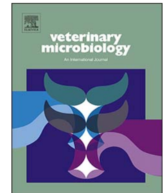
Veterinary Microbiology 214 (2018) 148–153

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic



Comparison of cytokine profiles in peripheral blood mononuclear cells between piglets born from *Porcine circovirus 2* vaccinated and non-vaccinated sows

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ARTICLE INFO

Keywords:
Porcine circovirus 2 (PCV2)
Sow vaccination
Maternally derived immunity

ABSTRACT

This study was aimed to evaluate the effect of *Porcine circovirus 2* (PCV2) sow vaccination on cell-mediated immune responses in sows and their progeny. At 7 weeks before farrowing, fifteen PCV2 PCR negative pregnant sows with medium-low antibody values were selected and randomly distributed in two groups according to the antibody levels. Seven sows were vaccinated with a commercial PCV2 vaccine and eight were injected with phosphate-buffered saline at 6 and 3 weeks before farrowing. Blood samples were taken from sows and their piglets (n = 90) during the study duration. PCV2 DNA and antibodies were tested in sera, and cytokine (IFN-α, IFN-γ, IL-12p40, TNF-α, IL-1β, IL-8, IL-4, IL-6 and IL-10) levels were assessed in supernatant from cultured peripheral blood mononuclear cells. All sows and piglets were negative by PCV2 PCR throughout the study. Significantly higher PCV2 antibody levels were detected in vaccinated sows after vaccination and in their offspring after colostrum ingestion compared to the non-vaccinated counterparts. Vaccinated sows did not show significant differences in cytokine secretion levels at farrowing compared to unvaccinated dams. In contrast, piglets from vaccinated sows had significantly higher levels of cytokines linked to Th1 memory cells (IFN-γ and TNF-α) in comparison to the ones from non-vaccinated dams. In conclusion, PCV2 sow vaccination, apart from triggering a humoral immunity response in sows and their progeny, might be associated to an increased transfer of cell-mediated immunity from the dam to the piglet.

1. Introduction

Porcine circovirus 2 (PCV2) is the etiological agent of several clinical or subclinical known as porcine circovirus diseases (PCVD) (Allan et al., 2002). Among these, PCV2-subclinical infection (PCV2-SI) is nowadays the most prevalent, representing the highest proportion of the negative economic impact at farm level in comparison to the other PCVDs (Alarcón et al., 2013).

The benefits of dam vaccination on their progeny have been demonstrated in terms of reduction of PCV2-systemic disease (PCV2-SD) prevalence, viremia and PCV2 load in tissues (Opriessnig et al., 2010; Pejsak et al., 2010; Fraile et al., 2012; O'Neill et al., 2012). However, this strategy does not provide full protection against PCV2 infection in the offspring, as PCV2 vertical transmission in vaccinated sows can occur (Madson et al., 2009a, 2009b; Hemann et al., 2014).

PCV2 vaccination elicits both humoral and cellular immune responses against PCV2 (Fort et al., 2009; Martelli et al., 2011; Seo et al., 2014). In sows, the goal of vaccination before farrowing, is the protection of the offspring by means of maternal immunity transfer through colostrum. Several studies have shown the maternal antibody transfer from sows to piglets (Kurmann et al., 2011; Fraile et al., 2012; Sibila et al., 2013; Oh et al., 2014; Dvorak et al., 2017). Nevertheless, the passive transfer of the PCV2-specific cellular immune response to the offspring has hardly been investigated. To our knowledge, only one peer-reviewed study has demonstrated that maternally derived colostral lymphocytes from PCV2 immunized sows may be transferred to the progeny (Oh et al., 2012). In that study, the participation of these lymphocytes in the adaptive immune response was measured by *in vivo* delayed type hypersensitivity (DTH) responses, *in vitro* lymphocyte proliferation and the presence of PCV2-specific IFN-γ-secreting cells (IFN-γ-SCs) in new-born piglets. However, in this context, information on cytokine profiles in piglets after colostrum intake and the influence

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https://doi.org/10.1016/j.vetmic.2017.12.011
Received 25 August 2017; Received in revised form 15 December 2017; Accepted 18 December 2017
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of sow vaccination on these profiles is not available. Therefore, the objective of the present work was to assess the effect of sow vaccination against PCV2 on humoral and cell-mediated immunity in sows and their offspring.

2. Material and methods

2.1. Farm selection

The study was conducted in a commercial farm with 1060 sows (Large White x Landrace) located in Spain. This farm was a two-site herd with all-in/all-out management and 4-week batch farrowing system. PCV2 vaccination in sows and piglets had never been applied in the herd under study. Sows were routinely vaccinated against *Porcine reproductive and respiratory syndrome virus*, *Aujeszky's disease virus*, *Swine influenza virus*, *Porcine parvovirus*, *Erysipelothrix rhusiopathiae, Escherichia coli* and *Clostridium perfringens.* Piglets were vaccinated against *Mycoplasma hyopneumoniae* at 2 days pre-weaning. Weaning was performed at 3 weeks of age. Besides, PCVD clinical problems were never detected in this herd and the average of the reproductive parameters was within the Spanish one (www.bdporc.irta.es). Furthermore, no clinical signs of any other significant diseases were observed.

In order to evaluate the PCV2 antibody levels in the sow herd, blood samples from 30 sows from 2nd to 7th parity were taken and processed by ELISA (Ingezim Circo IgG 11.PCV.K1®, Ingenasa, Madrid, Spain). PCV2 antibodies were detected in 29 out of 30 (96.7%) sows, observing the highest ELISA S/P values in 5th parity sows (Fig. 1).

2.2. Study design

Fifteen healthy sows (parity 3–4th) with the same expected farrowing day were selected from the screened farm at 7 weeks pre-farrowing. These animals were individually ear-tagged and bled. Blood samples were tested by conventional PCV2 PCR (Quintana et al., 2002) and ELISA (Ingezim Circo IgG 11.PCV.K1®). All sows were PCR negative and showed low-medium (ranging from 0.27 to 0.85) ELISA S/P values. At 6 weeks pre-farrowing, sows were randomly distributed in two treatment groups according to S/P values. Seven sows were vaccinated by intramuscular injection with 2 mL of a commercial inactivated PCV2 vaccine (CIRCOVAC®, Ceva) at 6 and 3 weeks pre-farrowing. In parallel, eight non-vaccinated sows received 2 mL of phosphate buffer saline (PBS) at the same time points and by the same route. Animals with different treatments were comingled in the same gestation pens as well as in the same farrowing unit rooms. In sows, blood samples were taken in vacuum tubes by jugular venepuncture at 6 weeks pre-farrowing and at the farrowing week (Table 1).

At birth, all piglets from litters of studied sows were ear-tagged and registered. Cross-fostering was not allowed for the sows included in the study. At 48–72 h after birth, blood samples from six healthy and medium-sized piglets per litter were taken in tubes without anticoagulant (n = 90). In addition, from two of these six piglets selected per litter, blood samples were also taken in heparinized vacuum tubes (n = 30). Once in the laboratory, blood samples in heparin tubes were immediately processed to obtain peripheral blood mononuclear cells (PBMCs), while the ones in tubes without anticoagulant were centrifuged at 750g during 20 min to extract the sera. Sera were aliquoted and stored at −20 °C until testing.

Table 1
Study design.

Population	Sampling points				
	7 weeks pre-farrowing	6 weeks pre-farrowing	3 weeks pre-farrowing	Farrowing	48–72 h after farrowing
Sows	Clinical signs Blood sampling[a]	Clinical signs Treatment Blood sampling[b]	Clinical signs Treatment	Clinical signs Blood sampling[a,b]	Clinical signs
Piglets				Clinical signs	Clinical signs Blood sampling[a,b]

[a] Blood in tubes without anticoagulant.
[b] Blood in tubes with heparin.

Any abnormality related to general state, condition of the skin, hair and mucosa, respiratory, digestive and nervous signs, and locomotive problems was registered at different time points (Table 1) in both sows and piglets. Housing conditions, feeding system, feed characteristics and health management remained consistent along the course of the trial, and were the same for both experimental groups. The present study was approved by the Ethics Committee for Animal Experimentation from the *Universitat Autònoma de Barcelona* and the Animal Experimentation Commission from the local government (*Dpt. de Medi Ambient i Habitatge* from the *Generalitat de Catalunya*; Reference 9402).

2.3. DNA extraction and conventional PCR

DNA was extracted from 200 μL of serum by using the MagMAX™ Pathogen RNA/DNA Kit (Applied Biosystems) following the manufacturer's instructions. DNA obtained was suspended in 90 μL of elution solution. Then, PCV2 genome was detected by standard PCR (Quintana et al., 2002). Each extraction and PCR plate included negative and positive controls, where samples were replaced for diethylpyrocarbonate (DEPC)-treated water or known PCV2 infected sample, respectively.

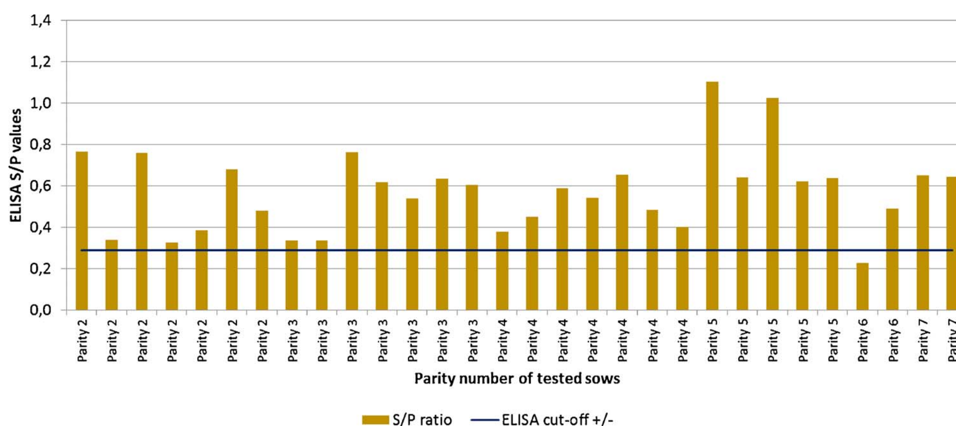

Fig. 1. Individual PCV2 ELISA S/P results in serum samples from sows with different parity number prior to the start of the study (farm screening).

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