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Evaluation of porcine circovirus type 2 double vaccination in weaning piglets that reared for gilts under field conditions

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Abstract

The objective of the present study was to evaluate the efficacy of a porcine circovirus type 2 (PCV2) double vaccination in weaning piglets reared for gilts under field conditions. The study was conducted at a Greek farrow-to-finish conventional pig farm with a previous history of PCV2 infections. The trial included 96 female piglets at 21 days of age, which were equally allocated to two different study groups. Piglets of the group-1 received a single PCV2 vaccination at 21 days of age, while piglets of the group-2 were double vaccinated against PCV2, at 21 and 42 days of age. The results indicated that the piglets of group-2 had better growth performance, as they showed higher body weight (BW) and average daily weight gain (ADWG). In addition, ELISA tests showed that the double- vaccinated piglets presented a better humoral response against PCV2, as higher levels of IgG antibodies were detected in them than the piglets of the group-1. In conclusion, the current results suggested that a double PCV2 vaccination of piglets, reared for gilts, on a PCV2-affected farm could lead to higher protection against the virus.

Introduction

Porcine circovirus-associated disease (PCVAD), including several syndromes (e.g., respiratory, reproductive) in pig, is caused by porcine circovirus type 2 (PCV2). Nowadays, PCVAD is one of the most important infectious diseases for swine industry worldwide, causing significant economic losses. It plays a key role in the development of systemic diseases characterized by severe immunosuppression (PCV2-SD, previously known as postweaning multisystemic wasting syndrome-PMWS) as well as enteric disease (PCV2-ED), lung disease (PCV2-LD), and reproductive disease (PCV2-RD).[1] However, PCV2 causes the most commonly subclinical infections in pigs worldwide (PCV2-SI).[2,3] Yet, even though the pathogenesis of PCV2-RD has not been fully understood, published studies have reported that porcine embryos are susceptible to PCV2 infection, leading to embryonic death, returns to estrus, fetus mummification and litters with stillborn and weak piglets.[4-6]

Vaccination protocols remain the main preventive measure for controlling and monitoring PCV2 infection in pig farms. Many studies have shown beneficial effects from the use of commercial PCV2 vaccines under field conditions, including reduction of the impact of PCV2-SD and secondary co-infections, improvement of performance parameters, reduction of PCV2 viremia and shedding.[3,7-10] The most common age of piglet vaccination against PCV2 is at 3 - 4 weeks of age, approximately on the day of weaning.[11-14] Routine use of commercial vaccines in farms affected by PCV2-SD has led to PCV2-SI cases, as most pigs without characteristic clinical signs also suffer from PCV2-SI.[15] However, routine PCV2 vaccination has been reported to be economically beneficial, improving performance para-meters in PCV2-SI field cases without obvious clinical signs.[16-18]

Despite widespread vaccination against PCV2, PCV2-SD or PCV2-SI are still observed under field conditions worldwide,[19-21] indicating that PCV2 continues to play an important role in the global swine industry. Gilts and sows up to second parity are more likely to spread PCV2 and contribute to the virus persistence in conventional sows' herds.[22] Therefore, vaccination of gilts is often applied to eliminate the risk of PCV2-RD in first litters, aiming to increase the level of maternal immunity transferred to piglets and to protect them against PCV2 infection until their vaccination.

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Pig farms suffering from PCVAD have a higher infection pressure especially at the end of the nursery period (nine weeks of age) and at mid-fattening (15 weeks of age) and lower IgG shortly before weaning (at three weeks of age).[23] A recent study reported a slight or no seroconversion after three to four weeks post-PCV2 immunization in conventional pig farms, while natural exposure to PCV2 occurs in the growing to fattening stage, and viremia may last until slaughtered age.[24] Based on the above studies, it seems that PCV2 vaccination of gilts is very important for commercial farms with nucleus herd, as in many cases gilts could be co-existed with growing and fattening pigs. Once gilts enter the breeding stock, implementing a strategic PCV2 vaccination of them is crucial to prevent, the virus from spreading to the breeding and finishing herd. The objective of this study was to investigate the effects of a PCV2 double vaccination in weaning piglets that were reared for gilts in a field environment model.

Materials and Methods

Ethical statement. All applied procedures in animals during this trial were in accordance with National and European Animal Welfare requirements.[25,26] All animal care and handling procedures were approved by the Committee on Research Ethics and Conduct of the University of Thessaly (Approval number 36/10.11.2020).

Trial farm/history. The current study was conducted in a farrow-to-finish conventional pig farm, located in Thessaly (Greece). The capacity of the farm was 550 sows (commercial hybrids of Large White x Landrace), as well as its own grandparent nucleus of 40 sows to produce gilts. The mean replacement rate of gilts for this farm was about 35.00%. The grandparent sows of the nucleus herd were separately housed, but on the same premises as the commercial herd. Semen was introduced from boar stud for artificial insemination. Routine vaccination program of breeding stock included vaccinations against porcine reproductive and respiratory syndrome virus (PRRSV), Aujeszky's disease virus, Swine influenza virus, porcine parvovirus, *Erysipelothrix rhusiopathiae*, *Escherichia coli* and *Clostridium perfringens*. Weaners were vaccinated against *Mycoplasma hyopneumoniae* (*M. hyo*) and PCV2 at 21 days of age. Weaning was performed at the age of 25 - 28 days and pigs were moved to fattening units at the age of 10 weeks. In addition, there was no evidence of any major swine disease and the immunity status of the herd against PRRSV was determined as "positive-stable" (II-A) according to the classification previously described.[27]

The farm had a previous history of PCV2 infections over the last decade. The PCV2 circulation was confirmed by a cross-sectional seroprofiling, including 10 pigs per batch of four age groups [gilts, weaners (three, seven weeks of age), growers (11, 15 weeks of age), finishers (19, 23 weeks of age) and gilts (23, 26 weeks of age)]. Blood samples were tested by real-time polymerase chain reaction (RT-PCR) and ELISA to detect viral nucleic acid and antibodies (IgGs), respectively. PCV2 genome was detected in 40.00, 35.00, 30.00, 25.00, 20.00 and 15.00% of the sampled pigs at seven, 11, 15, 19, 23 and 26 weeks of age, respectively. Seroconversion was detected from seven weeks of age onwards. Therefore, as no PCVAD clinical signs were evident on the farm, PCV2-SI was confirmed. The clinical signs of PCV2 outbreaks in the history of this farm included mainly respiratory distress in the nursery associated with severe financial losses as well as a very low incidence of reproductive failure in breeding stock (increased number of mummies, stillborn and weak born piglets, increased returns to estrus due to embryonic deaths). Following the diagnosis of PCV2 infection in the herd, a vaccination schedule against PCV2 was applied to weaners around the day of weaning and to gilts twice before the first insemination.

Animals. The study included 96 female piglets at 21 days of age, selected from sows of the grandparent nucleus of the farm (parity one to four). Piglets were assigned to two different groups of 52 piglets, so that littermates were spread evenly over the groups, with equal numbers of piglets derived from sows of parity one – four per group. At three weeks of age, piglets of control group (group-1) were vaccinated intramuscularly (IM) against PCV2 with a single dose vaccine in the neck behind the ear (2.00 mL of Suvaxyn Circo; Zoetis Belgium SA, Louvain-la-Neuve, Belgium) on the day of weaning (21 days of age). Piglets of the experimental group (group-2) were IM vaccinated twice with 2.00 mL of the same vaccine at three and six weeks of age (21 and 42 days of age). The vaccine Suvaxyn Circo used in our study is a single-dose, ready-to-use combo vaccine for PCV2 and *M. hyo*. The manufacturer's recommendation for this vaccine is the IM injection of 2.00 mL from the age of three weeks. According to manufacturer, the vaccination offers immunity three weeks after receiving the vaccine and the duration of immunity is twenty-three weeks. The feed provided to the farm animals was home mixed and was a corn/barley/wheat - soybean-based meal. The study groups were housed in the same barns due to the environmental challenge model used in this study. The animals of all groups were kept under similar conditions in terms of climate, ventilation, temperature, and air humidity.

Sampling. Blood samples were obtained from 50.00% of the animals per group (24 animals per group) via puncture of the jugular externa vena with a 19-gauge needle, using S-Monovette (Sarstedt, Germany) without anticoagulant for serum retrieval. Samples were collected at different age stages (65 days, 110 days, and 170 days). Each time they were collected, all samples and placed in a cooler with icepacks avoiding direct contact with the tubes and were transferred to the laboratory. Blood samples were centrifuged at 300 *g* for 10 min and serum was used

to detect antibodies against PCV2. Pigs were tested for the presence of anti-PCV2 IgG antibodies by a commercial blocking ELISA (SERELISA® PCV2 Ab Mono Blocking; Synbiotics Europe SAS, Lyon, France). Samples titers were calculated based on single dilutions using the calculation sheet supplied by the manufacturer. Moreover, pools of four samples (six pool samples per group) were subjected to nucleic acid extraction, using the QIAamp® cador® Pathogen Mini Kit (Qiagen, Hilden, Germany), under the manufacturer's recommendations. Extracts were tested for PCV2 using a previously described TaqMan probe-based real-time polymerase chain reaction (PCR).[28]

Clinical observations. All animals were observed daily and scored weekly for clinical signs and weighed several times during the study as previously described.[29] Briefly, scoring was defined as: 0 (normal), 1 (rough haircoat), 2 (rough haircoat and coughing or dyspnea), 4 (severe cough or dyspnea and abdominal breathing) and death. Observers were blinded to the vaccination status. The mortality rate was also calculated.

Growth performance parameters. The live body weight (BW) of each group one pig was measured at 21 [0 dpv1 – 0-day post vaccination one (dpv1)], 65 (44 dpv1), 110 (89 dpv1) and 170 (149 dpv1) days of age. The BW of each group two pig was measured at 21 (dpv1), 65 [44 dpv1 - 23 dpv2 (23 days post vaccination two)], 110 (89 dpv1 - 68 dpv2) and 170 (149 dpv1 - 128 dpv2) days of age. The average daily weight gain (ADWG; g pig^{-1} per day) was analyzed over four time periods: (1) between 21 and 42 days of age, (2) between 43 and 65 days of age, (3) between 66 and 100 days of age and (4) between 111 and 170 days of age. ADWG at the various production stages was calculated as the difference between the initial and final weight divided by the stage duration. Data for dead or removed pigs were included in the calculation.

Statistical analysis. The statistical analysis was performed in the R programming language[30,31] with the SignTest function[32] that applies the statistical sign test. Program and the statistical test are both cited in the test. Non-parametric statistical methods (Sign test) were also applied to assess potential differences between group 1 and group 2, in terms of clinical scoring, BW, and ADWG. Median antibody levels from vaccine 1 and vaccine 2 were compared with the non-parametric Sign test for paired samples (10) in the R programming language. A *p-value* less than 0.05 was considered significant for all tests performed.

Results

Clinical Scoring. All test results indicate no significant statistical difference between the two groups at all three-time points for clinical scoring of respiratory signs and mortality rate (Table 1).

Growth performance parameters. Median BW and ADWG between groups during the trial period are shown in Table 2. A statistically significant difference in BW between the two groups at all three-time points, except from the time of 21 days, was noticed. Test results indicate a statistically significant difference for ADWG between the two groups at 111 - 170 days and 21 - 170 days, while for 21 - 42 days, 43 - 65 days and 66 - 100 days the test shows no significant statistical difference.

Table 1. Clinical scoring and antibody levels between the experimental groups during the trial period. Data are presented as median (range).

Age	Group 1	Group 2	*p-value*
Clinical scoring			
65 days	0.00 (0.00 - 2.00)	0.00 (0.00 - 1.00)	0.63
110 days	0.00 (0.00 - 2.00)	0.00 (0.00 - 1.00)	0.22
170 days	1.50 (0.00 - 4.00)	0.00 (0.00 - 1.00)	0.18
Antibody levels			
65 days	99.00 (23.00 - 2,762.00)	166.50 (15.00 - 3,272.00)	< 0.001
110 days	1,988.50 (1,447.00 - 2,876.00)	4,226.50 (3,022.00 - 6,720.00)	< 0.001
170 days	1,771.50 (22.00 - 9,214.00)	4,061.50 (710.00 - 9,126.00)	0.04

Table 2. Median body weight (BW) and average daily weight gain (ADWG) between the groups during the trial period. Data are presented as median (range).

Age	Group 1	Group 2	*p-value*
Body weight (kg)			
21 days	6.70 (6.20 - 7.20)	6.65 (6.40 - 6.90)	1.00
65 days	27.00 (25.70 - 29.30)	28.50 (27.60 - 30.20)	< 0.001
110 days	38.05 (36.50 - 39.70)	41.60 (39.70 - 43.50)	< 0.001
170 days	103.75 (102.50 - 106.20)	108.10 (104.50 - 109.40)	0.01
ADWG (g pig^{-1} per day)			
21 - 42 days	345.00 (328.20 - 352.00)	349.75 (328.00 - 355.00)	0.39
43 - 65 days	646.50 (634.50 – 655.00)	650.75 (648.50 - 657.20)	0.39
66 - 110 days	846.25 (839.50 - 850.50)	850.50 (847.50 - 852.50)	0.07
111 - 170 days	686.75 (679.50 - 785.50)	717.75 (685.50 - 725.50)	0.04
21 - 170 days	754.25 (742.50 - 760.50)	761.75 (756.00 - 765.00)	0.01

Detection of anti-PCV2 IgG antibodies by ELISA. The median reported antibody levels, range, and the observed p-value from the Sign test for the two groups are presented in Table 1. All test results indicate a significant statistical difference in antibody levels between the two groups in all three-time points. The results indicated that the double vaccinated piglets (group 2) had a better humoral response against PCV2 and improved growth performance, as they showed higher BW and ADWG (Fig. 1).

Detection of PCV2-RNA by RT-PCR. No samples were detected positive by RT-PCR for both groups during the 65- and 110-day trial period. However, one of the six pools in group-1 was detected positive at 170 days.

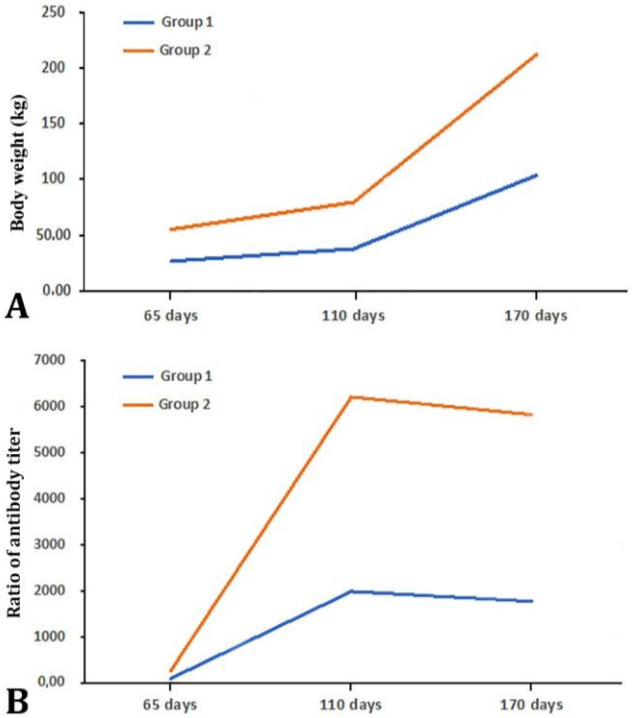

Fig. 1. A) Median body weight changes, and **B)** Antibody levels, during the trial period. Better humoral response (antibodies levels) against PCV2 and improved growth performance (BW and ADWG) in double vaccinated group-2. Serum samples were considered to be positive for anti-PCV2 antibody if the reciprocal ELISA titer was > 350.

Discussion

The present field trial was based on the hypothesis that a double PCV2 vaccination with a single dose vaccine of piglets reared for gilts on a PCV2-SI affected farm could lead to higher protection against PCV2-SI infection and better growth performance. Under the field conditions of the present study, doubled vaccinated piglets showed better growth performance and elicited a humoral immune response against PCV2. Similar findings have been reported in previous studies, using a single dose vaccine against PCV2 and *M. hyo*.[13,14,29,32,33] However, our findings delivered from a field trial, including the first time of the double vaccination against PCV2, at a PCV2-SI affected farm. Our results could support the findings of Cybulski *et al*. for the necessity of vaccination in replacement gilts against PCV2 in farms with a lack of or very low PCV2 circulation.[2] Moreover, our results are in consistent with previous studies that tested the same vaccine on PCV2-viremic and -seropositive piglets born from naturally PCV2- infected sows and elicited a high level of humoral immune response in vaccinated piglets.[29,34,35]

In the present field trial, piglets were vaccinated at three weeks of age, which is the most common age for piglet vaccination against PCV2.[11-14,36] Almost or completely asymptomatic subclinical PCV2 infection remains the most common disease leading to poor growth.[1,16,37,38] The routine use of commercial vaccines on PCV2-SD-affected farms has led to PCV2-SI cases, as most of the pigs without characteristic clinical signs also suffer from PCV2-SI.[15] However, PCV2 routine vaccination has been reported to be economically beneficial, improving performance parameters in PCV2-SI field cases without obvious clinical signs.[16-18]

The decrease in PCVAD outbreaks since 2008 is attributed to the successful introduction of efficacious PCV2 vaccines on the market. So far, there are five commercial PCV2 vaccines on the European market. In agreement with our study, previous field and experimental trials comparing the ADWG between vaccinated and unvaccinated animals, from wean-to-finish, for four commercial PCV2 vaccines, reported that the use of all products resulted in significantly higher ADWG values.[39] However, no significant differences were reported in the ADWG between the four different commercial vaccines.[40] In the study of Segalés *et al.* on three farms, using the same vaccine as in our study (i.e., Suvaxyn Circo), reported improved ADWG values in vaccinated pigs compared to unvaccinated pigs in a herd with clinical signs of PMWS.[12] The originality of our study is that for the first time, double vaccination with a single dose PCV2 vaccine was tested in weaning piglets reared for gilts. Our results showed that double PCV2 vaccination in weaning piglets has beneficial effects on ADWG values in the growing and finishing stages. In contrast, other studies with vaccination of weaning piglets with a single *M. hyo* and PCV2 vaccine reported improved ADWG values only during the finishing stage of the whole study period.[13,14,41]

The PCV2-RD has only been described in very high health status or newly established farms and in gilts who are fully sensitive to PCV2 infection.[42] Due to the common vaccination and the general decrease in infectious pressure, replacement gilts may remain naive until late age and get infected upon introduction into the breeding herd,[43] causing reproductive disorders and *in utero*

infection of the piglets.[5,44] The PCV2 vaccination of sows is proposed to be applied in farms, where despite the piglet vaccination, the PCV2-SD or PCV2-SI infection occurs soon after weaning.[3] However, the economic benefits of PCV2 vaccination of sows in pig farms with proven absence or limited PCV2 circulation are questionable in improving reproductive performance.[2,38] Even on these farms, previous studies indicated that PCV2 vaccination of replacement gilts should be maintained.[2] Gilts are often vaccinated prior to the first insemination (twice one - two months prior) to reduce the risk of PCV2-RD. The aim of this vaccination is to increase the level of maternal immunity transferred from gilts to piglets and thus protect piglets from PCV2 infection until they are vaccinated. Nevertheless, the present study did not extend to the first farrowing to evaluate the level of IgG antibodies in their offspring and reproductive parameters. However, the increased level of anti-PCV2 antibodies in double vaccinated piglets reared for gilts in our field trial could lead to increased maternal immunity transferred from these gilts to piglets.

In conclusion, our findings suggest that the double vaccination of piglets reared for gilts in a PCV2-SI-affected farm with a single dose vaccine against PCV2 has beneficial effects on humoral immunity and growth performance. The limitation of our study is that it was conducted only on one PCV2-SI-affected farm, with its own grandparent nucleus herd. Also, the impact of double PCV2 vaccination in piglets that were reared for gilts on their reproductive performance was not analyzed. Therefore, further studies should be performed in terms of the evaluation of reproductive parameters and litter characteristics of double PCV2 vaccinated piglets with a single-dose vaccine.

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Conflict of interests

The authors declare no conflict of interest of any kind arising out of this manuscript.

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