One dose of a porcine circovirus 2 (PCV2) sub-unit vaccine administered to 3-week-old conventional piglets elicits cell-mediated immunity and significantly reduces PCV2 viremia in an experimental model

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**Abstract**

The immunogenicity and efficacy generated by one dose of a PCV2 sub-unit vaccine (Porcilis PCV\(^{®}\)) were evaluated in 3-week-old conventional piglets. Vaccination induced both humoral and cell-mediated responses against PCV2, which were increased after the challenge with a PCV2 genotype “b” isolate. High levels of maternally derived antibodies (IPMA \(\geq 10\) log\(_2\)) at the time of vaccination were found to interfere with the active seroconversion, whereas titres below 8 log\(_2\) allowed the development of a proper antibody response. Nevertheless, the immunity induced by one dose of the product was partly protective against PCV2 infection, since viremia, shedding and viral load in tissues were significantly reduced in vaccinated pigs compared to controls.

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1. Introduction

Porcine circovirus type 2 (PCV2) is the causative agent of post-weaning multisystemic wasting syndrome (PMWS) [1] and has also been associated to other swine diseases such as porcine dermatitis and nephropathy syndrome (PDNS), porcine respiratory disease complex (PRDC) and reproductive failure, now collectively referred as porcine circovirus diseases (PCVD) [2]. For years, control of PMWS or other PCVD has been limited mainly to improved management strategies and control of concomitant diseases. At present, four vaccines against PCV2 have been introduced in the international market. All the vaccines are non-replicative (sub-unit or inactivated) and are targeted for weaned piglets (three vaccines) or sows (one). In all cases, the reports from field trials suggest that commercially available PCV2 vaccines significantly contribute to decrease mortality rates and to improve growth performance in PCV2-affected farms [3,4], thereby reducing the economical impact that PCVD, and particularly PMWS, have on pig production worldwide [5,6].

The protective immunity induced by commercial vaccines is thought to rely on the development of a strong humoral response, whereas little information is available on the role of cell-mediated immunity. Recently, Goubier et al. [7] reported the presence of PCV2-specific interferon-gamma (IFN-\(\gamma\)) secreting cells (IFN-\(\gamma\)-SC) in colostrum of PCV2 naturally infected and vaccinated sows, being their numbers significantly higher in the latter ones and proven to be passively transferred to their offspring. However, in that study, no conclusion could be established on their protective effect against PCV2 infection, since virus-specific IFN-\(\gamma\)-SC could be only detected in piglets within the first 5 days post-farrowing. The development of PCV2-specific IFN-\(\gamma\)-SC has been also described to occur in caesarean derived, colostrum deprived (CDCD) piglets after PCV2 infection, and suggested to mediate viral clearance in combination with neutralizing antibodies (NA) [8].

Protection provided by colostral antibodies against PCV2 infection or development of PMWS is documented in the literature [9–11], and seems to be associated with their neutralizing activity [12–14]. Conversely, the potential interference of the vaccine produced by maternal-derived antibodies is still unclear. Under experimental conditions, some reports indicated that high levels of maternal antibodies may partially interfere with the development of a humoral response after vaccination. Nevertheless, such interference was not enough to significantly hamper vaccine efficacy [14,15]. On the other hand, under field conditions, the lack of vaccine interference with maternal-derived immunity has been described [4], but there is at least one study reporting decreased vaccine efficacy to be dependant on the antibody levels at the time of vaccination [16].
In a previous work we evaluated the humoral response and the efficacy of a PCV2 sub-unit vaccine using a two-dose schedule in commercial pigs [14]. The results of that study showed that double vaccination was able to prevent viremia. In the present study we evaluated whether the degree of immunity induced after one-dose schedule of the same product was efficient enough in protecting 3-week-old piglets against PCV2 infection. For that, vaccine immunogenicity was assessed in terms of development of humoral and cell-mediated immune responses, and PCV2 infection by measuring PCV2 viremia, shedding and viral load in tissues. Besides, the potential effect of maternally derived antibodies on vaccine efficacy was also investigated.

2. Materials and methods

2.1. Animals and housing

One hundred and six 2-week-old male piglets were randomly selected from a PCV2-seropositive, conventional 2000-sow farm located in the North-eastern Spain. Piglets were selected from a total of 35 sows (1–6 piglets/sow). The herd was free from Aujeszky’s disease virus, Brachyspira hyodysenteriae, Mycoplasma hyopneumoniae, and seropositive but stable to porcine reproductive and respiratory syndrome virus. PCR [17] was performed on blood, nasal and faecal swabs to confirm that piglets were not infected with PCV2. Selected animals were transported to the experimental facilities in the Centre de Recerca en Sanitat Animal (CReSA) and let to acclimatize for 7 days before PCV2 vaccination. During that period, animals were weighted and bled to assess their serological status to PCV2 using a previously described immunoperoxidase monolayer assay (IPMA) technique [18]. Piglets were then distributed into four groups balanced according to weights and titres of PCV2 IPMA antibodies.

2.2. Vaccine and PCV2 inoculum

The vaccine product was a commercial PCV2 genotype “a” [19] based sub-unit vaccine (Porcilis PCV®, Intervet International, The Netherlands). In the present experiment the vaccine was administered as a single dose at 3 weeks of age.

PCV2 strain I-12/11 (PCV2 genotype “b”) was used as inoculum [14]. This strain was isolated from lymphoid tissues of a field case of PMWS in year 2003 in The Netherlands. The virus was propagated in PCV-free PK15 cells to a titre of 10^5 TCID50/ml.

2.3. Experimental design and sampling

At 21 days of age, 51 piglets were intramuscularly vaccinated (vaccinated, V), whereas the remaining animals (n = 55) received PBS (non-vaccinated, NV). Three weeks after immunization (42 days of age), piglets were challenged intranasally with either 2 ml of a suspension containing 10^5 TCID50 of PCV2/ml (challenged, C; n (V–C) = 26, n (NV–C) = 18) or 2 ml of sterile PBS (non-challenged, NC; n (V–NC) = 25, n (NV–NC) = 37). After inoculation, pigs were monitored for 21 days. During that period pigs were clinically examined on a daily basis and rectal temperatures were recorded three times per week. Body weight was measured before the immunization, at challenge and at weekly intervals thereafter. Blood samples were taken at the time of vaccination, at challenge and at days 7, 14 and 21 post-infection (PI). Sera were stored at –80 °C until used. For the evaluation of cell-mediated immunity, blood samples with lithium heparin were taken from 24 pigs (six from each of the four experimental groups) and isolation of peripheral blood mononuclear cells (PBMC) was performed by density gradient centrifugation using Histopaque 1.077 (Sigma–Aldrich).

To assess PCV2 shedding throughout the experiment, nasal and faecal swabs were also taken at all sampling days, re-suspended in 1 ml of PBS and stored at –80 °C until further analysis. At day 21 PI, all pigs were euthanized with an intravenous overdose of sodium pentobarbital and subjected to necropsy. For histopathological studies, samples of tonsil and mediastinal, superficial inguinal and mesenteric lymph nodes were collected and fixed in 10% buffered formalin.

Animal care activities and study procedures were conducted in accordance with the guidelines of the Good Experimental Practices, under the approval of the Ethical and Animal Welfare Committee of the Universitat Autònoma de Barcelona.

2.4. Pathological studies

Formalin-fixed, paraffin-embedded tissue samples were cut at 4 μm thick, stained with hematoxylin–eosin stain and examined for lesions compatible with PMWS. For each individual and tissue, lesions were scored from 0 (no lesions) to 3 (severe lesions) [20]. The final histopathological score was the average of the four studied tissues (tonsil, superficial inguinal, mesenteric and mediastinal lymph nodes). In situ hybridization (ISH) was also performed on the same tissues for the detection of PCV2 genome [20]. The amount of PCV2 DNA was semi-quantified using a score from 0 (lack of PCV2 genome) to 3 (high amount of PCV2 genome) in each individual examined tissue. The final ISH score was the average of the four studied tissues.

2.5. Detection of PCV2 DNA

Serum samples, nasal and faecal swabs were analysed for the presence of PCV2 genome by means of a real-time quantitative PCR (Q-PCR) using the method reported by Olvera et al. [21]. Viral concentrations were expressed as PCV2 DNA copy numbers per ml of serum or ml of PBS (nasal and faecal swabs) [22].

2.6. Serology

Sera were examined for PCV2 antibodies by means of an IPMA technique at all sampling days. PCV2 neutralizing antibodies (NA) were determined at day of vaccination, at challenge and at the end of the study using the technique described by Fort et al. [13]. With the aim to estimate the decay of passively acquired antibodies to PCV2, an additional analysis by an indirect enzyme-linked immunosorbent assay (ELISA) for PCV2 antibodies (Ingenasa-PCV, Ingenasa) was performed using serum samples of NV–NC.

2.7. Rate of decay of passively acquired PCV2 antibodies

Half-life of maternal-derived antibodies was defined as the time needed for a decline of the levels of maternally derived antibodies resulting in a 50% decrease in ELISA optical density. Thus, for each individual and pair of consecutive time-points, decline rate was calculated according to the following formula: T1/2 = (t1 − t2)[ln2/ln(ODt1–ODt2)], where t1 and t2 represent consecutive sampling times (in days) and ODt1 and ODt2 are the optical densities obtained in the ELISA. Assuming a constant rate of decay, this rate can be calculated from the following equation: 0.5 = (1 – RD)^1/2 where RD is the rate of decay and T1/2 is the average half-life of maternal-derived antibodies.

2.8. Determination of IFN-γ-SC specific to PCV2

An ELISPOT using commercial monoclonal antibodies (BD Pharmingen) to determine frequencies of IFN-γ-SC in isolated PBMC was performed at day of vaccination, day of challenge and...
at days 7, 14 and 21 Pt. The PCV2 strain used for challenge and an extract of recombinant capsid (Cap) protein expressed in baculovirus and produced in *Trichoplusia ni* insect larva [23] were used as stimuli. Supernatant of non-PCV2 infected PK-15 cells (mock-stimulus) and wild type baculovirus extract (Ni) were used as their corresponding negative controls. Briefly, 100 μl containing 5 x 10⁵ PBMC in RPMI 1640 medium supplemented with 10% foetal calf serum (FCS) were seeded into a 96-well plate pre-coated overnight with anti-IFN-γ antibody at 5 μg/ml (BD Pharmingen, clone P2G10) together with 100 μl of either phytohaemagglutinin (PHA) (10 μg/ml), PCV2 (multiplicity of infection of 0.05), mock, Cap or Ni protein (0.6 μg/ml). Cultures were done in duplicate. After 20 h of incubation at 37 °C in 5% CO₂ atmosphere, cells were removed and plates were incubated with a biotinylated antibody (BD Pharmingen, clone P2C11) at 0.5 μg/ml for 1 h at 37 °C, followed by 45 min incubation with 0.5 μg/ml of streptavidin-HRP (Biosource). Insoluble TMB (Calbiochem) was used to reveal the reaction. For each individual and stimuli, the ELISPOT count was reported as the average number of spots of replicates minus the average number in the corresponding negative control wells (mock-stimulated cells or Ni-stimulated cells). Results were expressed as the number of responding cells per million of PBMC.

2.9. Statistical analyses

Prior to the analyses, values of the number of copies of viral DNA/ml and serological titres (IPMA and VNT) were transformed to log₁₀ and log₂ values, respectively. Normality of the distribution of the examined variables was evaluated by the Shapiro–Wilk test. One-way ANOVA and the Tukey’s test were used for mean comparison of the normally distributed variables between groups. Non-parametric Kruskal–Wallis and Mann–Whitney tests. The distribution of the examined variables was evaluated by the Shapiro–Wilk test. One-way ANOVA and the Tukey’s test were used for mean comparison of the normally distributed variables between groups. Non-parametric Kruskal–Wallis and Mann–Whitney tests. The Chi-square test was applied to evaluate the proportion of positive samples. Statistical analyses were performed using SPSS v.15. The significance level (α) for all analyses was set at 0.05.

3. Results

3.1. Clinical presentation and histopathological evaluation

None of the pigs developed clinical disease or fever during the whole study. No statistically significant differences in body weight were observed among groups at any of the sampling times.

In the histopathological examinations, mild PMWS-like lesions (lymphocyte depletion and granulomatous inflammation) were observed in 3/18 (16.7%; CI95%: 4.4–42.7%) NV–C pigs and in 1/26 (3.9%; CI95%: 0.2–21.6%) V–C pigs, with no significant differences among groups. Also, PCV2 was detected in lymphoid tissues by ISH in 7/18 (38.9%; CI95%: 18.3–63.9%) NV–C pigs, whereas no positive tissues were observed in pigs from V–C group (0/26) (p < 0.05).

3.2. Detection of PCV2 DNA in sera, nasal and faecal swabs

The proportion of PCV2 Q-PCR positive pigs in sera, nasal and faecal swabs for each of the challenged groups during the study is shown in Table 1.

In the NV–C group, viremia was firstly detected at day 7 Pt in 2/18 (11.1%; CI95%: 1.9–36.1%) pigs (viral load of 6.0 and 6.6 log₁₀), being the proportion of viremic pigs increased up to 7/18 (38.9%; CI95%: 18.3–63.9%) pigs at day 14 Pt and 10/18 (55.6%; CI95%: 31.3–77.6%) by the end of the study. Mean viral loads were 5.3 ± 1.0 log₁₀ and 4.8 ± 0.6 log₁₀ PCV2 copies/ml, respectively. In contrast, of the 26 V–C pigs, only two developed viremia throughout the study and in both cases PCV2 DNA was only detected at day 7 Pt (viral load of 4.3 and 3.9 log₁₀); from then onwards, all pigs from V–C group remained negative.

One week after challenge, all pigs from the NV–C group (18/18), and 21/26 (80.8%; CI95%: 60.0–92.7%) in the V–C one were positive by Q-PCR in nasal swabs (mean viral loads 5.3 ± 1.4 log₁₀ and 4.8 ± 0.6 log₁₀) and, at day 14 Pt, all pigs from both groups were nasal shedders. However, by the end of the study the proportion of positive pigs increased to 17/26 (65.4%; CI95%: 44.4–82.1%) in the V–C group, whereas 15/18 (83.3%; CI95%: 57.7–95.6%) NV–C pigs were still positive (non-significant). At that time, viral quantity detected in nasal cavity of V–C pigs was significantly lower compared to NV–C counterparts (4.5 ± 1.0 log₁₀ versus 5.3 ± 1.2 log₁₀; p < 0.01).

PCV2 load in faeces was lower than in nasal swabs. In the NV–C group, both the percentage of faecal shedders and viral load detected in positive pigs increased from 2/18 (11.1%; CI95%: 1.9–36.1%) at day 7 Pt (mean viral load: 4.6 ± 0.2 log₁₀) to 9/18 (50.0%; CI95%: 26.8–73.3%) at day 21 Pt (mean viral load: 5.0 ± 0.7 log₁₀). Out of 26 V–C pigs only one (3.9%; CI95%: 0.2–21.6%) had detectable PCV2 DNA in faeces (21 days Pt, viral load: 4.5 log₁₀). These differences between NV–C and V–C groups were significant (p < 0.01).

Serum samples, nasal and faecal swabs from V–NC and NV–NC pigs were all PCV2 negative throughout the study.

3.3. Humoral immune response to PCV2

The evolution of serological titres to PCV2 measured by IPMA and VNT tests of the different animal groups is displayed in Fig. 1.

Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum (%)</th>
<th>Nasal swabs (%)</th>
<th>Faecal swabs (%)</th>
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<tr>
<td></td>
<td>7 d PI</td>
<td>14 d PI</td>
<td>21 d PI</td>
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<tr>
<td>Vac</td>
<td>Ch</td>
<td></td>
<td></td>
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<tr>
<td>NV–C</td>
<td>0/18</td>
<td>11.1/18</td>
<td>38.9/18</td>
</tr>
<tr>
<td>V–C</td>
<td>0/26</td>
<td>7.7/26</td>
<td>0/26</td>
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<td></td>
<td>0/18</td>
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<td></td>
<td>0/26</td>
<td>80.8/26</td>
<td>100/26</td>
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<tr>
<td></td>
<td>0/18</td>
<td>11.1/21</td>
<td>16.7/31</td>
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<td>0/26</td>
<td>0/26</td>
<td>0/26</td>
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<tr>
<td>NV–C</td>
<td>0/18</td>
<td>11.1/26</td>
<td>16.7/31</td>
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<td>V–C</td>
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Percentage of PCV2 Q-PCR positive pigs in serum, nasal and faecal swabs from day of PCV2 vaccination to 21 d Pt, in non-vaccinated and vaccinated challenged groups.
3.4. Rate of decay of passively acquired PCV2 antibodies

NV–NC pigs allowed the calculation of the decay rate of maternally derived antibodies. In order to improve the calculation accuracy, serum samples from NV–NC pigs were analysed using an indirect ELISA (Ingenasa-PCV, Ingenasa). Correlation between IPMA and ELISA results was 0.722 (p < 0.001). Thus, for NV–NC group, the average half-life was calculated to be 15.9 days and the antibody decay ratio was 0.044. If a linear correlation is assumed to exist between optical densities and antibody titres, the decay in the maternal antibody titres would be approximately 4.4% per day.

3.5. Effect of maternally derived antibodies on PCV2 infection

Since not all PCV2 inoculated pigs became viremic, the serological profiles of pigs that became viremic and those that did not were compared. For that, NV–C pigs were classified into two categories (viremic and non-viremic) depending on whether or not they had become viremic at any time during the experiment. Then, antibody titres (IPMA and NA) at challenge were compared. Non-viremic pigs had significantly higher IPMA titres than viremic animals (10.6 ± 1.3 log2 versus 5.5 ± 3.3 log2, p < 0.01). For NA, the picture was similar (8.0 ± 2.1 log2 in non-viremic versus 3.9 ± 1.7 log2 in viremic animals, p < 0.01).

3.6. Effect of maternally derived antibodies on PCV2 vaccination

Since a significant correlation was determined between antibody titres (both IPMA and NA titres) at challenge and prevention of viremia, the possible interference of maternal antibodies with the development of an active humoral immune response after vaccination was examined. Animals from the V–NC group were categorized according to their serological profiles obtained using both IPMA and VNT in response to vaccination (seroconversion or lack of seroconversion). Antibody titres at the time of vaccination were compared between responding and non-responding pigs. Results indicated that pigs that did not seroconvert against PCV2 had significantly higher IPMA titres (p < 0.01) at the time of vaccination (10.0 ± 1.3 log2) compared to those animals that seroconverted (8.0 ± 1.6 log2). For NA, differences were also significant (8.8 ± 1.8 log2 versus 5.3 ± 1.6 log2, respectively; p < 0.01). Fig. 2 shows the antibody-profile of PCV2 IPMA (2A) and NA (2B) titres of V–NC pigs according to their humoral immune response after vaccination.
3.7 Cell-mediated immune response to PCV2 using whole virus or Cap protein

Development of PCV2- and Cap-specific IFN-γ-SC for each experimental group is shown in Fig. 3. Before vaccination, frequencies of IFN-γ-SC for PCV2 or Cap were negligible \((0–6/10^6\) PBMC). At challenge, 4/6 pigs in V–C group and 5/6 in V–NC responded to the whole virus, with average frequencies of PCV2-specific IFN-γ-SC of \(20 \pm 12/10^6\) PBMC and \(18 \pm 10/10^6\) PBMC, respectively. Two weeks after challenge, no response was observed in V–NC animals \((0–2\) PCV2-IFN-γ-SC), while 6/6 pigs responded in the V–C group \((average frequency of PCV2-specific IFN-γ-SC 35 \pm 22/10^6\) PBMC), being this difference significant \((p < 0.05). In the V–C group, IFN-γ responses remained constant at day 21. For NV–C group, frequencies developed in V–C pigs while decreased to \(11 \pm 15/10^6\) PBMC at day 21 PI and \(11 \pm 8/10^6\) PBMC at day 21 PI; contrary to the results observed in vaccinated groups, in NV–C group frequencies developed in response to the Cap protein were lower in comparison to those obtained using the whole virus.

4. Discussion

PCV2 vaccines seem to be very efficient based on the results from experimental infections and field reports [4,14,15]. However, several aspects related to the practical use of PCV2 vaccination for piglets such as the interference of maternal-derived antibodies, the need for a second dose and the precise mechanisms involved in post-vaccination immunity are still poorly known. In the present study, with the aim to investigate whether the administration of one dose of a PCV2 sub-unit vaccine (Porcilis PCV®) might be efficient enough to control PCV2 infection, conventional piglets were vaccinated at 21 days of age and challenged 3 weeks later with a genotype “b” PCV2 isolate. Vaccine efficacy was assessed in terms of reducing PCV2-associated lesions and viral load in serum, nasal and faecal swabs. To evaluate vaccine immunogenicity, both humoral and cell-mediated responses were measured. Besides, the potential interference effect that maternal antibodies might have on the immunity induced by one-dose schedule vaccination, and therefore on its efficacy on controlling PCV2 infection, was also investigated.

PMWS is considered a multifactorial disease in which the participation of PCV2 is the essential element [2]. However, the disease is hardly reproducible under experimental conditions. A meta-analysis performed by Tomás et al. [24] indicated that the highest likelihood to experimentally reproduce PMWS includes the use of PCV2-seronegative piglets younger than 3 weeks of age. However, such models do not fit with the natural conditions in which pigs develop PMWS in the nurseries or fattening units. Therefore, conventional colostrum-fed piglets were used in the present study to test the vaccine product mimicking field conditions. It was assumed that chances to reproduce PMWS by using such type of animals and PCV2 alone as inoculum were low and, therefore, the evaluation of vaccine efficacy in terms of protection against clinical disease was not feasible. This situation applies to this study but also earlier ones in which only subclinical infections were developed [25,14,15]. As performed in those studies, parameters related to PCV2 infection such as viremia, shedding, presence of microscopic PMWS-like lesions in lymphoid tissues and viral load within lesions were considered for the assessment of vaccine efficacy in the present work.

A previous experiment performed in conventional pigs with two doses of Porcilis PCV®, administrated at 4 and 6 weeks of age, resulted in prevention of viremia [14]. In the present study, in which a single dose of the same vaccine was evaluated, 24 out of 26 V–C animals did not develop viremia either. In addition, the two pigs that were found PCV2 PCR positive in serum at day 7 PI were negative at day 14 PI, and remained so until the end of the study. In contrast, in the NV–C group, 10 out of 18 pigs were PCV2 positive in sera throughout the study. Comparison of the Q-PCR results in sera between those two groups indicated that a single dose vaccination was able to reduce the proportion of viremic pigs, and, when not fully prevented, viremia was shortened. In terms of vaccine efficacy, this represents 86.1% PCV2 viremia reduction. Additionally, a
positive effect of vaccination on PCV2 shedding was also observed, significantly reducing the proportion of PCV2 faecal shedders and viral load in nasal cavity at day 21 PI, in comparison to the NV–C group. Herein, it was shown that one dose was effective in reducing the effects of subclinical PCV2 infection, limiting virus replication and thereby decreasing the amount of virus released. Nevertheless, further studies are needed to perform side by side comparison between one and two dose regimens as well as to assess one dose vaccination efficacy on clinical PCVD control.

At challenge, development of a humoral immune response to the vaccine was observed since significantly higher IPMA titres in vaccinated pigs compared to controls were observed. In contrast, although NA titres were also higher in vaccinated groups at the same time, no clear seroconversion for the latter ones was observed until day 21 PI. Also, in the V–C group, a boost on PCV2 antibodies was detected firstly on day 14 PI using IPMA technique, whereas neutralizing response was mostly low and even undetectable in some pigs by the end of the study. These results support earlier data reporting low and delayed production of NA to occur sporadically in some pigs in response to both, PCV2 infection [13] and PCV2 vaccination [14,26]. In a recent study by Opiressnig et al. [15], no clear seroconversion for NA after a single PCV2 vaccination was observed in the presence of high level of antibodies at the time of immunization. The present data show that, although one dose was able to induce a neutralizing immune response in most of the pigs, mean NA titres developed (6.1 ± 1.7 log2) were apparently lower in comparison to the levels detected using the same technique in a previous work, in which piglets were given the same product twice (7.5 ± 1.2 log2) [14]. These results suggest that two doses might be necessary to induce a strong neutralizing response in pigs that have high antibody titres at vaccination. Further analysis of the serological data showed interference of passively acquired antibodies with PCV2 infection and active seroconversion to vaccination. According to our results, animals with IPMA titres beyond 10.6 log2 (or NA > 8 log2) are likely to be protected against PCV2 viremia when PCV2 challenged oro-nasally with 105 TCID50, whereas those having titres below 5.5 log2 at IPMA or 3.9 log2 at NA are potentially more susceptible. Regarding the effect of maternal-derived antibodies on vaccination, the performed analysis suggests that IPMA titres equal or beyond 10 log2 (or NA > 8.8 log2) seem to interfere with the development of the humoral immune response after vaccination, while levels below 8 or 5.5 log2 at IPMA and VNT techniques respectively do not.

Ideally, PCV2 vaccination should be administered when residual maternal antibodies are minimal and before pigs become naturally infected. Since PCV2 infection and seroconversion dynamics greatly vary among farms [27], antibody-profiling of a representative number of piglets prior to vaccine implementation seems therefore a good tool to estimate proper vaccination timing. Based on our results, to ensure a humoral immune response development after vaccination, PCV2 IPMA titres at the time of vaccine administration should stay below 10 log2. Considering the fact that pigs with IPMA titres of 5.5 log2 or lower are potentially susceptible to PCV2 infection and that the half-life of passively acquired antibodies is around 15 days, an estimation of the pig age at vaccination can be established, and vaccination scheduled accordingly.

In the present study, we first describe the development of cell-mediated immunity in response to PCV2 piglet vaccination. Thus, at challenge (3 weeks after immunization), only vaccinated pigs showed induction of IFN-γ-SC specific to PCV2 and the Cap protein. This response was increased in the vaccinated pigs which were later challenged, highly suggesting the presence of effector memory T cells in immunized animals. These results are in accordance with those obtained by Shen et al. [28] using a murine model, in which it was demonstrated that protective immunity against PCV2 in mouse was mediated by Cap-specific CD8+ cells and seroneutralization responses. In pigs, the development of virus-specific IFN-γ-SC in response to PCV2 infection has been also demonstrated under experimental conditions using caesarean derived-colostrum deprived piglets [8]. In that study, it was also suggested that cellular responses could contribute, together with neutralizing antibodies, to the viral clearance. Interestingly, in the present study, the intensity of responses generated using either the Cap protein or the whole PCV2 as stimulus differed among groups. Thus, whereas in vaccinated groups (V–NC and V–C) responses to the Cap protein were similar or slightly higher than to the whole PCV2, in NV–C pigs, IFN-γ responses obtained by stimulating PBMC with the whole virus were higher than with the Cap protein. These findings suggest that infected animals in which PCV2 is replicating might respond strongly to other viral components different from the Cap protein. Kekarainen et al. [29] demonstrated the ability of PCV2 viral components to differently modulate cytokine responses in vitro. Thus, stimulation of PBMC with the whole PCV2 resulted in IL-10 induction, while no production was observed using PCV2 virus like particles (VLPs) or oligodeoxynucleotides containing CpG motifs (CpG-ODNs) based on the PCV2 genome. Also, PCV2 and some CpG-ODNs but not VLPs were found to inhibit IFN-γ and IL-2 produced in vitro during recall antigen responses. These results, together with our findings and the fact that the Cap protein has been demonstrated to be a good immunogen [30,14], suggest that this protein is a suitable stimulus for in vitro assays aimed to assess cell-mediated immune responses to PCV2 vaccination.

In summary, the results of the present study demonstrate that one dose administration of Portcils PCV® significantly reduced PCV2 viremia, shedding, as well as viral genome loads within PMWS-like lymphoid lesions. Also, high levels of maternally derived antibodies were seen to interfere with active seroconversion to vaccination, while apparently no effect was observed on vaccine efficacy. The data presented here provide new information regarding the mechanism by which PCV2 vaccines may confer protective immunity against PCV2, describing for the first time the development of cell-mediated immunity in response to PCV2 piglet vaccination.

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