Kafkas Univ Vet Fak Derg
20 (3): 465-468, 2014
DOI: 10.9775/kvfd.2013.10373

Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

SHORT COMMUNICATION

Immune Response and Production Perfomance in Piglets Vaccinated at 15 and 21 Days Old Against Circovirus Infection

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Makale Kodu (Article Code): KVFD-2013-10373

Summary

The aim of this research was to determine the effect of vaccination on the amount of antibody titers specific for PCV2, and to determine the effect of vaccination on characteristics of pig production. The first group (A) was vaccinated at 15 days old, the second (B) at 21 days old while the third (C) was the control group. Group B piglets attained the best results, so the vaccination of piglets at 21 days old would have an advantage compared to vaccination at 15 days old, although we note that at 15 days old, there is a far greater influence of maternal antibodies on the creation and development of immune responses in the piglets after vaccination.

Keywords: *PCV2, Immunity, Antibodies, Piglets, Vaccine*

Domuzlarda Circovirus Enfeksiyonuna Karşı 15 ve 21 Günlükken Aşı Olan Domuz Yavrularının Bağışıklık Yanıtı ve Üretim Performansı

Özet

Bu incelemenin amacı, PCV2 Virüsüne özel antikor titresi seviyesine ve domuzların üretim özelliklerine aşılamanın etkisini belirlemektir. Birinci, A grubu, 15 günlükken aşı olmuştur, ikinci (B), 21 günlükken, üçüncü, C grubu ise, kontrol grubuydu. B grubu domuz yavrularında çok daha iyi sonuçlar alınmıştır. Dolayısıyla, domuz yavrularının 21 günlükken aşılanmasının, 15 günlükken yapılan aşılamadan daha başarılı olduğu anlaşılmıştır. Ancak, belirtmek gerekir ki maternal antikorların, 15 günlük olan domuz yavrularının kendi bağışıklık yanıtının gelişmesi ve oluşması üzerine etkisi çok daha büyüktür.

Anahtar sözcükler: *PCV2, Bağışıklık, Antikorlar, Domuz yavrusu, Aşı*

INTRODUCTION

Porcine circovirus type 2 (PCV2) is a widespread virus of domestic and wild pigs and is the primary cause of this pig disease group [1]. Increasing interest in circovirus infections began after the onset of the Post Weaning multisystemic wasting syndrome (PMWS) in Canada in 1991, and retrospective studies have demonstrated their presence in the late 1960s [2]. The group of circovirus diseases, in addition to PMWS, encompasses reproduction disorders, Porcine Dermatitis Nephropathy Syndrome (PDNS), and also a respiratory and enteric form of this disease [3]. The introduction of PCV2 vaccine significantly changed the impact of circovirus on pig production at the global level [4,5]. Vaccination of sows and piglets increases PCV2 antibody titers in serum and colostrums and protects piglets from PMWS development [6,7]. High titers generally provide solid protection against PCV2 infection, whereas lower titers do not provide protection against these infections. Time of vaccination is often problematic, as the large number of papers indicating the possibility of interference of colostral and vaccination antibodies indicates, and which is supported by the large difference in the time of the vaccine application suggested by the pharmaceutical companies [8].

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The aim of this study was to examine the importance of piglet vaccination through following the antibody titers in the serum of vaccinated and non-vaccinated piglets and the production performance of pigs. The second objective was to determine the optimal piglet vaccination time.

MATERIAL and METHODS

Experimental Animals

The research was performed on a pig farm, capacity of 2500 sows, with intensive, enclosed growth conditions, in which the presence of PCV2 infection was demonstrated. The experiment was conducted on 900 piglets divided into 3 groups of 300 randomly chosen piglets. The first group (A) was vaccinated at 15 days old, the second group (B) at 21 days old, and the third group (C) was the control, unvaccinated group. All experimental animals were clinically healthy and in good shape. The piglets were selected by random sampling method. After weaning, all piglets were housed in one building and were separated into boxes of 10 piglets each.

Vaccine

Vaccination of pigs against circovirus infections used the commercial vaccine, licensed for 2 week old piglets and older. Commercial vaccine, is a recombinant ORF2 sub-unit vaccine containing PCV2 antigen. Vaccine (1 ml) was administered intramuscularly in the neck of experimental piglets in group A (on the 15th day) and group B (on the 21st day).

Blood Sampling

Blood samples were taken from 90 piglets (30 piglets from each experimental group) to obtain blood serum samples for the determination of antibody titers. Sampling was first performed in piglets on the day of vaccination (on 15th day of age for group A, 21st day for group B, and 21st day for group C), and then on the 7th, 14th, 21st, 28th, 35th, 60th, 90th and 120th days after the first sampling. Blood was taken by puncture of the brachiocephalic plexus of animals. Samples were collected in approximately 9 ml volumes in vacutainers with coagulation activator and were delivered in a cool-box to the laboratory. Serums were separated after coagulation and centrifugation, and were stored at -20°C until testing.

Determining the Antibody Titers Specific for PCV2

Detection of antibodies specific for PCV2 was performed by an indirect ELISA method - INGEZIM CIRCO IgG (Ingenasa, Spain) according to the manufacturer's instructions. The titer of each sample was calculated according to the formula (Titer = 53 (e 3.2x)), where e is an irrational constant and represents the base of the natural logarithm (2.718), and x is the S/P value of the sample. During statistical processing of the obtained results, the values of antibody titers specific for PCV2 were normalized using $\log_2$ values.

Determining the Effect of Vaccination Against PCV2 on Pig Production Characteristics

The following parameters were analyzed: mortality, average daily weight gain (ADWG), the percentage of culls, feed conversion rate (FCR), weight at slaughter. Mortality was calculated by the ratio of the number of dead pigs and the number of pigs tested and is expressed as a percentage. Pigs marked as rejects were those pigs which had a 20% lower body weight than the average weight of pigs at slaughter. All parameters were analyzed from the moment of vaccination to 175th day of life.

Statistical Analysis

One-way ANOVA test and Turkey`s test were used for mean comparison of the normally distributed variables between groups. Statistical significance of differences between means was determined at the level of $P<0.05$.

RESULTS

By observing the studied population of pigs in whole, from the beginning to the end of the study, it was noted that: the lowest average values of antibody titers in vaccinated groups were determined on the 35th day while the maximum values occurred on the 90th day after vaccination. In the control group, from the beginning of the trial, the average titer decreased continuously until the 60th day, after which the antibody titer specific for PCV2 tended to rise. There were no significant differences ($P>0.05$) in the average level of antibodies in piglet serum measured from the start of observations until the 28th day between the vaccinated groups (A and B) and the unvaccinated control piglets *(Fig. 1)*.

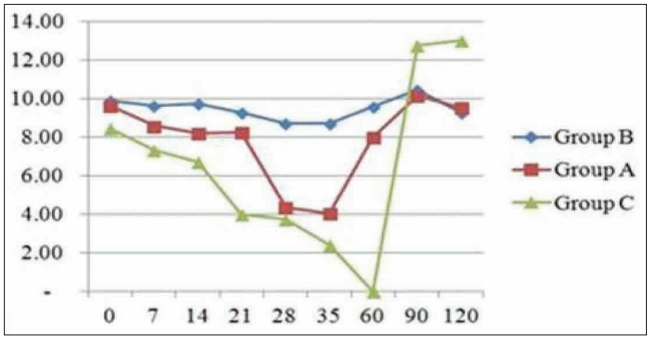

Fig 1. Comparative view of the average values of PCV2 specific antibody titers in the blood serum of piglets from the beginning of the study to 120th day after vaccination

Şekil 1. Deneyin başlamasından aşılamadan sonra 120. gününe kadar domuz yavrularının kan serumunda bulunan PCV2 Virüsüne karşı spesifik antikorların titresinin ortalama değerlerinin karşılaştırmalı görüntüleri

Table 1. *Comparative view of the production performance of pigs in all groups*

Tablo 1. *İncelenen tüm gruplarda domuzların üretim özelliklerinin karılaştırmalı verileri*

Item	Group A	Group B	Group C
ADWG (g/day)	746	750	690
FCR (kg)	3	3.01	3.09
Weight at slaughter (kg)	101.3	102	97
Mortality (%)	7.33	6.33	9
Cull (%)	11.66	11.33	17.33
Tested number	300	300	300

The data obtained on production characteristics of pigs vaccinated at 15 or 21 days old, and from the control group are shown in *Table 1*.

DISCUSSION

In our study, all piglets showed the presence of antibodies specific to PCV2, on the day of vaccination (titers ranged from 8.47 $\log_2$ in the control group to 9.91 $\log_2$ in Group B; *Fig. 1*). Seven days after vaccination, a slight decrease in specific antibodies in the blood serum of piglets was noticed *(Fig. 1)*, but complete absence of antibodies was not found in any piglet. This can certainly be explained, on one hand, by the antigen stimulation of applied vaccine, and, on the other hand, by immunosuppressive action of existing colostral or already created postinfectious antibodies in the blood serum of the piglets examined, which is in full accordance with the results of other authors [9,10]. The highest antibody titer (9.63 $\log_2$) was found in piglets 7 days after vaccination in group B (vaccinated at 21 days old), which may be related to the fact that this group had the highest average titer even before vaccination (9.91 $\log_2$; *Fig. 1*). The biggest decrease of immunoglobulin was observed in group C (1.14 $\log_2$). In group A, we measured a decrease of 1.04 $\log_2$, while in group B the decrease was only 0.28 $\log_2$. The above-mentioned decrease of specific antibodies lasted until the 90th day after vaccination, after which there was a jump in average serum titer levels to 10.46 $\log_2$ in Group B piglets. Levels again declined to 9.27 $\log_2$by day 120 *(Fig. 1)*. These phenomena are certainly attributable to postinfection antibody levels, which occured somewhere between the 35th and 60th days after vaccination *(Fig. 1)*. The most drastic changes occured in the blood sera of unvaccinated pigs (Group C), in which, on the 60th day after vaccination, no specific antibodies were found *(Fig. 1)*, which clearly indicates that there was a complete catabolism of colostral antibodies and the disappearance of passive immunity. In the same group, the average antibody titer on the 90th day suddenly jumped to 12.78 $\log_2$. Possible reasons for this sudden increase in titers of antibodies against PCV2 in blood serum of piglets are the widespread presence of this infection on the study farm, as well as the fact that our pig population initially lacked any immunity to the PCV2 infection. Group A piglets generally followed the same pattern of immunoglobulin changes, as was found in Group B piglets, although the changes were much less pronounced *(Fig. 1)*. The reasons for this are due to the fact that on the 15th day of age (when Group A piglets were vaccinated), large amounts of colostral immunoglobulins were still present, and these followed a similar pattern of changes to those we observed in the control group. This could explain the fact that in the sera of Group A piglets, even 35 days after vaccination, it was not possible to identify a single piglet with antibody titers above 9 $\log_2$, which undoubtedly corresponds to complete catabolism of colostrum antibodies and the lack of creating their own immunoglobulin. In this sense, vaccination of pigs at 21 days old would be advantageous compared to vaccination at 15 days old, although the period of 21 days is quite long, which could lead to infection of pigs with negative consequences in terms of health and achieved economic parameters (total weight gain, food consumption efficiency, etc.). The results we obtained are compliant with those of Krakowka et al.[9], and Meerts et al.[11], who stated that the conversion occurs 10 to 28 days after inoculation. Some other authors [12,13] suggest that the conversion of antibodies at 21 days after infection is lower than in subclinical infected pigs. This could be explained only by the level of colostral antibodies at the time of inoculation. On the other hand, Forth et al.[14], have shown that the anti-PCV2 IgG occurs between 7th and 14th day after inoculation of antigen, reaches its peak on the 21st day after inoculation and rises until the 69th day after inoculation. This is not consistent with our results, which clearly show that low levels of antibodies had a positive effect, and that the negative effect is observed only in the presence of high levels of maternal antibodies [10], although Ritzman et al.[15] in a study carried out on 1519 piglets demonstrated that the vaccine was effective regardless of the level of maternal antibodies.

Results to date have also shown a different number of pigs lagging in the growth. In the current study, 17.33% of pigs in the control group had insufficient weight gain. Overall, this resulted in 32.8% and 34.6% more unvaccinated piglets which did not thrive compared to vaccinated Group A or Group B piglets, respectively *(Table 1)*. Differences in the number of rejects caused quite different levels of mortality *(Table 1)*. In the control group of piglets, the mortality level was 9%, while that of group A was 7.33%, and that of group B was 6.33%. The differences found could be linked to differences in serological response, or the number of reject piglets in tested groups. Generally, however, during the course of our study, positive effects of the applied vaccine were noted. They are largely based on preventing larger oscillations in the level of antibodies specific for PCV2. The identified differences are likely the result of the presence of maternal antibodies in the blood serum of tested piglets at the time of the vaccination.

By preventing major fluctuations, we can prevent the possibility that a certain number of piglets, at any given moment, are without immunological protection and thus create the possibility of infection.

Overall, our study has shown that, with the vaccine studied, much better results are attained by vaccination of piglets at 21 days old, compared to 15 days old, there is a far greater influence of maternal antibodies on the creation and development of each piglet's own immune responses after vaccination.

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