Virological and serological characterization of vaccinated and non-vaccinated piglet subpopulations coming from vaccinated and non-vaccinated sows

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Abstract

The present study describes the virological and serological profiles of PCV2 vaccinated (V) and non-vaccinated (NV) piglet subpopulations coming from V and NV sows in a PCV2 subclinically infected farm. Four hundred seventy-six piglets born from V or NV sows were further subdivided in a total of four groups: NV sows-NV pigs (NV-NV), NV sows-V pigs (NV-V); V sows-NV pigs (V-NV) and V sows-V pigs (V-V). Seventy-five pigs were randomly selected at the beginning of the trial from each group and they were bled at 4, 8, 12, 16, 21 and 25 weeks of age. All animals included in the trial were weighed at 4 and 25 weeks of age and their average daily weight gain (ADWG) was calculated. Serum samples obtained at different time points were used to assess PCV2 infection (viremia) and the level of antibodies by means of immunoperoxidase monolayer assay (IPMA) against this pathogen. IPMA titres (classified in high, medium or low) and PCR results (positive or negative) were analyzed using a multiple correspondence and K-means cluster analysis. According to these tests, animals included in the study were classified into the following four clusters: 1) 93 piglets that were viraemic mainly from 12 to 25 weeks of age and with PCV2 antibody titers increasing over time; 2) 75 piglets with late PCV2 infection and seroconversion (later than 16 weeks of age); 3) 26 piglets with high but decreasing PCV2 antibody titers and low percentages of PCV2 PCR positive serum samples; and 4) 105 piglets with medium and high IPMA titers throughout the trial and sporadic PCR positive samples. The defined subpopulations of piglets were observed in all experimental groups (NV-NV, NV-V, V-NV and V-V) although in variable percentages. Thus, animals from clusters 1 and 2 belonged mainly to the NV-NV and V-NV groups and animals from clusters 3 and 4 were distributed mainly into the NV-V and V-V groups. Finally, the ADWG of pigs belonging to clusters 3 and 4 was significantly higher ($p=0.02$) than that of pigs belonging to clusters 1 and 2. Within each cluster, no statistically significant differences were found in ADWG between treatment groups.

Key words: Porcine circovirus type 2 (PCV2); vaccination; sow; piglet; individual variation; average daily weight gain; subpopulations

1. Introduction

Porcine circovirus type 2 (PCV2)-systemic disease (SD) is considered one of the major swine diseases worldwide. Infection with PCV2 is a necessary but not sufficient condition for pigs to develop the disease. Thus, management factors are important for disease development (Woodbine et al., 2007, Alarcon et al., 2011) and secondary pathogens, such as *Mycoplasma hyopneumoniae*, porcine reproductive and respiratory syndrome virus (PRRSV) and porcine parvovirus (PPV), are also considered infectious risk factors for disease triggering (Opriessnig et al., 2004). Traditionally, PCV2-SD control was based on counteracting infectious and non-infectious triggering factors, such as management improvement, control of co-infections and changes of the boar genetic background (Grau-Roma et al., 2011). However, PCV2 vaccines have demonstrated to be very efficient to control PCV2-SD and PCV2 infections under experimental and natural conditions (Beach and Meng, 2012).

PCV2 vaccines can be applied in sow and /or in piglets (Grau-Roma et al., 2011). PCV2 sow (and/or gilt) vaccination leads to an increase of PCV2 antibody titres in sow serum, a reduction in viremia as well as viral shedding in milk and colostrum and an improvement of piglet production records (mortality rates and average daily weight gain [ADWG]) (Gerber et al., 2011; Kurmann et al., 2011). Piglet PCV2 vaccination results in a significantly reduction of viral-induced microscopic PCV2-SD lymphoid lesions, decrease mortality and cull rates, decrease the frequency of co-infections and improve ADWG (Segalés et al., 2009, Martelli et al., 2011). Indeed, a meta-analysis study of 66 published field trials found a positive effect of all PCV2 commercially available vaccines on productivity, with no statistically significant differences among them (Kristensen et al., 2011). Alternatively, a not yet extensively studied strategy is the double (sow and piglet) PCV2 vaccination (Opriessnig et al, 2010, Pejsak et al, 2010, Fraile et al, 2012a). This strategy achieved the best productive results although no statistically significant differences were observed in comparison with a protocol based only in piglet vaccination (Fraile et al 2012a). On the other hand, a recent publication (Oh et al., 2014) confirmed that the combination of sow and pig (at 49 days of age) vaccination significantly reduced PCV2 viremia, induced higher neutralizing antibody titres, and resulted in higher proportion of CD4(+)CD8(+)IFN-γ(+) lymphocyte subsets in piglets compared to the other groups of animals tested (non-vaccinated piglets coming from vaccinated sows, only vaccinated piglets at 21 or 49 days of age, and piglets vaccinated at 21 days of age coming from vaccinated sows). Thus, the combination of sow and pig (49 days of age) vaccination could be more effective for controlling

PCV2 infection if PCV2 the infection occurs during the growing-finishing period in herds. From an economical perspective, PCV2 vaccination is considered a worldwide great success. However, these vaccines are imperfect in the sense that they prevent clinical signs but not infection (Kekarainen et al., 2010). Moreover, the seroconversion elicited by the vaccine can be affected by the antibody titres (Fraile et al 2012a,b) and the vaccine efficacy may depend on the timing of natural infection (Beach and Meng, 2012). As a consequence, in a PCV2 vaccinated community, subpopulations of animals not equally protected in front of a natural PCV2 challenge may exist. Optimization of PCV2 vaccination protocols would probably require a better characterization of piglet subpopulations coming from piglet, sow or both vaccination programs. To the authors' knowledge, this kind of information is not yet available in the literature. Thus, the main goal to this research work was to characterize, by means of PCV2 antibody and virological profiles, the different piglet subpopulations generated by piglet and/or sow vaccination. A secondary objective was to describe the ADWG observed in the different piglet subpopulations.

2. Material and methods

2.1. Study design

Data analysed in this study was taken from a previously published field study (Fraile et al., 2012a). Briefly, the study was conducted in a 1500-sow Spanish farm with clinical history of PCV2-SD in which no PCV2 vaccination had been ever used. Pigs included in the study were born from Landrace (50%) x Large white (50%) sows mated with Pietrain (100%) boars. One month before the beginning of the trial, PCV2-SD was diagnosed (fulfilling the internationally accepted disease case definition) (Segalés et al., 2005) in 5 out of 10 animals showing a wasting condition. One week before mating, a population of 57 sows was distributed into two groups: vaccinated (V, n=26) and non-vaccinated sows (NV, n=31). V sows received 2 ml of Porcilis® PCV (MSD Animal Health, The Netherlands) and NV received 2 ml of phosphate buffer saline (PBS). The parity average was not significantly different between V (3.1) and NV (3.2) sows; animals with different treatments were mingled in the same farrowing and gestation units. Blood samples were taken from the sows 2 weeks pre-mating and at 3, 10 and 17 weeks post-mating. All healthy piglets (n=476) born from these 57 sows were included (at 4 weeks of age) in a sow and/or piglet vaccination strategy as follows: NV sows-NV pigs (NV-NV, n=134), NV sows-V pigs (NV-V, n=135); V sows-NV pigs (V-NV, n=104) and V sows-V pigs (V-V, n=103). The housing and husbandry conditions were standard for pigs reared under intensive conditions in Spain. Briefly, animals were housed in

confinement with controlled environmental conditions (temperature and ventilation). In particular, cross-fostering was not allowed for the litters under study and the weaning age was close to 4 weeks of age (27±2 days). At weaning, V piglets received 2 mL of Porcilis® PCV by intramuscular route and NV ones received 2 mL of PBS by the same route. Piglets with different treatment were mingled in the same growing and finishing units. Mortality during the study was recorded. No evidence of PCV2-SD was observed in the studied batch. The sampling size of the study was carried out to be able to detect differences in ADWG close to 20 g/day between vaccinated and non-vaccinated piglets (Fraile et al, 2012a). Nevertheless, the final number of piglets included in this study allowed having statistical power to detect differences between the proportion of the piglets presents in the different groups equal or higher than 15%.

From each treatment, a group of seventy-five pigs was randomly selected and bled at 4 (27±2 days of age), 8, 12, 16, 21 and 25 weeks of age. In addition, these animals were weighed at 4 and 25 weeks of age and their ADWG was calculated. Data analysis was done considering these 75 pigs per treatment group. This number of animals allowed describing precisely the virological and serological profile for each treatment. Treatments, housing, husbandry and slaughtering conditions conformed to the European Union Guidelines and Good Clinical Practices, and were identical for all experimental groups.

2.2. PCR to detect PCV2 nucleic acid

Serum samples were processed by standard PCV2 PCR (Quintana et al., 2002). In the statistical analyses, a variable including PCR result (positive or negative) and sampling week was generated. Thus, variable PCRXS or PCRWX represented PCR results of sows at X weeks pre- or post-mating, and PCR results of piglets at X weeks of age, respectively.

2.3. Serology to detect PCV2 antibodies

Presence of antibodies against PCV2 were tested using immunoperoxidase monolayer assay (IPMA) (Rodríguez-Arrioja et al., 2000). IPMA results were expressed as log2 titre values. Animals were classified taking into account their antibody titre as low (<10 log2 IPMA titers), medium (between 10 and 12 log2 IPMA titres) or high (>12 log2 IPMA titres).

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143 *2.4. Statistical analysis*

144 Statistical analyses were carried out using the SAS system V.9.2 (SAS institute Inc, Cary, NC, USA) and
145 the SPAD v5.0, C.I.S.I.A., Saint Mandé, France. For all analyses, the individual pig was considered as the
146 experimental unit. The significance level (α) was set at 0.05.

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148 *2.4.1. Multiple correspondence analysis*

149 Multiple correspondence analysis (MCA) is a multivariate, descriptive and exploratory technique that is
150 used to visualize relationships within a set of categorical variables (Greenacre et al, 2006), without any
151 dependent variable or assumptions on data distribution. This technique was carried out to summarize the
152 values of piglet IPMA (low, medium, high) and piglet PCV2 detection by PCR in serum (positive or
153 negative) at each sampling time.

154

155 The remaining variables (piglet and/or sow PCV2 vaccination and IPMA and PCR results in sows at each
156 sampling time) were included in the analysis as supplementary variables. In a MCA analysis, these
157 variables were not used in determining the locations of the other, but were displayed on the output and
158 helped in the interpretation of the active variables (PCR and IPMA in piglets).

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160 In the MCA output, relationships between different categories of the selected variables are typically
161 represented as points in a two-dimensional space; the first and second dimensions are represented in the X
162 and Y axes of the graph, respectively. Supplementary variables are represented in different symbol and
163 color than the active ones. The size of the categories of the active variables is proportional to the number
164 of observations within this category. The plot can be interpreted by considering which variable categories
165 are plotted closely together; relatedness between variables is considered in both the 1st dimension along
166 the X axis, and in the 2nd dimension along the Y axis. The distance of an object from the origin of the plot
167 reflects variation from the "average response pattern", which relates to the most frequent category for
168 each variable. Thus, objects with many characteristics corresponding to the most frequent categories will
169 be near the origin of the axes (X=0, Y=0). In contrast, objects with unique characteristics are located far
170 from the origin of the axes. For example, the most frequent combinations between piglet IPMA (low,
171 medium or high) and PCR results (positive or negative) were plotted close to each other, whereas those

rare combinations were plotted further apart (Ribbens et al, 2008). For each variable and in each dimension, a discrimination measure is computed and compared using bivariate t-tests. This measure can be regarded as squared component loading and it is also the variance of the quantified variable in the dimension. Finally, with the MCA analysis, it will be also generated factors (combination of variables) to explain the whole observed variance.

2.4.2. Cluster analysis

The K-means cluster analysis is a multivariate method used to divide the heterogeneous group of piglets into homogeneous subgroups using non-hierarchical methods, via an iterative process that continues until the sum of squares to the assigned cluster centres is minimized. A K-means cluster analysis (Lebart, et al, 2000) was conducted to reveal populations of piglets with similar IPMA and PCR results based on the factors derived from the MCA. The clusters obtained are represented in a tree dendrogram (Hartigan, 1975).

The distribution of the experimental groups (NV-NV, NV-V, V-NV and V-V) within each cluster was analysed using a chi-squared test. Finally, An ANOVA test was used to compare the production performance (ADWG) between clusters and, within each cluster, the ADWG was also compared taking into account the experimental group as explanatory variable (NV-NV, NV-V, V-NV, V-V).

3. Results

3.1. Multiple correspondence analysis

The results of the MCA analysis are represented in Figure 1. The total variance explained by this analysis was 29.8%; with 16.5% explained by the ^{1st} dimension and 13.3% by the 2^{nd} dimension, respectively. Discrimination measures provided insight into the influence exerted by each variable in order to explain the variance observed in each dimension. Variables with the highest discrimination values are included in Table 1.

The 1^{st} dimension (X axis) separates PCR positive (X<0) from PCR negative animals (X>0). On the left side of 1^{st} dimension, positive PCR results at weeks 16, 21 and 25 were close to (and therefore associated

with) low values of IPMA at weeks 8 and 12, and high values of IPMA at weeks 21 and 25 due to its proximity in the plot. On the right side of the 1st dimension, there were higher values of IPMA at weeks 8 and 12. When supplementary variables were considered, the left side of this plot was dominated by NV piglets. On the contrary, the right side of the 1st dimension contained variables that were mainly associated with V piglets.

The 2nd dimension (Y axis) provides insight into early PCV2 detection by PCR (Y < 0 in the plot). Thus, PCR positive animals at weeks 8 and 12 have Y values less than -0.76. Such early infection was associated with lower values of IPMA at weeks 4 and 8 and higher values at weeks 16 and 21 (see the proximity in the plot between these parameters). When considering supplementary variables, lower quadrants were associated to viral detection by PCR in sows at weeks 10 and 17 post-mating and low IPMA values in sows at 10 weeks post-mating. The top (not early PCV2 detection in piglets) and bottom side of the plot is dominated by V and NV sows, respectively.

3.2.- Cluster analysis

The cluster analysis was performed considering the most significant 10 factors (combination of variables) obtained from the MCA analysis (which accounted for 77% of variance explained). Results are presented in a tree dendrogram (Figure 2) and in pie charts (Figure 3). The first division separates two groups of animals which were associated with the vaccination of the piglets: in the first group (A) there were most of the NV pigs (92%), whereas in the second group (B) most of the V ones (80.5%). Each one of these groups could be subdivided into two smaller groups, which were associated with both sow and piglet vaccination (Figure 3). Most of the piglets from the NV-NV group were included in clusters 1 and 2, with few animals in cluster 4. However, most of the piglets belonging to the V-V group were included in clusters 2, 3 and 4, with few pigs in cluster 1. On the other hand, most of the piglets of the V-NV group were included in clusters 2, 1 and 3, with few animals in cluster 4. Finally, most of the piglets of the NV-V group were included in clusters 4, 3 and 1, with few animals in cluster 2.

3.3.- Association of the clusters with PCR and IPMA values

The individual and average PCV2 antibody profiles and the percentage of PCV2 PCR positive animals of each cluster throughout the trial are described in Figure 4 (black and blue lines for individual and average values, respectively). The obtained clusters were the following: 1) 93 piglets (31%) that were viraemic

mainly from 12 to 25 weeks of age and with PCV2 antibody titers increasing over time (from 12 to 25 weeks of age); 2) 75 piglets (25%) with late PCV2 infection (later than 16 weeks of age) as well as seroconversion; 3) 26 piglets (9%) with high but decreasing PCV2 antibody titers and low percentages of PCV2 PCR positive serum samples; and 4) 105 piglets (35%) with medium and high IPMA titers throughout the trial and sporadic PCR positive samples. Finally, one piglet could not complete the trial and was excluded of the analyses.

3.4.- Association of the clusters to ADWG

The comparison of the ADWG of animals included in each clusters is detailed in Table 2. ADWG of pigs belonging to clusters 3 and 4 was significantly higher ($p=0.02$) than that of pigs belonging to clusters 1 and 2. Within each cluster, no statistically significant differences were found between the ADWG taking into account the experimental group (NV-NV, NV-V, V-NV, V-V) as explanatory variable.

Discussion

The vaccine used in this trial is licensed for piglets only, and therefore its use in sows was off label. A single dose of vaccine applied to piglets at weaning (independently of sow treatment) caused lower percentages of PCV2 infected pigs over the production period and a significant improvement of production parameters in comparison with non-vaccinated piglets (Fraile et al, 2012a). These results are in agreement with other studies already published testing one (Takahagi et al., 2010; Martelli et al., 2011) or two (Takahagi et al., 2010; Lyoo et al., 2011) doses of the same vaccine. In the present work, in which all the different vaccination protocols were compared contemporaneously (NV-NV, NV-V, V-NV and V-V) in a farm that never vaccinated before, the double PCV2 vaccination (sow and piglet) reduced the percentage of PCV2 infected pigs and reported also the best production parameters (although not significantly different from those of vaccinated pigs coming from non-vaccinated sows, NV-V group) (Fraile et al, 2012a). On the other hand, different subpopulations of piglets were detected in all experimental groups (NV-NV, NV-V, V-NV and V-V). This situation means that, in spite of the different vaccination treatments, there were a variable proportion of subpopulations of animals within each treatment.

Vaccination is one of the most efficient ways to prevent and control infectious diseases. However, vaccine efficacy for many diseases almost never reaches a value of 100% (Thursfield, 1997). Such vaccine "imperfection" was highlighted in the present study by the fact that a low percentage of vaccinated piglets, coming from vaccinated or non-vaccinated sows (NV-V and V-V), were viraemic mainly from 12 to 25 weeks of age and with PCV2 antibody titers increasing over the same period (cluster 1). Thus, the virological and serological profiles in this subpopulation of vaccinated piglets were similar to the virological and serological profiles observed in non-vaccinated ones. There are different reasons that could explain this finding. The first one could be a wrong application of the vaccine. This possibility is hardly probable in this case because animals were vaccinated using good clinical practices by experienced veterinarians. Another possible explanation could be the genetics of these piglets. The relationship of host genetics to responsiveness against vaccines has not been greatly explored, but it is likely to involve the major histocompatibility complex MHC (e.g. processed peptides may not bind or only bind at low affinity to particular alleles) and genes controlling cytokine profiles (e.g. Th1 versus Th2) or levels of proinflammatory cytokines. Such genes could account for low or non-responsiveness to vaccines (Glass, 2004). In fact, detection of "high" versus "low" responders in terms of cellular immunity measured as PCV2 specific IFN-γ secreting cells upon natural PCV2 infection has been described (Martelli et al., 2011). Therefore, it would not be surprising that host genetics would modulate the immune response mounted against PCV2 vaccination. Finally, lack of expected response to PCV2 vaccination could be attributed to interference with maternally derived immunity. Thus, interference with the efficacy on the PCV2 vaccine might depend on the level of maternally derived antibodies (MDA) at the time of vaccination. Animals with IPMA titers equal or beyond 10 log2 show interference with the development of the humoral immune response after vaccination, while piglets with levels below 8 log2 do not show interference (Fort et al., 2009, Martelli et al., 2011; Fraile et al., 2012a,b). In contrast, it has been demonstrated that, even in the presence of high levels of MDA, piglets immunized with PCV2 vaccines respond to vaccination with a significantly reduced viremia, number of PCV2 PCR positive pigs and mortality rate, and a significantly greater ADWG than those of the NV animals (Fraile et al., 2012a,b). In the present case, there was no association between the subpopulation of V piglets that seemed not to respond efficiently to vaccination and a high PCV2 antibody titer transferred by their dam through colostrum. This result reinforces that the possible interference between MDA and the response to PCV2 vaccination in piglets is probably of low relevance in terms of vaccine efficacy if vaccination is

applied later than 3 weeks of age as recommended by vaccine manufacturers (summary of product characteristics of PCV2 vaccines).

On the other hand, a small subpopulation of NV animals coming from NV sows showed medium and high IPMA titers throughout the trial and sporadic PCR positive samples (cluster 3). Moreover, these animals showed an excellent growth performance. Thus, these NV piglets resembled the V ones in terms of virological, antibody dynamics and production performance. There are two main reasons that could explain this apparent resistance to PCV2 infection. Firstly, it may happen that these animals did not get PCV2 infection throughout the trial but this possibility is hardly probable since PCV2 infection, either by PCR detection or seroconversion during the study period, was demonstrated in all the animals. Another potential explanation could be again the genetic background of these piglets as it has been stated above. In fact, it has been published that genetics plays a major role in respect to susceptibility or resistance to PCV2-SD clinical expression under field conditions (Lopez-Soria et al, 2011). This observation is consistent with other pig diseases such as porcine reproductive and respiratory syndrome virus (PRRSV). Thus, there has emerged a body of evidence associating host genetics with different outcomes following viral infections. As an example, pigs from lines or breeds with improved reproductive traits (Meishan and Large White) are more resistant to the effects of the PRRS virus than pigs from lines selected for lean growth rate (Duroc and Pietrain) (Petry et al, 2007; Reiner et al, 2010). In general terms, genetic variation in host resistance to infectious disease is ubiquitous (Bishop et al, 2005), but identifying genes or pathways that determine viral disease resistance/susceptibility is a complex process (Bishop and MacKenzie, 2003). Although pathways and mechanisms involved in specific disease/infection-resistance traits have not yet been fully characterized, genetic variation in disease resistance/susceptibility is likely to be polygenic, regulating aspects of both innate resistance and acquired immunity. So far, genomic regions that could be involved in the genetic resistance to PCV2-SD are not known.

The rationale of vaccinating sows is based on increasing PCV2 antibody titres in sow serum and reducing viremia as well as viral shedding in milk and colostrum (Gerber et al., 2011, Kurmann et al., 2011). Moreover, PCV2 antibody titres in piglets coming from V sows are higher compared to piglets coming from NV ones (Sibila et al., 2013). This vaccination strategy could be an efficient way to control PCV2-SD but it is critical to ensure enough colostrum intake during the first 24 hours of life. According to

obtained results, all 4 different subpopulations (clusters) were fairly well represented in the V-NV group of pigs. A plausible rationale for this observation in the first cluster (viraemic mainly from 12 to 25 weeks of age and with PCV2 antibody titers increasing over time) could be explained by a low colostrum intake during the lactation period with an early PCV2 infection, as suggested by Grau-Roma et al. (2011). In contrast, the second cluster (late PCV2 seroconversion) could be explained by a good colostrum intake that prevented an early PCV2 infection. Finally, the presence of animals belonging to the third (high but decreasing PCV2 antibody titers and low percentages of PCV2 PCR positive serum samples) and fourth clusters (medium and high IPMA titers throughout the trial and sporadic PCR positive samples) could be partially explained, as detailed before, by the individual genetic background of the pigs in terms of PCV2 disease/infection resistance.

In conclusion, the present study describes four subpopulations of V and NV pigs coming from V and NV sows according their serological and virological status. Those different profiles were variably represented in all the experimental groups, but the best serological/virological outcomes were mainly composed by vaccinated piglets (coming from V and NV sows). It is very likely that these subpopulations would be represented in all PCV2 vaccinated farms, although the different farm conditions, vaccination regimes, and piglet management during lactation may affect the proportion of pigs in each cluster.

Conflict of interest statement

None of the authors declares conflict of interests that could inappropriately influence or bias the content of the paper.

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Figure Legend

Figure 1. Multiple correspondence analysis. The plot is interpreted by considering which variable categories are plotted closely together; relationship between variables is considered in both the 1st dimension along the X axis, and in the 2nd dimension along the Y axis. The 1st dimension (X axis) separates PCR positive (X<0) from PCR negative animals (X>0). The 2nd dimension (Y axis) provides insight into early PCV2 detection by PCR (Y < 0 in the plot). Supplementary variables are represented in different symbol and color than the active ones. The size of the categories of the active variables is proportional to the number of observations within this category.

Figure 2. K-means cluster analysis using non-hierarchical methods providing homogeneous subgroups of animals according to PCV2 IPMA and PCR similarities. The clusters are represented in a tree dendrogram, and percentages indicate the number of pigs belonging to each of these subpopulations.

Figure 3. Distribution of the piglets within each cluster taking into account the experimental group (NV sows-NV pigs (NV-NV), NV sows-V pigs (NV-V); V sows-NV pigs (V-NV) and V sows-V pigs (V-V)).

Figure 4. PCV2 antibody titre (A) and the percentage of PCV2 serum positive animals by PCR (B) depending on the cluster throughout the trial. It is represented all the animals (black lines) and the average value for each week in each cluster (blue line).

508 Table 1 Discrimination measures of variables that were used in the multiple correspondence analysis.
509 These values correspond to a Student's t distribution (absolute values greater than 2 are statistically
510 significant).

	Discrimination measure	
Variable	1st Dimension	2nd Dimension
Low IPMA4	NS	-8.55
High IPMA4	NS	10.02
Low IPMA8	-7.80	-5.58
Med IPMA8	NS	8.29
High IPMA8	9.66	-3.23
Low IPMA12	-8.67	NS
Med IPMA12	-4.27	4.62
High IPMA12	11.05	-5.64
Low IPMA16	-6.76	10.41
Med IPMA16	4.16	NS
High IPMA16	NS	-11.45
Low IPMA21	NS	8.43
Med IPMA21	9.01	NS
High IPMA21	-6.95	-5.05
Low IPMA25	4.51	6.53
Med IPMA25	8.35	NS
High IPMA25	-9.53	NS
PCR8	NS	-5.64
PCR12	NS	-9.69
PCR16	-8.34	-4.47
PCR21	-9.40	NS
PCR25	-10.45	NS

511 NS= Not significant

512 Table 2. Average daily weight gain (Kg/day), SE and confidence interval (95%) calculated for pigs from

513 4 to 25 weeks of age taking into account the cluster they belong to.

514

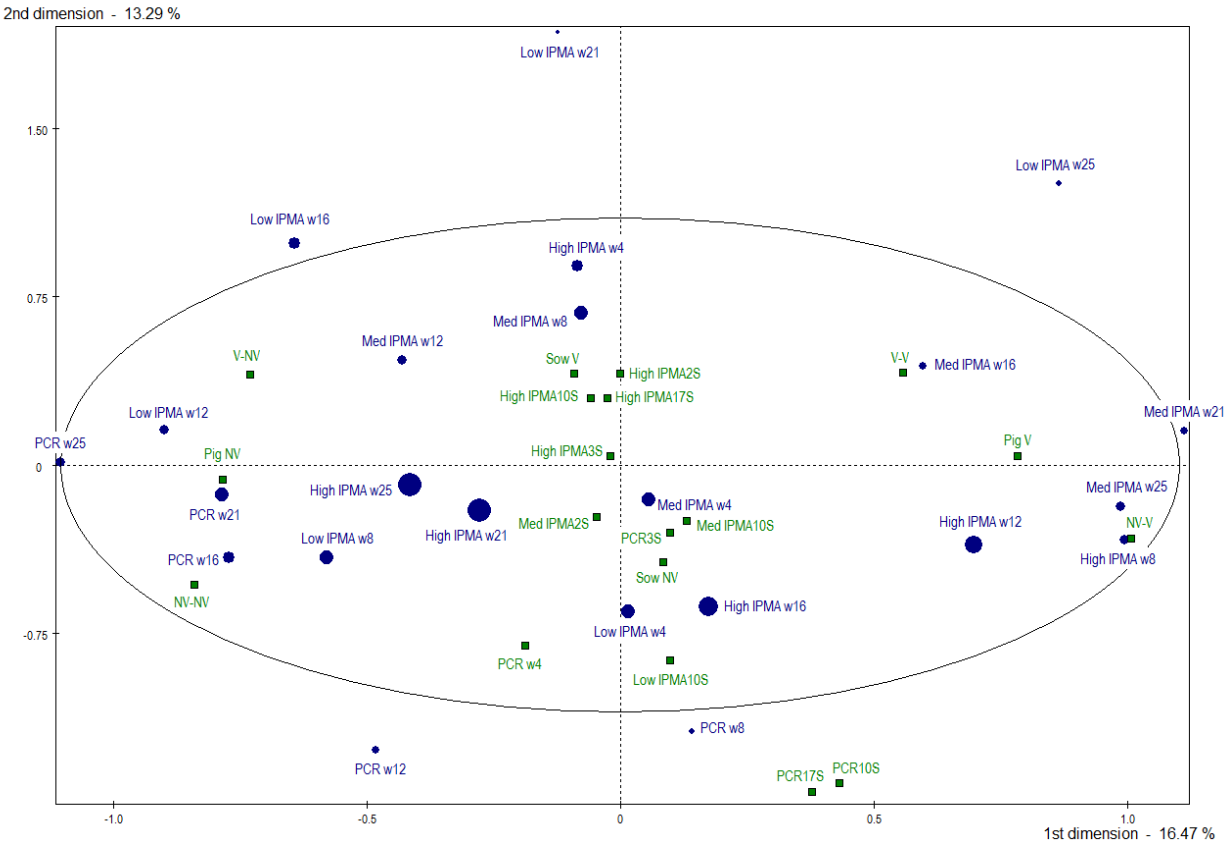
Cluster	Average	SE	Confidence interval (95%)	
			Lower limit	Upper limit
1	0.62[b]	0.008	0.60	0.63
2	0.60[b]	0.008	0.58	0.61
3	0.64[a]	0.014	0.61	0.66
4	0.63[a]	0.007	0.61	0.64

515

516 Different superscript means statistically significant differences between clusters ($p<0.05$).

Figure

Figure 1.



Blue variables: Used in the MCA analysis

Green variables: Supplementary variables that have not affected the MCA results.

PCRW4 was a supplementary variable because only four animals were PCV2 PCR positive at this age and it could not be included in the MCA analysis.

PCRwX means PCV2 positive piglets by PCR at week X

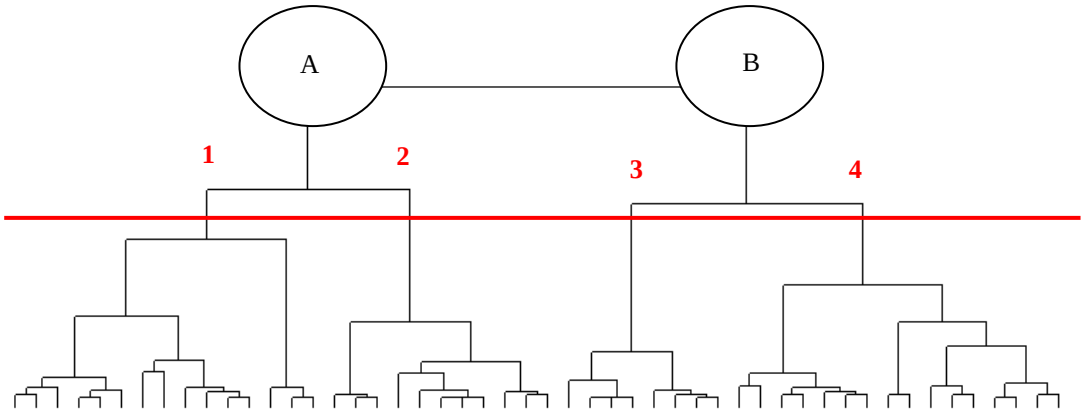
PCRXS means PCV2 positive sows by PCR at week X

Pig V and Pig NV mean vaccinated and non-vaccinated piglets, respectively.

Sow V and Sow NV mean vaccinated and non-vaccinated sows, respectively.

The 1st dimension (X axis) separates PCR positive (X<0) from PCR negative animals (X>0) and the 2nd dimension (Y axis) provides insight into early PCV2 detection by PCR (Y < 0 in the plot).

Figure 2.



Tree dendograma with a classification in 4 clusters

The first division separates two groups of animals which were associated with the vaccination of the piglets: in the A group (clusters 1 and 2) there were most of the NV pigs (92%), whereas in the B group (clusters 3 and 4) most of the V ones (80.5%).

Figure 3.

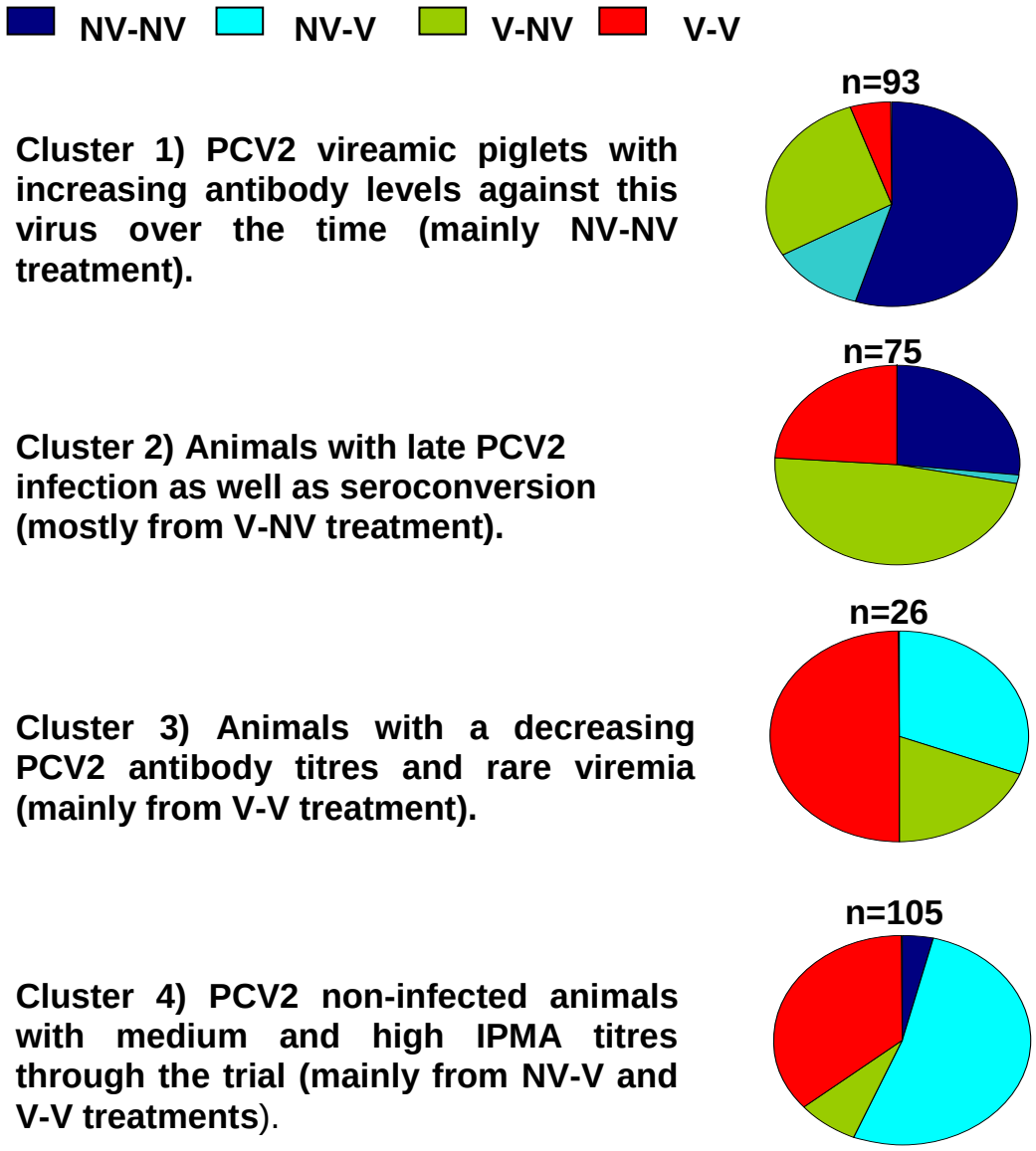
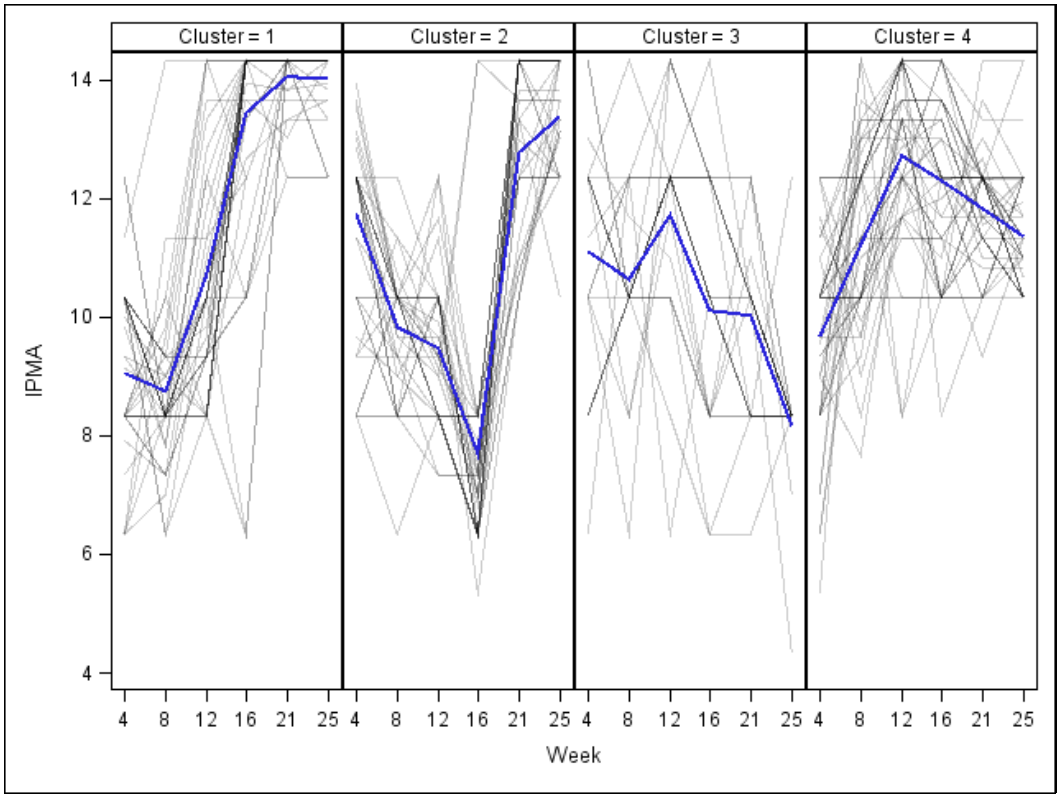
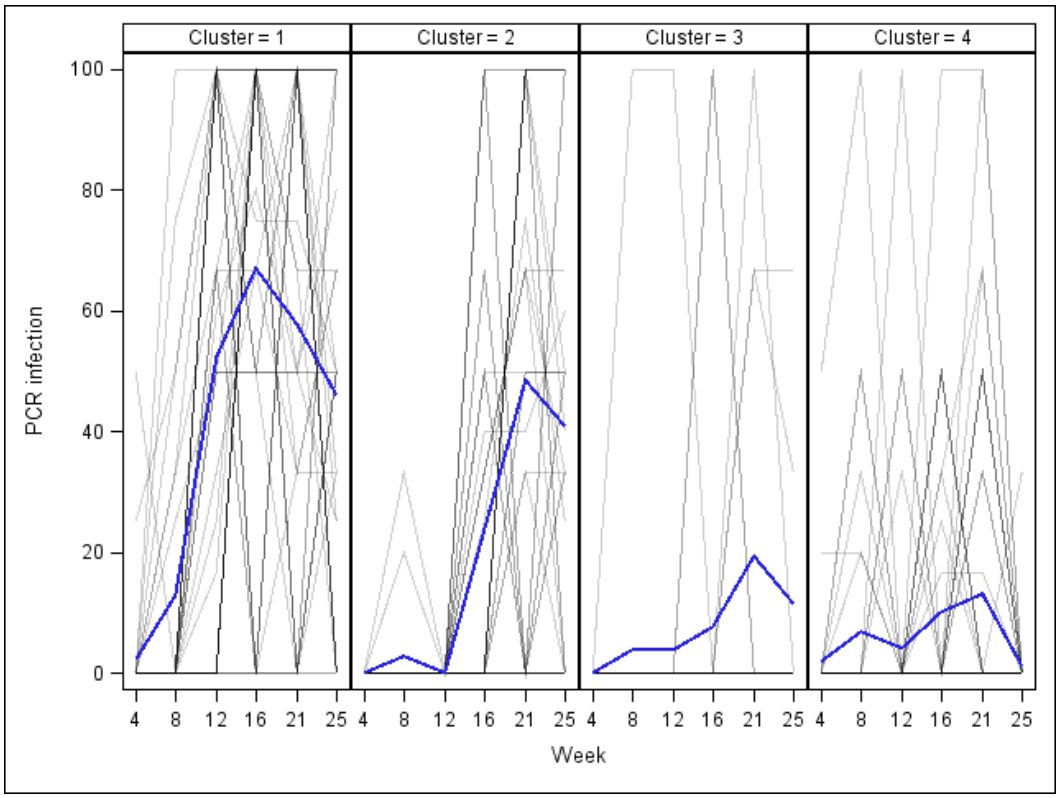

NV-NV
NV-V
V-NV
V-V
n=93
Cluster 1) PCV2 vireamic piglets with increasing antibody levels against this virus over the time (mainly NV-NV treatment).
n=75
Cluster 2) Animals with late PCV2 infection as well as seroconversion (mostly from V-NV treatment).
n=26
Cluster 3) Animals with a decreasing PCV2 antibody titres and rare viremia (mainly from V-V treatment).
n=105
Cluster 4) PCV2 non-infected animals with medium and high IPMA titres through the trial (mainly from NV-V and V-V treatments).

Figure 4.

A


Cluster = 1
Cluster = 2
Cluster = 3
Cluster = 4
IPMA
14
12
10
8
6
4
4 8 12 16 21 25
Week

B


Cluster = 1
Cluster = 2
Cluster = 3
Cluster = 4
PCR infection
100
80
60
40
20
0
4 8 12 16 21 25
Week