Influence of age on the effectiveness of PCV2 vaccination in piglets with high levels of maternally derived antibodies

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ABSTRACT

Two field studies were conducted to investigate the influence of age on the efficacy of vaccination against Porcine Circovirus Diseases (PCVD) in animals with high levels of maternally derived antibodies (MDA). A total of 416 piglets (Study 1) and 600 piglets (Study 2) were randomly allocated to one of three groups. Two groups in each study received a single dose of a PCV2 subunit vaccine, one group at 1 week old and the other at 3 weeks of age. The third group was left untreated. Animals vaccinated at 3 weeks of age showed a significantly higher average daily weight gain and significantly reduced viraemia following PCV2 infection than the respective control groups. This difference was not observed in pigs vaccinated at 1 week of age. Furthermore, only animals vaccinated at 3 weeks of age showed an increased serological response and a higher frequency of IgM-positive animals compared with controls. The data indicated that PCV2 vaccination in the presence of high MDA levels is efficacious when used in 3-week-old pigs. As the range of MDA titres of pigs vaccinated at both 1 and 3 weeks of age were comparable, the data suggest that PCV2 vaccine efficacy was independent of the level of MDA. It appears that other age-related factors affecting the active and passive transfer of immunity may perhaps have interfered with the efficacy of the vaccine in 1-week-old piglets. These findings have implications for future PCV2 vaccine testing and administration strategies.

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1. Introduction

Postweaning multisystemic wasting syndrome (PMWS) was first described in 1996 with respect to clinical cases observed 5 years earlier (Clark, 1996; Harding, 1996). Shortly afterwards porcine circovirus type 2 (PCV2) was identified as the causative agent for this disease syndrome. It soon became apparent that PCV2 was also associated with a number of other disease complexes which have been named collectively Porcine Circovirus Diseases (PCVD) (Segales, 2012). Vaccination against PCV2 along with good production practice is currently the only way to control this complex of diseases. Since 2007, four vaccines conferring either active or passive immunity have become commercially available. All of them are able to reduce viraemia and PCV2-related clinical effects such as decreased weight gain and mortality (Fachinger et al., 2008; Fort et al., 2009; Fraile et al., 2012; Seo et al., 2012).

The use of most PCV2 vaccines is recommended for piglets from 3 weeks of age onwards. However, vaccination of even younger animals is increasingly becoming an area of

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interest. It is widely believed that the biggest obstacle to the effective vaccination of neonates is the potential for passive interference by maternally derived antibodies (MDA) (Chappuis, 1998; Hodgins and Shewen, 2012; Salmon et al., 2009). Antibody responses in neonates have been reported to be diminished and less persistent in the presence of high MDA levels (Hodgins and Shewen, 2012; Siegrist, 2003).

The role of maternal lymphocytes on the effective vaccination of neonates is less clear. It has been found that piglets can ingest >130 million lymphocytes daily via colostrum (Evans et al., 1982; Le Jan, 1996) which can traverse the intestinal epithelium and localize in mesenteric lymph node and other tissues (Gans, 2012). These maternal lymphocytes seem to contribute to the defence against pathogens in neonates and may also have the capacity to interfere with vaccination (Hodgins and Shewen, 2012; Le Jan, 1996). In addition, studies in piglets have confirmed the uptake of cytokines such as IL4, IL6, IL10, IL12 and IFNγ via colostrum and their persistence for 1–2 weeks (Nguyen et al., 2007). These maternal cytokines may also modulate neonatal immune functions in the first weeks of life.

For how long, and to what extent these different passively transferred immune factors may interfere with the neonatal immune system is not completely clear. Interestingly, neonatal T-cell response are not necessarily suppressed in the presence of high MDA levels, but may in fact contribute to protection, and provide a long-lasting immunity (Gans, 2012). For example, piglets derived from Mycoplasma hyopneumoniae-vaccinated sows were able to develop a specific T-cell response following vaccination, even in the presence of high MDA levels (Bandrick et al., 2008). Similarly, a specific immunity could be induced in piglets with high MDA levels following vaccination against pseudorabies virus (Kimman et al., 1992; Kit et al., 1993).

With regard to PCV2, it is well-documented that vaccination of 3-week old piglets against PCV2 in the presence of high levels of MDA provides protection (Fachinger et al., 2008; Fraile et al., 2012; Kixmoller et al., 2008; Opriessnig et al., 2008). However, in at least two of the studies, the humoral immune response following vaccination was clearly diminished (Fraile et al., 2012; Opriessnig et al., 2008). These findings indicate that piglets are able to develop an active, most likely T-cell mediated immune response against PCV2 in the presence of high MDA levels. Furthermore, vaccination of 5-day old PCV2-seronegative piglets with two different PCV2 vaccines was shown to provide protection against an experimental PCV2 challenge 4 weeks later (O’Neill et al., 2011). This suggests that the immune system of 5-day old piglets is, in principle, mature enough to mount an active immune response against PCV2 in the absence of MDA. However, these two findings do not allow any conclusions about the development of an active immune response in neonates following single vaccination in the presence of other potentially interfering factors such as maternal lymphocytes and/or maternally transferred non-specific immune components.

It was the purpose of this study, therefore, to investigate the influence of age on the efficacy of PCV2 vaccination in piglets with high levels of MDA.

2. Materials and methods

2.1. Farm selection

Pigs in Study 1 (German Landrace × German Yorkshire [F] × Pietrain) were included on a breeding and nursery farm with 510 sows and a one week farrowing interval. Sows were routinely vaccinated against PPV, erysipelas and PRRSV and piglets were routinely vaccinated against M. hyopneumoniae at 10 days of age. Weaning was performed at 4 weeks of age and pigs were moved to the growing-to-finish farm with an all-in-all-out production system at an age of 10 to 11 weeks of age. The study site had a history of PCVD-related clinical signs which were most prominent during the middle of the fattening period when wasting, lameness, cough and diarrhoea were observed. Approximately 2% of animals showed signs of PDNS. The mortality rate varied between 2 and 5%. Vaccination of sows against PCV2 (Circovac®, Merial GmbH) was introduced but ceased 7 months before the start of the study. Before the start of the trial, the clinical diagnosis of PCVD was confirmed by the detection of high PCV2 viral loads in serum by qPCR and in situ hybridization.

Pigs in Study 2 (ADN [F] × Pietrain) were included on a breeding and nursery farm with 1800 sows and a two week farrowing interval. Sows were routinely vaccinated against PPV, erysipelas, PRRSV Escherichia coli and Clostridium perfringens. Animals were weaned at 3 weeks of age and transferred to the growing-to-finish farm with an all-in-all-out system at 12 weeks of age. The study site had a history of clinical signs of PMWS and PDNS which were observed from the middle of the fattening period onwards. The mortality rate during fattening was 5–6%. In an attempt to control losses caused by PCV2 on the fattening farm, the sows of the breeding farm had been vaccinated against PCV2 (Circovac®, Merial GmbH). This vaccination was discontinued eleven months before the start of the study. Six months before study initiation, the diagnosis of PMWS was verified on the basis of clinical signs, PCV2 viral load in serum as well as histopathology of various lymphnodes and lung that all occurred when animals were approximately 15 weeks old.

2.2. Experimental design

Both studies followed a randomized, negative-controlled and blinded, parallel study design. Improvement of Average Daily Weight Gain (ADWG) of the vaccinated groups compared to the control group during the fattening period was chosen as the primary efficacy parameter. In Study 1 a sample size of 124 animals in each group was calculated to detect a difference in means of 25 g/day with a power of 80% and an expected standard deviation of 70 g/day using an ANOVA with a consecutive two group t-test and a 5% two-sided significance level. As the standard deviation of Study 1 was higher than expected (83 g/day) the sample size of Study 2 was enlarged to 182 animals using an assumed standard deviation of 85 g/day. In each study 10% more animals were included in order to compensate for possible drop-outs. Viral load in serum and serology served as secondary parameters in both
studies. A sample size of 14 animals in each group and farrowing batch was calculated to detect a reduction of the mean viral load in serum at the peak of viraemia of 2.0 log_{10} copies per ml serum with a power of 80% and an expected standard deviation of ≤1.7 copies per ml serum. To compensate for possible drop-outs, one additional sample animal per group was included.

In total, 416 and 600 healthy piglets 3–10 days old with a body mass greater than 1 kg, were included in studies 1 and 2 respectively. Animals were derived from two farrowing batches (Study 1) or one farrowing batch (Study 2). On the day of inclusion, animals were distributed equally with regard to initial body weight and litter assignment between three treatment groups. The piglets were vaccinated with an inactivated subunit vaccine (Porcilis PCV1®; MSD Animal Health) on the day of inclusion (‘one week’ group, in this group the vaccine was used off-label) or 2 weeks later (‘three weeks’ group). To ensure blinding of both studies all unvaccinated animals of study 1 were treated with the control product (adjuvants only, having the same visual appearance as the vaccine) at both 1 and 3 weeks of age. As an independent administrator was available in Study 2, animals of the control group were treated with the control product (sterile saline) at 3 weeks of age. Details of the study animals at the time of vaccination are shown in Table 1.

All animals in Study 1 were weighed at 1, 12 and 25 weeks of age and in Study 2 at 1, 11 and 26 weeks of age and the average daily weight gain (ADWG in g/day) analyzed over the periods “1 week old to end of nursery”, “end of nursery to end of fattening” and “1 week old to end of fattening”.

Dead animals and animals that had to be euthanized for reasons of animal welfare were recorded daily by the stock keeper.

At the time of animal inclusion, 3 animals were selected at random from each of the first 14–15 litters per farrowing batch (Study 1: two farrowing batches, Study 2: one farrowing batch.). Blood samples were collected in Study 1 at 3, 7, 11, 14, 18, 22, and 25 weeks of age and in Study 2 at fortnightly intervals. Blood samples were allowed to clot and were transported to the laboratories within 36 h, under cool conditions. Serum was prepared from the clotted blood samples and stored at −20°C until tested.

2.3. Serological analyses

The quantification of anti-PCV2 antibodies in serum samples was performed at the Service Lab of MSD Animal Health by an indirect ELISA developed in-house using a fixed amount of baculovirus-expressed PCV2 ORF2 as antigen and biotin-labelled PCV2-specific monoclonal antibody in combination with peroxidase-conjugated streptavidin as detection system. This ELISA can detect antibodies against PCV2 in a range of 2–16 log_{2}. Comparison of the in house ELISA with a commercially available ELISA (Symbiotics SERELISA®PCV2 Ab Mono Blocking ELISA) showed a good correlation with slightly higher Ab titres in the SERELISA (up to a titre of 12 log_{2}) compared to the in-house ELISA (Martens et al., 2010).

The percentage of animals with IgG and IgM antibodies against PCV2 was determined by a commercially available ELISA test (Ingezim Circovirus IgG/IgM, Ingenasa).

2.4. Quantification of viral DNA

For the quantification of the PCV2 viral load in serum, samples from 17 animals collected at 3, 7, 11, 14, 18, 22 and 25 weeks of age were analyzed at the Clinic for Swine at the University for Veterinary Medicine Vienna (Study 1) and samples from 15 animals collected at 17, 19, 21, 23 and 25 weeks of age were analyzed at Virology R&D MSD AH, Boxmeer (Study 2) by a PCV2-specific real-time polymerase chain reaction (PCR).

In summary, viral DNA was extracted from serum using the High Pure PCR Template Preparation Kit (Study 1) or the DNA/Viral NA SV 1.0 kit (Study 2) (both Roche Diagnostics GmbH). In Study 1, the amplification was performed by a commercial test kit (PCVP-TaqVet™ Porcine Circovirus 2, Laboratoire Service International). In Study 2, the amplification was performed in reaction mixture containing 10 μl extracted DNA, 1.5 μl (15 mM) of forward primer (5’-TggCCCCgCAGTATTCTgATT-3’), 1.5 μl

---

**Table 1**

Descriptive data of vaccinated and control animals on the day of vaccination.

<table>
<thead>
<tr>
<th>Study</th>
<th>No of animals</th>
<th>No of males/females</th>
<th>Body weight (kg)</th>
<th>PCV antibody titre (log_{10})</th>
<th>% IgG-positive animals</th>
<th>% IgM-positive animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study 1</td>
<td>138</td>
<td>70/68</td>
<td>2.17</td>
<td>13.0±10.2^b</td>
<td>100/96^a</td>
<td>0/0^a</td>
</tr>
<tr>
<td>Study 2</td>
<td>200</td>
<td>94/106</td>
<td>2.26</td>
<td>10.6±9.1^b</td>
<td>87/87^b</td>
<td>0/0^a</td>
</tr>
</tbody>
</table>

For control group at 1 week: 139/139, 68/71, 73/66. For vaccination group at 1 week: 200/200, 81/119, 83/117.

^a At 1 week of age.

^b At 1/3 weeks of age.
(15 mM) of reverse primer (5’-ggggAAAggTGcACGACTG-3’), 2.0 μl (20 mM) DLHP probe (5’-FAM-CCAGCAATCAGACCCCgTTggATAT-TAMRA-3’), 5.0 μl dNTPs (SphaeroQ), 1.0 μl SuperTaq (SphaeroQ) and 29 μl PCR buffer. The reactions were performed in a real-time thermocycler with the following cycling times: 1 cycle at 50°C for 120 s, 1 cycle at 95°C for 600 s, 40 cycles at 95°C for 15 s and at 60°C for 60 s. During validation of the PCR, the limit of quantification was found to be 10⁴ template copies per millilitre serum for both assays.

2.5. Data analysis and statistical evaluation

The statistical unit was the individual pig. Homogeneity of the study population with regard to initial weight was analyzed by analysis of variance (ANOVA) and sex distribution by Fisher’s exact test. Average daily weight gain was analyzed using analysis of variance with the factors “treatment group”, “sex”, “farrowing batch” (Study 1 only) and “initial body weight” as a covariate. The vaccinated groups were compared with the control group using ANOVA derived Dunnett tests. The Wilcoxon Mann–Whitney test was used in order to assess differences with regard to parameters of viraemia and serology. Groups were tested with respect to mortality rates by Fisher’s exact test. The statistical analyses were performed using SAS software release 8.2 (2001) (SAS, Cary, NC; SAS Institute Inc.).

3. Results

3.1. Average daily weight gain

Table 2 shows the ADWG (least square mean) per treatment group in both studies for the intervals between the three different weighing times. Until the end of the nursery phase, the majority of the vaccinated groups showed a reduced ADWG compared to the control group. During fattening, animals vaccinated at 3 weeks of age gained significantly more weight (study 1: 25 g/day, \( p = 0.0061 \); Study 2: 30 g/day, \( p = 0.0167 \)) than the respective control animals. By contrast, the ADWG of piglets vaccinated at 1 week of age was not significantly different from that of the respective control animals.

3.2. Mortality

During the suckling period of Study 1, no difference in the mortality rate of the different treatment groups was seen whereas animals vaccinated at 1 week of age in Study 2 showed a higher mortality rate than animals of the other two groups (Table 3). This higher mortality rate was caused by an acute outbreak of E. coli diarrhoea which was not

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Average daily weight gain.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (weeks)</td>
<td>Weight gain</td>
</tr>
<tr>
<td>Study 1</td>
<td></td>
</tr>
<tr>
<td>1–12</td>
<td>ADWG (g/day)</td>
</tr>
<tr>
<td></td>
<td>Confidence Interval</td>
</tr>
<tr>
<td></td>
<td>Difference®</td>
</tr>
<tr>
<td></td>
<td>p-value®</td>
</tr>
<tr>
<td>12–25</td>
<td>ADWG (g/day)</td>
</tr>
<tr>
<td></td>
<td>Confidence Interval</td>
</tr>
<tr>
<td></td>
<td>Difference®</td>
</tr>
<tr>
<td></td>
<td>p-value®</td>
</tr>
<tr>
<td>1–25</td>
<td>ADWG (g/day)</td>
</tr>
<tr>
<td></td>
<td>Confidence Interval</td>
</tr>
<tr>
<td></td>
<td>Difference®</td>
</tr>
<tr>
<td></td>
<td>p-value®</td>
</tr>
<tr>
<td>Study 2</td>
<td></td>
</tr>
<tr>
<td>1–11</td>
<td>ADWG (g/day)</td>
</tr>
<tr>
<td></td>
<td>Confidence Interval</td>
</tr>
<tr>
<td></td>
<td>Difference®</td>
</tr>
<tr>
<td></td>
<td>p-value®</td>
</tr>
<tr>
<td>11–26</td>
<td>ADWG (g/day)</td>
</tr>
<tr>
<td></td>
<td>Confidence Interval</td>
</tr>
<tr>
<td></td>
<td>Difference®</td>
</tr>
<tr>
<td></td>
<td>p-value®</td>
</tr>
<tr>
<td>1–26</td>
<td>ADWG (g/day)</td>
</tr>
<tr>
<td></td>
<td>Confidence Interval</td>
</tr>
<tr>
<td></td>
<td>Difference®</td>
</tr>
</tbody>
</table>

a ADWG of vaccinated group minus ADWG of control group.

b Compared to control group.

c \( n = 131, 126, 128 \) animals for 1–12, 12–25 and 1–25 weeks in Study 1 and 191, 188, 188 animals for 1–11, 11–26 and 1–26 weeks of age in Study 2.

d \( n = 132, 128, 131 \) animals for 1–12, 12–25 and 1–25 weeks in Study 1 and 181, 177, 177 animals for 1–12, 12–26 and 1–26 weeks of age in Study 2.

e \( n = 134, 128, 128 \) animals for 1–12, 12–25 and 1–25 weeks in Study 1 and 190, 187, 187 animals for 1–12, 12–26 and 1–26 weeks of age in Study 2.

f Significant with \( p < 0.05 \) (compared to control group).

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However, the study reached a significant percentage (Study 2.7 vs 12.5 log2) of fatting period. Positive findings showed a peak of viraemia (peak at 2.0 log2 at age 17 weeks of age (Study 2). From 22 weeks of age (Study 1) and 23 weeks of age (Study 2) onwards, the antibody titres of the control groups began to increase again.

At the time of vaccination, the antibody titres of the groups vaccinated at 3 weeks of age were in the high range (Study 1: 11.0 log2; Study 2: 9.1 log2) and comparable with the titres of the groups vaccinated at 1 week of age (Study 1: 12.2 log2; Study 2: 10.6 log2) (Table 1 and Fig. 1).

Following vaccination, the groups vaccinated at 1 week of age showed a titre which was very similar to that of the

### Table 3
Mortality rate per group and observation period.

<table>
<thead>
<tr>
<th>Age (weeks)</th>
<th>Mortality</th>
<th>Control</th>
<th>Vaccination at 1 week</th>
<th>3 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1–4</td>
<td>No</td>
<td>2/138</td>
<td>(1.4%)</td>
<td>2/139</td>
</tr>
<tr>
<td></td>
<td>(%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5–12</td>
<td>No</td>
<td>3/135</td>
<td>(2.2%)</td>
<td>2/137</td>
</tr>
<tr>
<td></td>
<td>(%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13–25</td>
<td>No</td>
<td>4/133</td>
<td>(3.0%)</td>
<td>5/135</td>
</tr>
<tr>
<td></td>
<td>(%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1–3</td>
<td>No</td>
<td>1/200</td>
<td>(0.5%)</td>
<td>9/200</td>
</tr>
<tr>
<td></td>
<td>(%)</td>
<td></td>
<td></td>
<td>1/200</td>
</tr>
<tr>
<td>4–11</td>
<td>No</td>
<td>8/199</td>
<td>(4.0%)</td>
<td>10/190</td>
</tr>
<tr>
<td></td>
<td>(%)</td>
<td></td>
<td></td>
<td>8/199</td>
</tr>
<tr>
<td>12–26</td>
<td>No</td>
<td>2/190*</td>
<td>(1.1%)</td>
<td>4/180</td>
</tr>
<tr>
<td></td>
<td>(%)</td>
<td></td>
<td></td>
<td>3/190*</td>
</tr>
</tbody>
</table>

* One animal lost to follow up.
* Significant with \( p = 0.0200 \) (compared to control group).

seen in Study 1. During the nursery as well as during the fattening period, the mortality rate was comparable across all the groups in both studies.

### 3.3. PCV2 viraemia

PCV2-positive control animals were found in the late phase of fattening (Fig. 1). Peak levels of viraemia were reached at 22 weeks of age in Study 1 and \( \geq 25 \) weeks in Study 2. In Study 1, both vaccinated groups showed a significantly reduced viral load and lower percentage of positive animals at the peak of viraemia (\( p < 0.0001 \)). However, both the viral load and the percentage of viraemic animals were higher in the group vaccinated at 1 week of age, compared to the group vaccinated at 3 weeks of age. In Study 2, only animals vaccinated at 3 weeks of age (not those vaccinated at 1 week of age) showed a significantly reduced viral load and a lower percentage of viraemic animals at the peak of viraemia (3 week group: \( p = 0.0004 \); 1 week group: \( p = 0.6516 \) compared to the control group).

### 3.4. Serological response following vaccination and PCV2 infection

At the start of the study, the antibody titres against PCV2 of all treatment groups were high with a mean of 12.5 log2 in Study 1 and a mean of 10.5 log2 in Study 2 (Fig. 2). Thereafter, antibody titres of both control groups progressively declined, reaching minimum levels of 2.7 log2 at 18 weeks of age (Study 1) and 2.0 log2 at 17 weeks of age (Study 2). From 22 weeks of age (Study 1) and 23 weeks of age (Study 2) onwards, the antibody titres of the control animals began to increase again.

At the time of vaccination, the antibody titres of the groups vaccinated at 3 weeks of age were in the high range (Study 1: 11.0 log2; Study 2: 9.1 log2) and comparable with the titres of the groups vaccinated at 1 week of age (Study 1: 12.2 log2; Study 2: 10.6 log2) (Table 1 and Fig. 1).

Following vaccination, the groups vaccinated at 1 week of age showed a titre which was very similar to that of the
of age was comparable to the control group in Study 1 while it was delayed and lower than in the control group in Study 2. Animals vaccinated at 3 weeks of age showed an increase comparable with that in the control group in Study 1 and a greater increase in titre in Study 2.

3.5. IgG and IgM specific serological response against PCV2

In both studies the percentage of animals with IgG antibodies specific for PCV2 was above 87% in all groups at 1 and 3 weeks of age (Fig. 3) and decreased considerably between 7 and 9–13 weeks of age. Interestingly, the percentage of positive animals in those groups vaccinated at 3 weeks of age was still slightly higher than in the other two groups within this period. From 18 weeks of age (Study 1) and 21 weeks of age (Study 2) onwards, the percentage of IgG-positive animals increased markedly, in parallel with the increase in the total PCV2 antibody titres (compare Figs. 2 and 3).

There were no IgM-positive animals in the vaccinated groups at the time of vaccination in either study. Between 2 and 9 weeks post vaccination, only the groups vaccinated at 3 weeks of age showed an increased proportion of IgM-positive animals. By contrast, the first IgM-positive animals in the control groups and the groups vaccinated at 1 week of age appeared around the time of exposure to PCV2 (as determined by qPCR). Overall, control animals showed the highest proportion of IgM-positive animals at the respective peaks of viraemia, followed by the groups vaccinated at 1 week of age and then the groups vaccinated at 3 weeks of age.

4. Discussion

The purpose of these two field studies was to investigate the influence of age on the effects of single vaccination in piglets with high levels of MDA. In order to be able to also determine the duration of protection in animals vaccinated in the presence of high MDA levels, study sites were chosen with a known late onset of PCV2 viraemia. However, at least in Study 2, viraemia occurred at such a late stage of fattening (21 weeks of age) that it is unclear whether or not the peak of PCV2 viraemia had actually been reached by the time of the last blood sampling (25 weeks of age).

At the time of vaccination, the mean MDA titres (as measured by ELISA) of all the vaccinated groups ranged between 9.1 and 12.2 $\log_{2}$, which may be considered “high”. In fact, a threshold of approximately $10 \log_{2}$ has been used repeatedly to distinguish between “high” MDA and “moderate” and “low” MDA titres (Fachinger et al., 2008; Fort et al., 2009; Larochelle et al., 2003). Furthermore, the high MDA titres in these two studies were in line with the high, and comparable, percentage of IgG-positive animals vaccinated at one and three weeks of age (>85%), and the linear decline in the PCV2 MDA titres of the control animals over a prolonged period (up to 17 and 18 weeks of age). Considering that sows in both studies were last vaccinated against PCV2 11 and 7 months respectively before the start of the study, the high MDA titres in piglets suggest recent PCV2 infections on both farms and indicate...
that the source of high MDA titres was a combination of vaccine and naturally-derived antibodies.

Even in the presence of high levels of MDA, piglets vaccinated at 3 weeks of age responded to vaccination with a significantly reduced viraemia and a significantly increased weight gain, compared to the respective control animals. Protection of pigs vaccinated at 3 weeks of age in the presence of high MDA levels has also been described for a number of other PCV2 vaccines and is therefore not an uncommon finding (Fachinger et al., 2008; Fraile et al., 2012; Kixmoller et al., 2008; Opriessnig et al., 2008). However, in most of these studies, PCV2 infection occurred earlier and therefore the effect of high MDA levels on the duration of protection could not be adequately assessed.

Interestingly, it has been reported that high MDA levels do interfere with the active humoral immune response following vaccination with different PCV2 vaccines (Fort et al., 2009; Fraile et al., 2012). For the tested PCV2 vaccine, it could be shown earlier that antibody titres of animals vaccinated in the presence of low \(4 \log_2\) or moderate \(8 \log_2\) MDA titres increased post vaccination to levels of 12–13 \(\log_2\) (Fort et al., 2009; Martelli et al., 2011), whereas antibody titres of animals vaccinated in the presence of high \(10 \log_2\) MDA titres remained unchanged (Fort et al., 2009). Also in the present studies, a decline of the antibody titres could be observed at 4 weeks post vaccination, though the decline was less pronounced than in the control animals. Accordingly it might be speculated that, even in the presence of high MDA titres, at least a weak, active humoral immune response was induced by vaccination. This is supported further in the observed increase in the percentage of IgM-positive animals reaching a maximum

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at 4 weeks post vaccination in the groups vaccinated at 3 weeks of age, whereas control animals remained IgM-negative. In this context, it is notable that the mean PCV2 titres of animals vaccinated at 3 weeks of age had fallen to a level of 6.6 log2 (Study 1) and 4.1 log2 (Study 2) at 18 and 19 weeks post vaccination. Since PCV2 titres (as measured by IPMA) of 5.5 log2 or lower have been suggested to render pigs potentially susceptible to PCV2 infection (Fort et al., 2009), it is remarkable that pigs were still protected from a subsequent PCV2 infection in both studies. As suggested earlier (Fraile et al., 2012), these data may therefore provide supportive evidence that PCV2 vaccine efficacy depends not only on the humoral, but also on the development of the cellular immune response. Indeed, the induction of increased levels of IFNγ in pigs starting at 2–3 weeks post vaccination has already been described (Fort et al., 2009; Martelli et al., 2011) and demonstrates the general capacity of the tested PCV2 vaccine to induce a cellular immune response. The finding that cellular immune responses may not be suppressed by the presence of high MDA levels, but can, in fact, contribute to protection, has also been described for other vaccines in other species (Salmon et al., 2009; Siegrist et al., 1998; Siegrist, 2003), and strongly suggests the need for a re-evaluation of vaccination strategies previously rejected based on serological data.

Whereas the tested PCV2 vaccine provided protection when administered once in 3-week old piglets, it appeared rather ineffective when administered once in 1-week old piglets. In fact, the ADWG during fattening, the serological profile over the time and the absence of an IgM response following vaccination was comparable to that of the control group and the level of viraemia was less reduced than in animals vaccinated at 3 weeks of age. As the MDA titres of piglets vaccinated at 3 and 1 weeks of age were both in a comparably high range, it is questionable whether the lack of efficacy of the vaccine in those animals vaccinated at 1 week old can be solely attributed to the level of MDA at the time of vaccination. The possibility that the immune system of 1-week old piglets was not mature enough also appears to be unlikely because in an earlier study, piglets which were vaccinated against PCV2 at the age of 5 days were protected from subsequent challenge (O’Neill et al., 2011). However, that study was performed in PCV2-seronegative piglets. An alternative explanation could be that maternal lymphocytes and cytokines present in colostrum, as well as elevated corticosteroid levels in the neonate, may have served to modulate neonatal immune function and suppress active cellular and humoral immune responses against PCV2 in 1-week old piglets. Most of the maternally transferred cytokines, as well as the elevated corticosteroid levels, have declined in piglets within the first 14 days after birth (Hodgins and Shewen, 2012; Nguyen et al., 2007) and little information is available about the survival time of maternal lymphocytes (Hodgins and Shewen, 2012). However, in view of these different factors interfering with the immune response, it could be speculated that the immune functions of piglets at 1 week of age are considerably different to those of 3-week old piglets. Further studies are certainly warranted to analyze in detail the differences between the immune functions of different ages of young piglets.

An additional limitation of the present studies is the absence of groups with low MDA levels for the purposes of comparison. Grouping of animals by either high or low MDA levels was not possible, as the majority of individual MDA titres on the two farms selected were in the high range at the time of vaccination. It therefore remains unclear whether the degree of protection would eventually be higher in animals with low MDA titles compared to those with high titles. Also, the data provide evidence that the cellular immune response may have contributed considerably to the protection of piglets vaccinated at 3 weeks of age, whereas it is not clear to what degree a similarly active cellular immune response was elicited in piglets vaccinated at 1 week of age. A comparative analysis of the cellular immune response in piglets vaccinated at 1 and 3 weeks of age could therefore be an interesting investigation in order to learn more about the functionality and capacity of the neonate immune system in swine, and contribute to the development of future vaccination strategies.

5. Conclusion

The data presented indicate that PCV2 vaccination of 3-week old piglets in the presence of high MDA levels provides long-lasting protection, as measured by an increased ADWG and reduced viral load following the onset of PCV2 viraemia. However, compared to earlier field studies in which animals were vaccinated with the PCV2 vaccine in the presence of low to moderate MDA (Martelli et al., 2011), the humoral immune response to vaccination was reduced, suggesting interference of the active humoral immune response by MDA present at the time of vaccination. Accordingly, the results provide evidence that other immune functions, such as a cell-mediated immune response, may have contributed considerably to protection in this age group. In animals vaccinated at 1 week of age in the presence of comparably high MDA levels, the vaccine was ineffective. This indicates that the level of MDA is not the sole factor in the potential interference with vaccine efficacy. In fact, other factors, such as maternal PCV2-specific lymphocytes and maternally transferred non-specific cytokines, may play an equally important role, and should certainly be taken into account when assessing vaccine efficacy in neonates.

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