



RESEARCH ARTICLE

REVISED Longitudinal comparison of the humoral immune response and viral load of Porcine Circovirus Type 2 in pigs with different vaccination schemes under field conditions [version 2; referees: 2 approved, 1 approved with reservations]

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Abstract

Background: Porcine Circovirus type 2 (PCV2) infections are distributed worldwide and cause Porcine Circovirus Associated Disease (PCVAD). To minimize the impact of PCV2 infection on swine health and production, different vaccination schemes have been used since 2006. However, the association between vaccination schemes, virus load and disease under field conditions are not completely understood. Therefore, the objective of this study was to compare the effect of two different PCV2 vaccination schemes on the humoral response and PCV2 load in pigs after weaning under field conditions.

Methods: Two commercial pig farms (Farm A and B), endemically infected with PCV2, which were using two different PCV2 subunit vaccinations schemes for sow, gilts and piglets, were selected. We designed a longitudinal study and measured IgG levels by ELISA and virus load by quantitative PCR in pigs after weaning. Forty 3-week old piglets were randomly selected at weaning and followed for 20 weeks. IgG levels and virus loads were compared within and between farms and considered statistically different if the non-parametric Wilcoxon-test p value was lower than 0.05.

Results: We found that low virus loads were maintained in pigs from both farms regardless of the vaccination scheme used ($p > 0.05$). However, there was significant difference in the mean IgG levels observed over time ($p < 0.05$) while there were no significant differences in viral loads. This suggests that different humoral immune response is not associated with different virus loads observed over time.

Conclusions: These results are important because they can help to prevent PCV2 infections using different vaccination schemes to minimize the effect of PCVAD on swine health and production.

Keywords

Porcine Circovirus type 2 (PCV2), PCV2 vaccines, IgG anti PCV2, viral loads.

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REVISED Amendments from Version 1

Revisions to the text were made according to the reviewers' observations. Suggestions were taken into account as it has been shown that the level of viremia has decreased in North America using vaccination, particularly in the United States. Also on citing and including in the bibliography studies with a greater number of strains phylogenetic analyzes. It was stated that PCV2 circulation was not demonstrated in the study, which was not true, because the qPCR technique demonstrated that circulation, this is clarified and discussed. Regarding the humoral response, although there were differences in the antibody response between the two farms studied, it cannot be stated that one was unprotected since the neutralizing antibodies were not evaluated.

It provides and clarifies information on aspects such as aspects of production of farms, vaccination schemes and weaning age. As for the methodology, an error was found in the data in Table 1. The table has been updated with the right values for each group. However, the statistical difference noted within and between farms are correct. Figure 1 was elaborated, taking into account the reviewer's comment, with the same units of measurement in the two graphs

See referee reports

Introduction

Porcine circovirus type 2 (PCV2) belongs to the *Circoviridae* family. It is a non-enveloped icosahedral virus with a single-stranded circular DNA genome that contains 1766 to 1768 nucleotides (Fenaux *et al.*, 2004; Guo *et al.*, 2010). The PCV2 genome contains four open reading frames (ORFs), namely ORF1, ORF2, ORF3 and ORF4 (Allan *et al.*, 2012; Xiao *et al.*, 2015). ORF1 encodes the Rep and Rep' proteins required for viral replication, ORF2 encodes the immunogenic capsid protein (Cap) (Fenaux *et al.*, 2004), ORF3 encodes a protein involved in apoptosis (ORF3 protein) (Liu *et al.*, 2005) and ORF4 encodes a protein that affects the activity of CD4+ and CD8+ cells (He *et al.*, 2013). Additionally, the nucleotide diversity of ORF2 sequences allows to differentiate five different PCV2 genotypes denominated PCV2a, PCV2b, PCV2c, PCV2d (formerly known as mutant PCV2b) and PCV2e (Davies *et al.*, 2016; Franzo *et al.*, 2015b; Xiao *et al.*, 2015). PCV2a and PCV2b are distributed worldwide, although PCV2b is more prevalent than PCV2a (Opriessnig *et al.*, 2013). Until 2015, PCV2c was only reported in Denmark (Dupont *et al.*, 2008); however, it is now reported in feral pigs in Brazil (Franzo *et al.*, 2015a). Additionally, PCV2d is found in several countries, including China, Brazil, and USA (Davies *et al.*, 2016; Franzo *et al.*, 2015a; Guo *et al.*, 2010; Xiao *et al.*, 2015; Zhai *et al.*, 2011). Moreover, the distant PCV2 genotype (PCV2e) is found in China (Wang *et al.*, 2009) and the USA (Davies *et al.*, 2016). In Colombia, PCV2 infections have been described since 2002 and have been recently characterized (Rincón Monroy *et al.*, 2014).

Several syndromes collectively named Porcine Circovirus Associated Disease (PCVAD) are associated with PCV2 infections, and high PCV2 viral loads have been associated with disease severity (Olvera *et al.*, 2004). PCVAD include PCV2-subclinical infection (PCV2-SI), PCV2 systemic disease (PCV2-SD, initially named as post-weaning multisystem

wasting syndrome (PMWS), PCV2-reproductive disease (PCV2-RD), porcine dermatitis and nephropathy syndrome (PDNS), respiratory complex and enteritis (Segalés, 2012; Shen *et al.*, 2010). PCV-SD is considered the most economically significant condition for the swine industry among all PCVAD (Segalés, 2012).

PCVAD prevention is mainly based on vaccination against PCV2 infections (Feng *et al.*, 2014; Fort *et al.*, 2009), this has led to a decrease in the prevalence of the virus and in the levels of viremia (Dvorak *et al.*, 2016). PCV2 vaccination is effective in reducing viral load, viral shedding, and PCV2-SD associated lymphoid lesions (Cline *et al.*, 2008; Fachinger *et al.*, 2008; Fort *et al.*, 2008; Park *et al.*, 2014). Vaccination can also induce neutralizing antibodies and IFN γ secreting cells (IFN γ SCs), which facilitates viral clearance (Fort *et al.*, 2009; Martelli *et al.*, 2011). Additionally, PCV2 vaccination can minimize the effect of PCV2 infection on swine health improving average daily weight gain (ADWG) and reducing mortality, especially in the presence of co-infection with other viruses (Fachinger *et al.*, 2008; Horlen *et al.*, 2008; Kixmüller *et al.*, 2008; Park *et al.*, 2014).

There are at least four different types of commercial PCV2 vaccines based on the PCV2a genotype worldwide (Opriessnig *et al.*, 2014; Park *et al.*, 2014) that are effective at reducing the impact of PCV2a and PCV2b infections (Fort *et al.*, 2008). One inactivated vaccine contains whole PCV2 as the antigen, and is recommend for 3-week old piglets or breeding females (Beach & Meng, 2012; Segalés, 2015). In contrast, chimeric PCV1-2 vaccine contains the immunogenic capsid gene of PCV2a cloned into the genome backbone of the non-pathogenic PCV1 (Segalés, 2015). Moreover, subunit recombinant vaccines express the capsid protein within a baculovirus system (Shen *et al.*, 2010; Triple & Rowland, 2012) and are recommended for pigs between 2 and 4 weeks of age. However, off-label use of the chimeric vaccines in sows and gilts can result in the reduction of viremia and increased ADWG in the offspring (Fraile *et al.*, 2012; Segalés, 2015). Vaccination of sows seeks to reduce viremia and viral loads in piglets through neutralizing antibodies present in colostrum, and could improve the productive performance of their offspring after weaning (Beach & Meng, 2012; Gerber *et al.*, 2011; Pejsak *et al.*, 2010). Moreover, vaccination of the piglet is used to induce active humoral and cellular immunity, reduce viral loads, shorten duration of viremia, and improve productive performance (Fachinger *et al.*, 2008; Fraile *et al.*, 2012; Lyoo *et al.*, 2011; Takahagi *et al.*, 2010). Currently, it is feasible to vaccinate sows, piglets, or both (Fraile *et al.*, 2012; Opriessnig *et al.*, 2010), although the interference between maternally derived antibodies and active immunity of the piglet is under debate (Fraile *et al.*, 2012).

Although it is well known that vaccination reduces the clinical presentation of the disease, limited information is available regarding the effect of different PCV2 vaccination schemes on virus load and humoral immune response over time under field conditions. Therefore, the objective of this study was to compare the effect of two different PCV2 vaccination schemes on

the humoral response and PCV2 load in pigs after weaning. Our results indicated that different vaccination schemes against PCV2 induce different humoral immune responses overtime without a difference in the viral load observed. These results are important because they can help to prevent PCV2 infections and minimize the effect of PCVAD on swine health and production.

Methods

Farms and sample selection

For this study two commercial pig farms in Colombia (Farm A and B), endemically infected with PCV2, were conveniently selected. Farm A was 500-sow farrow-to-finish but with the nursery all in all out but it is close to site1; the site 3 is distant with continuous flow management. Farm B was 250-sow farrow-to-wean farm, with two additional sites for the nursery and finishing stages of production. Sites 1, 2 and 3 are geographically distant and the nursery or site two is all in all out; the site 3 is distant from site 2 but management is in continuous flow. Farm A vaccinated all sows and gilts (replacement animals for the breeding stock) against PCV2 every six months and all piglets on a weekly basis at 3 weeks of age. In contrast, Farm B vaccinated all gilts at arrival and piglets at 3 and 5 weeks of age on a weekly basis.

Forty 3-week old piglets were randomly selected at weaning in each farm. Each pig was ear tagged and randomly assigned to two treatments groups: non-vaccinated pigs (n=10) and PCV2 vaccinated pigs (n=30). Piglets with different treatments were comingled among other pigs after weaning based on the farmer's production system. Animal care and procedures at the farms were in accordance with the guidelines of the "Porcine Animal Welfare" guide (Pork Colombia, former Colombian Association of Pig Farmers), which is based on the concept of the five freedoms (established by the Welfare Council of Farm Animals, 1992 in the United Kingdom). The pens are in cement with plastic Slat zones, water troughs with water ad libitum, feeders and a rest area in straw. The densities were managed according to the weight of the pigs following the recommendations of [guideline 2008/120/EC](#). Pigs were injected intramuscularly on the right side of the neck at 3 weeks of age (weaning) with 1ml of commercial subunit vaccine A (VAC-A) in Farm A or 2ml of commercial subunit vaccine B (VAC-B) in Farm B. Additionally, pigs in Farm B were boosted with VAC-B at 5 weeks of age. Individual blood samples (10 ml) were collected by jugular venipuncture at 3, 7, 11, 15, 19 and 23 weeks of age (W3, W7, W11, W15, W19, and W23, respectively).

ELISA and quantitative polymerase chain reaction (qPCR)

IgG antibodies against PCV2 were evaluated by ELISA using the INGEZIM Circo IgG1.1® assay (Ingenasa-Spain) at 450nm on a BioTek® Power Wave XS OD system with a cutoff value of 0.3, according to the manufacturer's instructions.

Additionally, PCV2 viral loads were estimated over time using quantitative polymerase chain reaction (qPCR) ([Olvera et al., 2004](#)) in a Light Cycler® 480 II-Roche thermal cycling system. Briefly, DNA extractions were first performed from all serum samples collected using QIAamp DNA kit (QIAGEN®). Then PCV2 rep coding region of PCV2 was amplified using

PCV2-ABF 5'GCCAGAATTCAACCTTMACYTTYC 3' and PCV2-ABR 5'GCGGTGGACATGMTGAGATT 3' primers, as previously described ([Rincón Monroy et al., 2014](#)). PCR reactions were carried out in 20µl containing 5µl of DNA mixed with 15µl of real-time PCR master mix (Light Cycler® 480 SYBR Green I Master-Roche mix + 1µM of each primer) at 95°C for 1 minute followed by 40 cycles of 95°C for 1 minute, 61°C for 25 seconds and 72°C for 5 seconds. Additionally, a plasmid (PCR blunt vector plasmid) containing the complete PCV2 genome was used as positive control (kindly donated by Dr. Carl A. Gagnon, Swine and poultry infectious diseases research center -CRIPA, Université de Montréal, St-Hyacinthe, Québec, Canada). Ten-fold dilutions of the plasmid (from 10⁹ to 10¹ PCV2 plasmid copies/ml) were used as standard curve for PCV2 quantification. The cut-off level to diagnose animals as PMWS positive was established at 10⁷ PCV2 genomes/ml, according to previous studies ([Olvera et al., 2004](#)). Piglets with viral loads lower than 10⁷ were considered asymptomatic animals ([Olvera et al., 2004](#)). Data analysis was done using the corresponding software (Light Cycler® 480 II-Roche).

Statistical analysis

Mean IgG and PCV2 copies/ml were compared within and between VAC groups and considered statistically different if the non-parametric Wilcoxon-test p value was lower than 0.05. Additionally, the linear association between ELISA titers and the viral load was estimated at each sampling event and considered statistically significant if the null hypothesis of slope equal to 0 was rejected. The software used was R statistics version 3.4.1.

Results

Anti PCV2-IgG response

All piglets had IgG antibodies against PCV2 at weaning and there was no statistical difference between treatment groups within farms before vaccination ([Table 1](#)). However, at 3 weeks of age the anti-PCV2 IgG levels were higher in piglets from Farm A (VAC-A) than in piglets from Farm B (VAC-B) (p<0.05). The anti-PCV2 IgG response after vaccination was different between farms. In Farm A, IgG levels were high at 3 weeks of age and then decreased over time without significant difference in the average level of anti-PCV2 IgG between vaccinated and non-vaccinated pigs from Farm A (VAC-A) at each sampling event over time ([Table 1](#), p>0.05). Additionally, the mean optical density values obtained from pigs in Farm A overtime demonstrated that there was no seroconversion ([Figure 1A](#)). Moreover, in Farm B IgG levels increased after vaccination until week 15 of age when they started to decrease, while non-vaccinated pigs from the same farm did not seroconvert ([Figure 1B](#)) and showed statistically lower IgG titers over time (p<0.05, [Table 1](#)) compared to vaccinated pigs within the same farm. Interestingly, none of the vaccinated or non-vaccinated pigs in this study had anti-PCV2 IgG levels greater than 0.41 after 23 weeks of age.

PCV2 viral loads

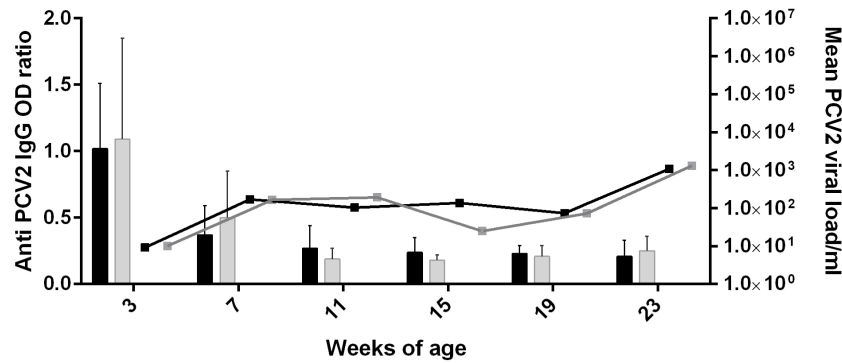
All serum samples from this study tested PCR positive for PCV2; however, none had a viral load greater than 10⁴ DNA copies/ml ([Figure 1A and B](#)). Hence, all pigs were considered PCR positive, but with low viral loads, and therefore PMWS

Table 1. Mean IgG levels distributed by week of age (3, 7, 11, 15, 19, and 23), farm (A and B), and treatment (vaccinated and non-vaccinated). Mean IgG levels are compared within farm (vaccinated vs. non-vaccinated) and between farms (VAC-A vs. VAC-B). Different letters within farm indicate a significant difference ($p < 0.05$) in the mean IgG level between vaccinated and non-vaccinated pigs. The significance level of difference in the IgG level between vaccinated pigs in farm A (VAC-A) and Farm B (VAC-B) by week are indicated with * ($p < 0.05$) and ** ($p < 0.01$). Pigs were vaccinated at week 3 and on farm B they received a booster at week 5.

Sampling - Weeks of age	Farm A				Farm B			
	Vaccinated		Non-vaccinated		Vaccinated		Non-vaccinated	
	IgG mean	Sd	IgG mean	Sd	IgG mean	Sd	IgG mean	Sd
3*	1.02 ^a	0.49	1.09 ^a	0.76	0.69 ^a	0.45	0.91 ^a	0.51
7**	0.37 ^a	0.22	0.50 ^a	0.35	0.98 ^a	0.56	0.44 ^b	0.35
11**	0.27 ^a	0.17	0.19 ^a	0.08	1.24 ^a	0.58	0.19 ^b	0.07
15**	0.24 ^a	0.11	0.18 ^a	0.04	1.35 ^a	0.61	0.24 ^b	0.13
19**	0.23 ^a	0.06	0.21 ^a	0.08	0.54 ^a	0.28	0.24 ^b	0.29
23**	0.21 ^a	0.12	0.25 ^a	0.11	0.41 ^a	0.17	0.22 ^b	0.36

Sd: Standard deviation

- A. Farm A: IgG in vaccinated Farm A: Viral load in vaccinated
 Farm A: IgG in non-vaccinated Farm A: Viral load in non-vaccinated



- B. Farm B: IgG in vaccinated Farm B: Viral load in vaccinated
 Farm B: IgG in non-vaccinated Farm B: Viral load in non-vaccinated

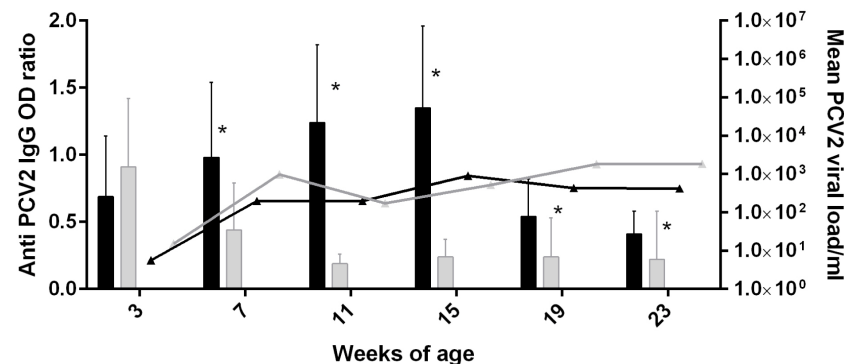


Figure 1. ELISA and PCV2 viral load comparison between vaccinated and non-vaccinated pigs in Farm A (panel A) and Farm B (panel B). Bars indicate the mean IgG level in vaccinated (black) and non-vaccinated (grey) pigs at 3, 7, 11, 15, 19, and 23 weeks of age. Lines indicate the mean PCV2 viral load in vaccinated (black) and non-vaccinated (grey) pigs at 3, 7, 11, 15, 19, and 23 weeks of age. * $p < 0.05$.

negative or asymptomatic during the study period. Additionally, there was no difference within farm in the viral load between vaccinated and non-vaccinated pigs and there was no difference found in the viral load between vaccinated pigs in Farm A (VAC-A) and vaccinated pigs in Farm B (VAC-B).

Dataset 1. Data of the results obtained in the study

<http://dx.doi.org/10.5256/f1000research.13160.d188246>

The data obtained and analysed for the ELISA and qPCRy tests are available in an attached document where are classified by farms. Likewise, the results of the negative controls used are included.

Discussion

To better understand the effect of PCV2 vaccination on the IgG response and PCV2 viral loads in pigs after weaning, we designed a longitudinal study and compared two different vaccination schemes under field conditions. We found that the PCV2 viral load in pigs after weaning was not associated to the vaccine scheme used in each farm studied. However, we found differences in the IgG levels between farms that could be associated with vaccination schemes. Understanding the effect of different vaccines and vaccine schemes on virus load and humoral response is important to design better health intervention to control PCV2 infection and minimize its effect on swine health and production.

PCV2 vaccination has proven to control the effect of PCV2 infection on swine health and production (Cline *et al.*, 2008; Horlen *et al.*, 2008; Kixmüller *et al.*, 2008) and there are different PCV2 vaccination schemes used in the contemporary swine industry. However, new PCV2 genotypes have been discovered (Davies *et al.*, 2016; Xiao *et al.*, 2015) and vaccine failure has been described (Fraile *et al.*, 2015; Wang *et al.*, 2009). In this study, we found low viral loads regardless of the vaccination scheme used in the farms studied. These findings were expected because vaccination can reduce the percentage of infectious pigs and the level of viremia in them (Cline *et al.*, 2008; Dvorak *et al.*, 2016; Fachinger *et al.*, 2008; Feng *et al.*, 2014; Opriessnig *et al.*, 2010). It is possible that viral loads remained low due to continuous vaccination of the herd regardless of the vaccination scheme. It was interesting to find that non-vaccinated animals maintained low viral loads within farms endemically infected with PCV2. We speculate that finding non-vaccinated pigs with low viral titers was the result of the overall herd immunity. This is in agreement with the findings by Feng *et al.* (Feng *et al.*, 2014), in which mass vaccination against PCV2 reduced viral loads at the population level. The presence of low viral loads in both vaccinated and unvaccinated pigs shows that the virus is circulating. Studies have shown that PCV2 is very stable in the environment, causing numerous routes of infection and that piglets can also be infected in the presence of maternal immunity (Dvorak *et al.*, 2013)

In this study, we found differences in the humoral response between vaccinated pigs from Farm A and Farm B over time. This result can probably be explained by the second dose (booster) used in piglets in Farm B, although this cannot be concluded from the results obtained since the levels of neutralizing antibodies were not evaluated. Studies show that vaccination against PCV2 does

not necessarily stimulate capsid-specific antibodies but does seem to be involved in the increase of neutralizing antibodies (Dvorak *et al.*, 2018). In our study, vaccination against PCV2 using two doses in piglets results in a higher antibody response than a single dose ($p < 0.05$), even though in terms of protection the two options have shown to be effective and control PCV2 viremia (Lyoo *et al.*, 2011). However, a single dose at 3 weeks of age might interfere with maternal antibodies as described before (Fort *et al.*, 2009; Fraile *et al.*, 2012; Martelli *et al.*, 2011). In our study, pigs from Farm A showed higher levels of maternal derived antibodies at weaning, did not seroconvert after a single vaccination, and showed low PCV2 loads over time. Pesjak *et al.* (Pesjak *et al.*, 2010) and Opriessnig *et al.* (Opriessnig *et al.*, 2010), demonstrated that the presence of maternal-derived antibodies do not affect the efficacy of PCV2 subunit vaccines and proved low concentrations of viral DNA in serum after vaccination (as seen in our study), absence of histological lesions, and improvement in the productive parameters. Moreover, the different humoral immune response between vaccinated and non-vaccinated pigs in Farm B corresponded to a classical pattern of antibody response due to vaccination. Furthermore, it is the classical profile of humoral response after weaning without virus circulating. The humoral immune profile of piglets and sows is determined by PCV2 circulation, vaccination schemes, and is associated with virus load in pigs after weaning.

Fraile *et al.* (Fraile *et al.*, 2015) defined four clusters of pigs based on PCV2 serological and PCR profiles. Cluster 1 is composed mainly by none vaccinated sows and none vaccinated pigs, in which viremic pigs are present with increasing antibody levels over time. Cluster 2 contains mostly vaccinated sows and non-vaccinated piglets in which late PCV2 infection and seroconversion is observed. Cluster 3 has mainly vaccinated sows and vaccinated pigs, viremia is rare and antibodies decrease over time; and cluster 4 is composed basically of non-vaccinated sows and vaccinated pigs in which infected animals are rare and high immunoperoxidase monolayer assay (IPMA) titers are observed. Regardless of the vaccination scheme used in our study (Farm A versus B) all pigs met the criteria of cluster 3, rare viremia and antibody induction over time, even though not all sows were vaccinated (Farm B).

The present study contributes to the understanding of PCV2 infection and control under field conditions. However, it is important to keep in mind that we assumed that farms were endemic infected with PCV2 although high viral loads were never observed. Therefore, we could not test if there was an appropriate protection induced by the vaccines or minimal virus challenge. Additionally, our low sample size for the non-vaccinated control groups ($n=10$) might had been insufficient to detect viremic pigs under very low prevalence of the virus at the population level.

Vaccination is a key intervention to control the impact of PCV2 on swine health and production. Our findings illustrated that, regardless of the vaccination scheme used, low viral loads of PCV2 were maintained, although a similar response was found in the unvaccinated group. This could indicate that when a farm

has a vaccination program established some time ago, it can contribute to the control of the virus. This can probably be explained by the presence of neutralizing antibodies in the control group that were not detected by the ELISA test. These results are important because they can help to prevent PCV2 infections and minimize the effect of PCVAD on swine health and production. Future studies are required to understand the epidemiology of PCV2 infection in positive farms with very low prevalence of PCV2 infections.

Ethical statement

The farms included in the study are associated with **Pork Colombia** and follow the guidelines of production, biosecurity and animal welfare required by this institution. Approval was requested from the farms where the study was conducted and they agreed to its completion. The veterinarians of each farm supervised and collaborated with the study. The Bioethics Committee of the Faculty of Veterinary Medicine and Animal Sciences of the National University of Colombia approved the procedures performed on the pigs (resolution OF-CBE-FMVZ-0006-10).

Every effort was made to reduce the suffering of the pigs to a minimum. Veterinarians trained in this procedure took the blood samples and the pigs were monitored for one hour after

taking the sample to control for any adverse effects on the procedure.

Data availability

Dataset 1: Data of the results obtained in the study. The data obtained and analysed for the ELISA and qPCR tests are available in an attached document where are classified by farms. Likewise, the results of the negative controls used are included. DOI, [10.5256/f1000research.13160.d188246](https://doi.org/10.5256/f1000research.13160.d188246) (Vargas-Bermudez *et al.*, 2017).

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Open Peer Review

Current Referee Status:   

Version 2

Referee Report 24 September 2018

doi:10.5256/f1000research.17540.r37654

 **Alvaro Rafael Ruiz-Garrido** 
 Facultad de Cs. Veterinarias, Universidad de Concepcion, Concepción, Chile

Regarding to the authors answer in 2.3.2, did not clarified the meaning of the * in the legend of the figure 1, as they say.



Competing Interests: No competing interests were disclosed.

Referee Expertise: Swine infection diseases and their effect in swine intensive production systems.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Referee Report 06 September 2018

doi:10.5256/f1000research.17540.r37656

 **Jesús Hernandez** 
 Laboratory of Immunology, Research Center for Food and Development (CIAD), Hermosillo, Mexico

I have no comments to make.

Competing Interests: No competing interests were disclosed.

Referee Expertise: Viral immunology

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Referee Report 13 February 2018

doi:10.5256/f1000research.14275.r30290



👤 **Jesús Hernandez** 

Laboratory of Immunology, Research Center for Food and Development (CIAD), Hermosillo, Mexico

The manuscript by Vargas-Bermudez *et al.* evaluated humoral response (IgG) and viremia in pigs from farms with two different vaccination programs. The authors concluded that viral loads were low regardless the vaccination schemes. The manuscript is well written, clear and provides interesting information.

Major concerns:

Conclusions

There are statements that are not supported by the results and have to be modified.

- “Another explanation for vaccinated and non-vaccinated pigs with low viral loads is that there was no PCV2 circulating in the farm and that continuous vaccination of the populations has indeed minimized PCV2 infection between pigs”.

Positive PCR indicate that PCV2 is circulating in the farm.

- “Our findings illustrated that different vaccination schemes against PCV2 can maintain low viral load in endemically infected populations regardless of the different humoral immune profiles observed over time”.

This statement is not correct, because non-vaccinated group maintained low viral loads.

Minor concerns:

Results

- Can you include the IgG values of week 5?
- It is not clear the weaning age. It is week 3?
- The inclusion of vaccination time in the table 1 could help in the interpretation.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Referee Expertise: Viral immunology

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 20 Aug 2018

Jairo Jaime, Universidad Nacional de Colombia

3. Jesus Hernandez:

3.1. Discussion.

3.1.1. In discussion, paragraph 2, you are correct in stating that: Another explanation for vaccinated and non-vaccinated pigs with low viral loads is that there was no PCV2 circulating in the farm and that continuous vaccination of the populations have indeed minimized PCV2 infection between pigs.

This correction coincides with 1.3.1. Mike Murtaugh

1.3.1. In Paragraph 2, you are right in stating that: Another explanation for vaccinated and non-vaccinated pigs with low viral loads is that there was no PCV2 circulating in the farm and that continuous vaccination of the populations has indeed minimized PCV2 infection between pigs. Evidently there was PCV2 circulation. The phrase was rectified and changed by: The presence of low viral loads in both vaccinated and unvaccinated pigs shows that the virus is circulating. Studies have shown that PCV2 is very stable in the environment, causing numerous routes of infection and that piglets can also be infected in the presence of maternal immunity (Dvorak et al., 2013).

3.1.2. In discussion, paragraph 6 states: Our findings illustrated that different vaccination schemes against PCV2 can maintain low viral load in endemically infected populations, regardless of the different humoral immune profiles observed over time.

This correction coincides with 2.2.7. (Alvaro Ruíz)

2.2.7. Paragraph 6 states: Our findings illustrated that different vaccination schemes against PCV2 can maintain low viral load in endemically infected populations, regardless of the different humoral immune profiles observed over time. This statement is not correct since the unvaccinated group maintained low viral loads. This statement was corrected as follows:

Our findings illustrated that, regardless of the vaccination scheme used, low viral loads of PCV2 were maintained, although a similar response was found in the unvaccinated group. This could indicate that when a farm has a vaccination program established some time ago, it can contribute to the control of the virus. This can probably be explained by the presence of neutralizing antibodies in the control group that were not detected by the ELISA test.

3.2. Results:

3.2.1. The IgG values can be included in week 5?

It was not evaluated in that week (which was when the booster was made (second dose) in farm B.

3.2.2. It is not clear the week of weaning, was week 3?

Yes it was in week 3, this was added in the text in methods (paragraph 2).

3.2.3. The inclusion of the vaccination time in table 1 could help the interpretation.

This recommendation is shared and included in the text of the table.

Competing Interests: No competing interests

Referee Report 09 February 2018

doi:10.5256/f1000research.14275.r30258



Michael P. Murtaugh , **Cheryl Dvorak**

Department of Veterinary and Biomedical Sciences, College of Veterinary Medicine, University of Minnesota, Saint Paul, MN, USA

The authors evaluated the effects of moderately different vaccination schemes on PCV2, concluding there was no substantial difference in the various vaccination schemes. The study is narrow in scope, having used two independent farms for the main treatment difference, and a different vaccine on each farm. These confounding factors limit the generalizability of the findings, which the authors are aware of. It is a common limitation in field studies that is a bit compensated for by the direct applicability of the findings. The lack of replication and the small sample sized are additional limitations that the authors acknowledge.

It was a bit surprising that the sow herds were either fully vaccinated or entering gilts were vaccinated. This practice is to my knowledge uncommon in North America since it is obvious that capsid vaccines used only in the farrowing room and maybe the nursery are highly effective in preventing PCVAD in growing pigs. It also is common knowledge that the level of PCV2 viremia in finishing pigs has been reduced tremendously since vaccines were introduced in North America in 2006 (Dvorak et al. National reduction in porcine circovirus type 2 prevalence following introduction of vaccination. *Vet Micro* 189 [2016] 86-90). For this reason, much of the preceding published literature may be out of date and not relevant to the present situation.

There is a wide variation in quality in the published literature that the authors might want to evaluate. Phylogenetics studies with hundreds or thousands of sequences (e.g. Davies. [2016] Diagnostic phylogenetics reveals a new porcine circovirus 2 cluster. *Virus Res.* 217:32-37) are superior to reports with tens of sequences which often miss important variants due to random chance. The authors also should refer to original and primary reports rather than reviews in citing new discoveries.

Minor comments:

Methods. Farms and sample selection, 1st paragraph – The phrase "vaccinated on a weekly basis" is confusing. Perhaps the phrase could be deleted or the vaccination scheme explained a bit more.

Discussion. It is incorrect to say that no PCV2 was circulating in the farm, since there were PCV2 positive animals in all treatment groups. It also is clear that PCV2 is environmentally stable, providing for numerous sources of infection in the environment of the pigs (Dvorak et al. [2013] Multiple routes of porcine circovirus type 2 transmission to piglets in the presence of maternal immunity. *Vet. Microbiol.* 166:365-374.)

Discussion. Third paragraph – "In this study, we found differences in the humoral response between vaccinated pigs from Farm A and Farm B over time, mainly explained by the second dose (booster) used in piglets in Farm B and the vaccination schemes used in gilts and the sows." Because of the confounding factors present in the experimental design it really is not possible to conclude that the booster was the reason for the differences in humoral response.

Interestingly, it has been shown previously that vaccination does not necessarily boost capsid-specific antibodies, but does seem to be involved in increasing neutralizing antibody titers. Perhaps, this is dependent upon the vaccine used, which would agree your data. (Dvorak et al. Effect of Maternal Antibody Transfer on Antibody Dynamics and Control of Porcine Circovirus Type 2 Infection in Offspring. *Viral Immunology* [2018] 31: 40-46.)

Discussion. Fourth paragraph – “Fraile et al....” I think you mean non-vaccinated not “none vaccinated”.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Referee Expertise: Viral immunology, veterinary immunology, molecular virology, phylogenetics, viral evolution, animal infectious diseases

We have read this submission. We believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however we have significant reservations, as outlined above.

Author Response 20 Aug 2018

Jairo Jaime, Universidad Nacional de Colombia

1. Mike Murtaugh

1.1. Introduction:

1.1.1. It has the reason that it has been shown that the level of viremia has decreased in North America using vaccination, particularly in the United States. Correction was made, thus leaving the phrase: PCVAD prevention is mainly based on vaccination against PCV2 infections (Fort et al., 2009; Feng et al., 2014), which has led to a decrease in the prevalence of the virus and viremia levels (Dvorak et al., 2016).

1.1.2. It has the reason that phylogeny should be compared with papers that support analysis of hundreds or thousands of sequences to obtain clearer clusters. In this case, the bibliography was corrected in the first paragraph of the introduction.

1.2. Methods:

1.2.1. The phrase ... at 3 and 5 weeks of age on a weekly basis. It was eliminated *on a weekly basis*.

1.3. Discussion:

1.3.1. In Paragraph 2, you are right in stating that: Another explanation for vaccinated and non-vaccinated pigs with low viral loads is that there was no PCV2 circulating in the farm and that continuous vaccination of the populations has indeed minimized PCV2 infection between pigs. Evidently there was PCV2 circulation. The phrase was rectified and changed by: *The presence of low viral loads in both vaccinated and unvaccinated pigs shows that the virus is circulating. Studies have shown that PCV2 is very stable in the environment, causing numerous routes of infection and that piglets can also be infected in the presence of maternal immunity (Dvorak et al., 2013).*

1.3.2. In paragraph 3, you are right that it cannot be said that: In this study, we found differences in the humoral response between vaccinated pigs from Farm A and Farm B over time mainly explained by the second dose (booster) used in piglets in farm B and the vaccination schemes used in gilts and sows. The sentence was rectified as follows: *In this study, we found differences in the humoral response between vaccinated pigs from Farm A and Farm B over time. This result can probably be explained by the second dose (booster) used in piglets in Farm B, although this cannot be concluded from the results obtained since the levels of neutralizing antibodies were not evaluated. Studies show that vaccination against PCV2 does not necessarily stimulate capsid-specific antibodies but does seem to be involved in the increase of neutralizing antibodies (Dvorak et al., 2018).*

Competing Interests: No competing interests

Referee Report 08 February 2018

doi:10.5256/f1000research.14275.r30289



Alvaro Rafael Ruiz-Garrido 

Facultad de Cs. Veterinarias, Universidad de Concepcion, Concepción, Chile

I think that the paper is very good, and should be published, but there are some things that must be improved before, as follows:

- Abstract

The authors should review the conclusions, because they did not observe statistical differences between the vaccinated and unvaccinated groups, in both farms, regarding to the viral loads. They just studied 2 variables (humoral response and viral load) and they only found that the humoral response is different depending of the vaccine used.

- Methods

The authors should explain better the differences that exist between both farms, from the infrastructural, animal's flow and management point of view.

It is lamentable that the authors do not have a replicate of the treatment in each farm, in order to give more power to the results.

- Statistical analysis

The authors should use a test of repeated samples ANOVA, if the assumptions allow it.

- Results

The authors should review the tables, because there are some differences in the results that are possible to obtain from the original data and the ones showed in tables (mean and standard deviation) and figures (mean) of the paper.

In Figure 1, the authors should use the same scale for the ELISA results of farm A and B. Additionally, they have to indicate if the * indicate statistical differences between vaccinated and unvaccinated animals of farm B or statistical differences between the different weeks of sampling of vaccinated animals of farm B.

- Discussion

The authors should discuss the phrase “However, at 3 weeks of age the anti-PCV2 IgG levels were higher in piglets from Farm A (VAC-A) than in piglets from Farm B (VAC-B) ($p < 0.05$).” that it is in the results, and the implication that the farms were different from the beginning, and how this can influence the results that they obtain.

As Figure 1 shows, the authors should give explanations for the serology decay of the vaccinated animals in farm B at the 19 and 23 weeks. Additionally, the authors should discuss why there is an increase, no statistical significance, in 3 of the 4 groups, at weeks 23, of the mean PCV2 DNA load, as show by Figure 1.

There is a little of over conclusions, since it states that “In our study, vaccination against PCV2 using two doses in pig- lets results in a higher antibody response than a single dose ($p < 0.05$), even though in **terms of protection the two options have shown to be effective and control PCV2 viremia**”; the authors cannot affirm this because there were no differences in viral load between control (un vaccinated) and vaccinated group.

The authors should indicate what is the meaning of IPM in the clusters 4.

The authors should review the sentence “Our findings illustrated that different vaccination schemes against PCV2 can maintain low viral load in endemically infected populations regardless of the different humoral immune profiles observed over time”. The authors did not observe statistic differences between the control group and the vaccinated group, so they can not affirm the above sentence.

It could have been interesting if the authors had measured the productive parameters of the treatment groups, to see any difference, but with the reduced number of animals (especially the control group) this was not possible.

The authors should discuss the differences between farms (including the Ig G level at the beginning of the essay), vaccine used, vaccine protocols and epidemiology of PCV2 at the population level and there effect on they results.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Partly

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Referee Expertise: Swine infection diseases and their effect in swine intensive production systems.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 20 Aug 2018

Jairo Jaime, Universidad Nacional de Colombia

2. Alvaro Ruíz Garrido

2.1. Abstract

2.1.1. Include in the abstract the variables handled (humoral response and viral load) indicating that there are only changes in the immune response.

In the Abstract, the paragraph of methods says what the reviewer is requesting: We designed a longitudinal study and measured IgG levels by ELISA and virus load by quantitative PCR in pigs after weaning. In the abstract also, in the paragraph of results, the following sentence was added: We found that low virus loads were maintained in pigs from both farms, regardless of the vaccination scheme used ($p > 0.05$). However, IgG levels were observed over time ($p < 0.05$) *while no significant differences were found in viral loads. This suggests that different humoral immune response is not associated with different virus loads observed over time.*

2.2. Methods:

2.2.1. Indicate the differences between farms in terms of infrastructure, animal flow and management. The sentence was rectified as follows:

For this study two commercial pig farms in Colombia (Farm A and B), endemically infected with PCV2, were conveniently selected. Farm A was 500-sow farrow-to-finish but with the nursery all in all out but it is close to site 1 and site 3 is distant with continuous flow management. Farm B was 250-sow farrow-to-wean farm, with two additional sites for the nursery and finishing stages of production. Sites 1, 2 and 3 are geographically distant and the nursery or site two is all in all out. Site three is distant from site two but management is in continuous flow.

2.2.2. In the statistical analysis because an ANOVA test of repeated samples was not done. We decided to not use ANOVA because the data was not normally distributed and it was not

independent at all (vaccinated and not vaccinated animals were in the same farm). Hence we used a non parametric test to compare the mean of to correlated samples.

2.3. Results

2.3.1. Review the tables since there is a difference of results between the original data and those of the tables (SD / media)

The reviewer is correct and the mean and SD for animals in farm A in Table 2 are incorrect. The table has been updated with the right values for each group. However the statistical difference noted within and between farms are correct.

2.3.2. In figure 1 use the same scale for the ELISA. The * indicates differences between vaccinates (farm B) or statistical differences over time.

In Figure 1, different scales were used so that the reader can better visualize the differences, however it is considered that the reviewer is right and the two graphs with the same scale were adjusted. The * indicates difference between vaccinated and unvaccinated in each evaluated week, not in time. This is clarified in the legend of the figure.

2.4.3. In paragraph 2, the sentence: However, at 3 weeks of age the anti-PCV2 IgG levels were higher in piglets from Farm A (VAC-A) than in piglets from Farm B (VAC-B) ($p < 0.05$). What implications does this have from the beginning and how can it influence the results?

The presence of higher levels of antibodies in farm A both in vaccinated and unvaccinated pigs at the beginning of the experiment (week 3) could be indicating that there was a greater transmission of maternal antibodies compared to farm B, without any of the farms this difference will affect the viral load.

2.2.4. In Figure 1. Why there is a decline in the serology of vaccinated pigs in farm B at 19 and 23 weeks. It should be analyzed why there is an increase, without statistical significance, in 3 of the 4 groups in week 23 of the average PCV2 DNA load?

The decay of antibodies at weeks 19 and 23 in farm B in the vaccinated group without modification of the viral load would indicate that the antibodies detectable by ELISA have been metabolized, but do not show that the pigs have lost protection. By week 23, an increase in viral loads (without statistical significance) could be established, which could indicate that viral loads were increasing while antibodies were decreasing and if the pigs were kept longer it is likely that viral loads increased to levels of risk. (This is better in the discussion)

2.3. Discussion

2.3.1. In paragraph 3 it cannot be stated: In this study, we found differences in the humoral response between vaccinated pigs from Farm A and Farm B over time mainly explained by the second dose (booster) used in piglets in farm B and the vaccination schemes used in gilts and sows.

This correction coincides with 1.3.2. Mike Murtaugh

1.3.2. In paragraph 3, you are right that it cannot be said that: In this study, we found differences in the humoral response between vaccinated pigs from Farm A and Farm B over time mainly explained by the second dose (booster) used in piglets in farm B and the vaccination schemes used in gilts and sows. The sentence was rectified as follows: In this study, we found differences in the humoral response between vaccinated pigs from Farm A and Farm B over time. This result can probably be explained by the second dose (booster) used in piglets in Farm B, although this cannot be concluded from the results obtained since the levels of neutralizing antibodies were not evaluated. Studies show that vaccination against PCV2 does not necessarily stimulate capsid-specific antibodies but does seem to be involved in the increase of neutralizing antibodies (Dvorak et al., 2018).

2.3.2. What does IPM mean?

It was corrected by IMPA (immunoperoxidase monolayer assay)

2.2.7. Paragraph 6 states: Our findings illustrated that different vaccination schemes against PCV2 can maintain low viral load in endemically infected populations, regardless of the different humoral immune profiles observed over time. This statement is not correct since the unvaccinated group maintained low viral loads. This statement was corrected as follows:

Our findings illustrated that, regardless of the vaccination scheme used, low viral loads of PCV2 were maintained, although a similar response was found in the unvaccinated group. This could indicate that when a farm has a vaccination program established some time ago, it can contribute to the control of the virus. This can probably be explained by the presence of neutralizing antibodies in the control group that were not detected by the ELISA test.

2.3.3. The authors should discuss the differences between farms (including the IgG level at the beginning of the trial), the vaccine used, the vaccine protocols and the epidemiology of PCV2 at the population level and its effect on the results.

It is likely that the level of neutralizing antibodies in both farms was sufficient to control the virus. The implication of these initial levels of antibodies in the results is not clear and could be on the response of the same. In farm A, it was able to influence the levels of antibodies to remain low throughout the experiment, while in farm B he was able to increase them. The foregoing is explained by the consumption of the antibodies against the vaccine challenge at 3 weeks (farm A).

Competing Interests: No competing interests

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