.5)	0 (0)	31
Colostrum deprivation					
NO	44 (72.1)	10 (16.4)	5 (8.2)	2 (3.3)	61
YES	18 (46.1)	8 (20.5)	4 (10.3)	9 (23.1)	39
Seronegative for PCV2					
NO	20 (87.0)	2 (8.7)	0 (0)	1 (4.3)	23
YES	42 (54.5)	16 (20.8)	9 (11.7)	10 (13.0)	77
Route of inoculation					
intranasal	53 (60.9)	15 (17.2)	8 (9.2)	11 (12.6)	87
other	9 (69.2)	3 (23.1)	1 (7.7)	0 (0)	13
Genotype					
1	10 (50.0)	4 (20.0)	4 (20.0)	2 (10.0)	20
2	48 (64.0)	13 (17.3)	5 (6.7)	9 (12.0)	75
Dose (TCID₅₀/pig)					
< 10E4	5 (41.7)	2 (16.7)	4 (33.3)	1 (8.3)	12
10E4 – 10E5	18 (66.7)	5 (18.5)	2 (7.4)	2 (7.4)	27
10E5 – 10E6	19 (65.5)	4 (13.8)	3 (10.3)	3 (10.3)	29
> 10E6	11 (57.8)	4 (21.1)	0 (0)	4 (21.1)	19
Type of experiment^a					
PCV2	42 (73.7)	8 (14.0)	5 (8.8)	2 (3.5)	57
ISV	14 (58.3)	6 (25.0)	0 (0)	4 (16.7)	24
CI	4 (25.0)	3 (18.8)	4 (25.0)	5 (31.2)	16
ISV-CI	2 (66.7)	1 (33.3)	0 (0)	0 (0)	3

Table 3. Eigenvalues, individual (% inertia) and cumulative (Cum. % inertia) proportion of explained inertia of the first seven axis of the multiple correspondence analysis.

Axis	Eigenvalue	% inertia	Cum. % inertia
1	0.40	18.7	18.7
2	0.30	14.2	32.9
3	0.25	11.9	44.7
4	0.19	9.1	53.8
5	0.18	8.6	62.4
6	0.16	7.6	69.9
7	0.14	6.4	76.3

673 Table 4. Results of the mixed classification method.

Number of experimental units within class	Percentages ^a		Number of cases within modality	Modality	Variables ^b
	Cla/Mod	Mod/Cla			
Class I: 14					
8	66.7	57.1	12	<10E4 TCID ₅₀	Dose of PCV2 inoculate
14	23.0	100.0	61	NO	Colostrum deprivation
9	37.5	64.30	24	4-5 wk	Age at inoculation
7	35.0	50.0	20	1	Genotype
Class II: 30					
26	83.9	86.7	31	>5 wk	Age at inoculation
22	81.5	73.3	27	10E4-10E5 TCID ₅₀	Dose of PCV2 inoculate
29	47.5	96.7	61	NO	Colostrum deprivation
28	37.3	93.3	75	2	Genotype
30	34.5	100.0	87	intranasal	Route of inoculation
25	40.3	83.3	62	0%	Success
15	50.0	50.0	30	>11	Sample size
	2.5 (6.9)	15.1 (25.8)			Percent (s.e.) of affected pigs
Class III: 14					
13	100.0	92.9	13	other routes	Route of inoculation
9	45.0	64.3	20	1	Genotype
8	33.3	57.1	24	4-5 wk	Age at inoculation
13	21.3	92.9	61	NO	Colostrum deprivation
Class IV: 42					
37	94.9	88.1	39	YES	Colostrum deprivation
29	96.7	69.1	30	1-7 days	Age at inoculation
17	89.5	40.5	19	>10E6 TCID ₅₀	Dose of PCV2 inoculate
40	53.3	95.2	75	2	Genotype
42	48.3	100.0	87	intranasal	Route of inoculation
9	81.8	21.4	11	>50%	Success
	23.8 (30.6)	15.1 (25.8)			Percent (s.e.) of affected pigs

674 ^a Cla/Mod: percentage of experiments classified in this class out of all the experiments
675 that display the characteristic, Mod/Cla: percentage of experiments that display a
676 determined characteristic within a class.

677 ^b s.e.: standard error

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Table 5. Differences of Least Squares Means between categories of variables significant in the individual and global models.

Variables	Estimate (β)	S.E.	t Value	P value	Exp(β)
Individual Modelisation					
Colostrum deprivation					
YES vs. NO	1.5	0.4	-3.7	0.0005	4.3
Seronegative for PCV2					
YES vs. NO	1.3	0.6	-2.0	0.0495	3.5
Age at inoculation					
1-3 wk vs. 1-7days	0.1	0.6	0.2	0.8163	1.1
1-3 wk vs. 4-5 wk	0.7	0.6	1.2	0.2460	2.1
1-3 wk vs. >5 wk	1.7	0.6	2.7	0.0094	5.5
1-7days vs. 4-5 wk	0.6	0.6	1.0	0.3156	1.8
1-7days vs. >5 wk	1.6	0.6	2.6	0.0113	4.8
4-5 wk vs. >5 wk	1.0	0.6	1.6	0.1268	2.7
Type of experiment^a					
CI vs. PCV2	1.3	0.4	-3.7	0.0006	3.6
ISV vs. PCV2	0.5	0.4	-1.2	0.2422	1.6
ISV-CI vs. PCV2	0.3	1.3	-0.2	0.8227	1.3
CI vs. ISV	0.8	0.5	1.8	0.0791	2.2
CI vs. ISV-CI	1.0	1.2	0.8	0.4257	2.7
ISV vs. ISV-CI	0.2	1.3	0.2	0.8751	1.2
Global modelisation					
Colostrum deprivation					
YES vs. NO	1.8	0.3	-5.4	<0.0001	5.8
Genotype					
1 vs. 2	1.2	0.4	3.5	0.0008	3.5

^a PCV2: PCV2 alone, ISV: PCV2 plus immunostimulator or vaccination, CI:

PCV2 plus co-infection, ISV-CI: combination of categories ISV and CI.

Figures

Figure 1. Scatter of the multiple correspondence analysis. Modalities of each variable are represented in a two dimensional plane along the horizontal axis (first axis, inertia = 18.7%) and the vertical axis (second axis, inertia = 14.2%). Black squares correspond to nominal active variables: age at inoculation (1-7 days, 1-3 wk, 4-5 wk, >5 wk), colostrum deprivation (CD_YES, CD_NO), seronegative for PCV2 (SN_YES, SN_NO), route of inoculation (intranasal, other routes), dose of inoculum (<10E4 TCID50, 10E4-10E5 TCID50, 10E5-10E6 TCID50, >10E6 TCID50), PCV2 genotype (Genotype1, Genotype2), type of experiment (PCV2: PCV2 alone, ISV: PCV2 + immunostimulator or vaccination, CI: co-infection, ISV-CI: combination of categories ISV and CI). White squares correspond to illustrative variables: percent affected (0%, 0-25 %, 25-50%, >50%), sample size (N≤6, 7<N<10, N≥11), research group (RG1 to RG12). Arrows indicate the direction of increase of quantitative variables (sample size and percent of affected pigs) along the factorial space.

