

# Efficacy of the Porcine circovirus 2 (PCV2) vaccination under field conditions

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## Keywords

Porcine circovirus type 2, Piglet, Vaccination, Field conditions, Vaccine efficacy.

## Summary

The objective of this study was to determine the effect of the porcine circovirus type 2 (PCV2) vaccination on the levels of viremia, the number of viremic-positive pigs, and production performance [i.e. nursery mortality, post-weaning mortality, and average daily weight gain (ADWG)] under field conditions. There were 140 farrow-to-finish pig herds involved in this study. The vaccination of piglets was implemented in 82 of the 140 herds. In each herd blood samples were collected from sows and pigs in different age category. In addition, a questionnaire regarding the production performance was provided for each herd. Results demonstrate that the vaccination of piglets prevented the development of viremia in 23.2% of herds. Significant decreases in the levels of PCV2 DNA in serum and in the number of viremic pigs were also noted. These results indicate that the vaccination of piglets against PCV2 is a useful tool in controlling the PCV2 infection in herds with a high risk of a wide range of viral and bacterial agents, poor management strategies, and a low level of biosecurity practices.

## Effetto della vaccinazione contro il Porcine circovirus 2 (PCV2) in condizioni di campo

## Parole chiave

Porcine circovirus di tipo 2, Maialini da latte, Vaccinazione, Condizioni di campo, Efficacia vaccinale.

## Riassunto

L'obiettivo di questo studio è stato determinare, in condizioni di campo, l'effetto della vaccinazione PCV2 sui livelli di viremia, il numero di suini viremico-positivi e i parametri di produzione. Questo studio ha interessato 140 allevamenti di maiali da ingrasso. La vaccinazione è stata attuata in 82 delle 140 mandrie. Sono stati raccolti campioni di sangue da tutte gli allevamenti e, per ciascun allevamento, è stato fornito un questionario relativo ai parametri di produzione. I risultati dimostrano che la vaccinazione dei maialini ha impedito lo sviluppo della viremia nel 23,2% degli allevamenti. Nel siero e nel numero di suini positivi sono stati inoltre osservati diminuzioni significative dei livelli di DNA di PCV2. Sorprendentemente, non è stata osservata nelle mandrie vaccinate l'influenza significativa della vaccinazione sui parametri di produzione.

## Introduction

Porcine circovirus type 2 (PCV2) is one of the smallest, non-enveloped, single-stranded DNA virus belonging to the *Circovirus* genus of the *Circoviridae* family (Meehan *et al.* 1998). Several studies have confirmed the association of the PCV2 infection with disease syndromes collectively named porcine circovirus diseases (PCVD). The

predominant clinical signs of PCVD are wasting and retarded growth among grow-finish pigs (Harding *et al.* 1998, Cino-Ozuna *et al.* 2011). Among PCVD porcine circovirus systemic disease (PCV2-SD) (previously named post-weaning multi-systemic wasting syndrome) is one of the most devastating and economically damaging diseases to the swine industry (Segalés 2012). In the European Union, the cost of PCV2-SD was assessed to be between

€ 562 million and € 900 million per year. The need for the introduction of effective control strategies is therefore essential (Segalés and Domingo 2002, Tucker 2006, Alarcon *et al.* 2013).

One of the most effective PCVD control strategies is the administration of PCV2 vaccines in piglets/weaners and/or sows, before farrowing. The vaccination of sows has been shown to reduce the prevalence of PCV2 viremia and improve the performance of their offspring (Pejsak *et al.* 2010, Seo *et al.* 2014). Vaccines are mainly administered to newborn piglets in herds with a high risk of PCV2 infection. Several studies have found that the vaccination of piglets against PCV2 significantly reduces the viral load in their blood and thus the prevalence of positive animals, which in turn is associated with a reduction of clinical signs and improved production performance (Fort *et al.* 2008, Horlen *et al.* 2008, Kixmoller *et al.* 2008, Martelli *et al.* 2011, Hemman *et al.* 2012, Heissenberger *et al.* 2013, Seo *et al.* 2014).

Previous studies investigating the efficacy of the PCV2 vaccination under field conditions were either performed within single farms with relatively healthy animals, good management practices, and high levels of biosecurity (Cline *et al.* 2008, Fachinger *et al.* 2008, Jacela *et al.* 2011) or with no data regarding management, biosecurity, and environmental conditions (Lyoo *et al.* 2011, Martelli *et al.* 2011, Heissenberger *et al.* 2013).

Little is known about the efficacy of the PCV2 vaccination with regards to levels of viremia and production performance on farms with low health status, poor management strategies, and inadequate biosecurity, sanitation, and environmental conditions. The aim of this study was to address this gap by assessing the efficacy of the PCV2 vaccination on farms with poor management strategies, low levels of biosecurity practices, and inadequate sanitation. Selected production performance (i.e. nursery mortality, post-weaning mortality, and ADWG), the level of PCV2 DNA in serum, and the proportion of viremic pigs were evaluated.

## Materials and methods

### Herd characteristics

The study design was discussed and approved by the Polish Institutional Ethics Committee (allowance number: 37/2014).

The study was conducted in 140 conventional, farrow-to-finish pig farms, with between 22 and 2,000 ( $200.67 \pm 336.39$ ) sows per farm. Eighty-two (58.6%) of the 140 farms administer vaccines against

PCV2 to piglets at the age of 3-4 weeks as a part of their regular farming practices. In 61.4% and 8.6% of herds, vaccinations against enzootic pneumonia and porcine pleuropneumonia were administered to piglets and/or growers, respectively. Immunization programmes for sows included vaccination against porcine parvovirus, *Erysipelothrix rhusiopathiae*, colibacillosis, and porcine reproductive respiratory syndrome virus (PRRSV) in 92.3%, 90.6%, 54.1%, and 9.3%, respectively. In none of the examined herds sows were vaccinated against PCV2 and swine influenza virus (SIV).

The herds were affected by several endemic diseases. Specific antibodies (avoiding vaccination interferences) against *Mycoplasma hyopneumoniae*, *Actinobacillus pleuropneumoniae*, SIV, PCV2, and PRRSV were detected in 85.2%, 96.1%, 87.9%, 100%, and 37.8% of the investigated herds respectively.

Low levels of biosecurity practice, inadequate sanitation, and poor management strategies were observed in all of the examined farms. Five established biosecurity measures – a fence around the farm, disinfection mats within the herd, the use of boots and clothes provided by the farm, a changing room with showers, and quarantine for purchased pigs – were only implemented in a very low percentage of investigated farms. Above mentioned biosecurity measures were applied in 4.9% of farms vaccinated piglets against PCV2 and in 3.5% of farms non-vaccinated piglets against PCV2. The adoption of an all in – all out (AIAO) pig flow at all production stages (i.e. farrowing, nursery, and finishing) was respected in 22% and 10% of farms with piglets vaccinated and non-vaccinated against PCV2, respectively. However, significant differences were not observed (data not shown).

### Serum samples

In total, 1,160 and 760 serum samples were collected from vaccinated and non-vaccinated pigs, respectively. Blood samples were taken randomly, from sows and pigs that were 4-5, 6-7, 8-9, 10-11, 12-13, 14-15, 16-17, 18-19, 20-21, and 22-24 weeks old. In a particular farm, the number of different age groups selected to collect the serum samples depended on the herd size and the types of batch farrowing system (1-, 2-, 3-, 4- week farrowing batch interval) operated in the farm. Six pigs in each age group were always sampled.

### Production performance

Data regarding production performance, including nursery mortality, overall post-weaning mortality, and ADWG were gathered using a questionnaire at each farm. To minimise confusion and maximise

the accuracy of responses, questions were written in clear and intelligible language. Aside from questions regarding production parameters, the questionnaire included queries about biosecurity measures.

### Polymerase chain reaction (PCR)

The individual sera were pooled 3:1 within each age category and tested by real-time PCR according to the method described by Opriessnig and colleagues (Opriessnig *et al.* 2003). Nucleic acids were isolated with a commercially available kit (MagNA Pure LC Total Nucleic Acid Isolation Kit, Roche) according to the manufacturer's recommendations. Results of real-time PCR were expressed as the Ct (threshold cycle) value.

### Statistical analysis

Samples with Ct values equal to or lower than 39 were considered 'positive'. A herd was classified as positive for PCV2 DNA if at least 1 serum sample taken from the herd had a positive PCR result. Differences in Ct among vaccinated and non-vaccinated pigs were determined by a Mann-Whitney U test. Differences in the prevalence of positive herds, as well as the proportion of viremic-positive pigs in vaccinated and non-vaccinated herds, were determined by a chi-squared test (statistical significance at  $p < 0.05$ ). A Mann-Whitney U test was applied in order to compare production performance.

## Results

### Association of vaccination with the levels of PCV2 DNA

Mean Ct values in serum samples from vaccinated pigs were significantly higher (in total and in

all different age categories) as compared to the non-vaccinated group ( $p < 0.05$ ). These results indicate lower viral loads (i.e. the level of virus in the blood) in pigs vaccinated against PCV2, compared to their non-vaccinated counterparts (Figure 1).

### Association of vaccination with the number of positive herds and the proportion of viremic pigs

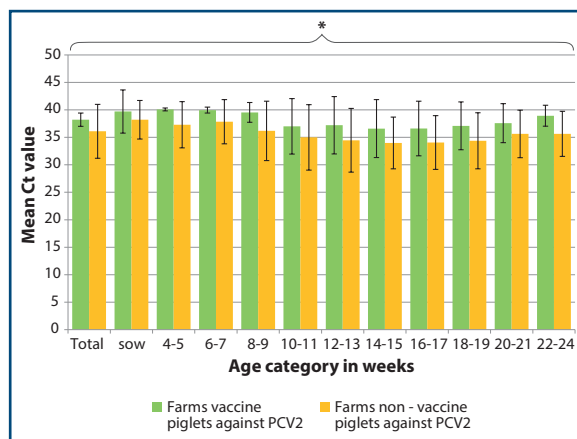
PCV2 DNA was detected in all of the non-vaccinated herds and in 68 (82.9%) out of 82 vaccinated herds. The total number of positive pigs was significantly reduced in vaccinated herds, compared to their non-vaccinated counterparts ( $p < 0.05$ ). The same trends were observed in all different age categories (Figure 2). The lowest prevalence of PCV2 DNA was observed in pigs aged 4-5 weeks or 6-7 weeks, from vaccinated and non-vaccinated herds, respectively. The highest prevalence of the PCV2 virus was observed in pigs from 14 to 21 weeks of age, in the case of both vaccinated and non-vaccinated herds.

### Association of vaccination with production performance

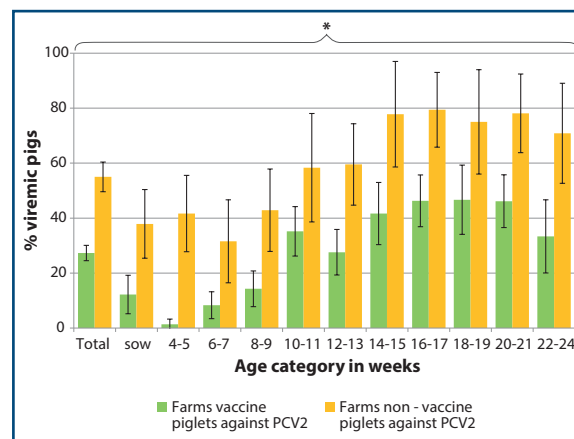
None of the evaluated production parameters were significantly affected in vaccinated herds compared to non-vaccinated herds ( $p > 0.05$ ) (Table I).

## Discussion

In recent years, a wide range of commercial PCV2 vaccines, designed to diminish the negative impact of PCVD in pigs have become available. Previous experimental and field reports have proved that PCV2 vaccines are capable of decreasing the prevalence of PCV2 DNA and viral load in serum and improving production parameters (Fachinger *et al.* 2008, Fort



**Figure 1.** Mean Ct ( $\pm$  SD) values in pigs sera (in total and in different age categories) when tested by PCV2 real time PCR. \*  $p < 0.05$  between bars.



**Figure 2.** Proportion of PCV2 viremic pigs ( $\% \pm$  CI) (in total and in different age categories). \*  $p < 0.05$  between bars.

**Table 1.** Production parameters from herds vaccinated and non-vaccinated against PCV2.

Production parameters	Vaccination against PCV2				p value
	Yes		No		
	Mean	SD	Mean	SD	
Nursery mortality (%)	2.8	1.5	3.2	1.5	0.184
Overall postweaning mortality (%)	5.0	3.0	5.3	3.0	0.408
Average daily weight gain (g)	642.8	43.5	628.1	47.9	0.273

*et al.*, 2008, Horlen *et al.* 2008, Fort *et al.* 2009, Pejsak *et al.* 2010, Martelli *et al.* 2011, Heissenberger *et al.* 2013). However, previous studies were conducted under laboratory or field-experimental conditions, mainly in herds with a reasonably good health status, effective management, and high level of farm biosecurity strategies.

Under the conditions of this study, PCV2 vaccination in piglets aged 3 weeks prevents the infection in 23.2% of vaccinated herds. By contrast, the PCV2 infection was detected in 100% of non-vaccinated herds. In addition, significantly lower amounts of PCV2 DNA were detected in sera, and a lower proportion of viremic pigs were observed in vaccinated herds. This trend has been observed in vaccinated pigs of all different age groups, including sows that were not vaccinated against PCV2. These results suggest that the implementation of the PCV2 vaccination substantially reduces the viral burden in the housing facilities. Decreasing the prevalence of PCV2 in the sow sector is important because it reduces the risk of foetal infection (Pensaert *et al.* 2004, Sarli *et al.* 2012). Similarly, findings from US showed a decrease of PCV2 infectious pressure in the American pig population 5 years after the implementation of a widespread PCV2 vaccination programme (Shen *et al.* 2012).

In the vaccinated herds, only 1.3% of 4-5 week-old piglets were PCV2 positive, while in non-vaccinated herds, 41.7% ( $p < 0.05$ ) of pigs of the same age were PCV2 positive. These results suggest that the vaccination of piglets significantly delayed the development of PCV2 viremia. This finding is in agreement with previous studies, demonstrating the effectiveness of PCV2 vaccines in controlling viremia in piglets (Fort *et al.* 2008, Seo *et al.* 2014). Moreover, the postponed onset of viremia in piglets also diminished the risk of PCV2-SD developing (Rose *et al.* 2003, Lopez-Soria *et al.* 2005).

In both vaccinated and non-vaccinated herds, the highest percentage of PCV2 positive pigs was observed among pigs from 14 to 21 weeks of age. This finding indicates that the best time to detect PCV2 DNA in blood, under field conditions, in both

vaccinated and non-vaccinated herds, is at this age. It has previously been shown that blood is a suitable specimen for PCV2 detection using PCR (Shibata *et al.* 2003, Grau-Roma *et al.* 2008).

Numerous studies have reported that the vaccination of pigs against PCV2 can improve ADWG and decrease mortality rates (Cline *et al.* 2008, Fachinger *et al.* 2008, Horlen *et al.* 2008, Lyoo *et al.* 2011, Martelli *et al.* 2011). This study also compared production performance between vaccinated and non-vaccinated herds. ADWG, nursery mortality, and overall, post-weaning mortality, were slightly better in herds practicing PCV2 vaccination in 3-week-old piglets, although without statistical significance ( $p > 0.05$ ). Discrepancies between the results obtained previously and the results of this study could be associated with differences in study design (i.e. field experimental studies versus a field cross-sectional study). Possible reasons for the lack of significance between vaccinated and non-vaccinated herds with regards to production performance may be a result of varied health status, management strategy, biosecurity and sanitation practices, environmental conditions, antibiotics usage, the breed of reared pigs, and the geographical location of the evaluated farms. In general, management strategies and biosecurity practices were poor in the farms included in this study.

Another potential reason for vaccine failure with regard to production performance were factors such as the lack of use of the recommended dosage of vaccine, administering vaccines to sick or immune-compromised pigs, non-compliance with proper vaccine schemes, and/or vaccinated pigs registering the presence of interfering, maternally-derived antibodies (MDA). In addition, the antigenic difference between the vaccine strain and field strain, possible strain mutations under field conditions, the adjuvant type, and the amount of PCV2 antigen in the vaccine could all have had an influence on the results reported here (Lefebvre *et al.* 2008, Opriessnig *et al.* 2009, Guo *et al.* 2010, Lyoo *et al.* 2011, Prpić *et al.* 2014). Nevertheless, further studies are required in order to explain the obtained findings. This relates especially to the analysis of the identification of possible risk factors affecting production performance in commercial pig farms under field conditions and how these risk factors are relate to PCV2 vaccination efficacy.

In summary, on the basis of the obtained results, we can conclude that under field conditions, the vaccination of piglets with a commercial vaccine reduces the viral load in blood and proportion of PCV2-viremic PCV2-positive pigs. Moreover, vaccination protected the animals against early infection, as shown by the delayed onset of viremia in vaccinated piglets. Although the PCV2 vaccination

was only administered to piglets, it also decreased the viral burden in sow sectors. These results indicate that the vaccination of piglets against PCV2 is a useful tool in controlling the PCV2 infection in herds with a high risk of a wide range of viral and bacterial agents, poor management strategies, and a low level of biosecurity practices.

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