

## SWINE HEALTH

**Title:** Head-to-head comparison of the level of protection and duration of immunity induced by different commercial and an autogenous PCV2 vaccine – **NPB #07-210**

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### Industry Summary

The study objectives were to compare the duration of immunity of commercially available, one and two dose, killed porcine circovirus type 2 (PCV2) vaccines. Sixty, 3.5-week-old pigs were randomly divided into 6 treatment groups: one dose vaccines (FDAH-1, BIVI-1), two dose vaccines (Intervet-2, FDAH-2), and non-vaccinated negative and positive controls. Tissue homogenate challenge was conducted 63 (two doses) or 84 (one dose) days post vaccination. Viremia was reduced by 78.5% in pigs vaccinated with one dose and by 97.1% in pigs vaccinated with two dose products and overall microscopic lymphoid lesions were reduced by 78.7% and 81.8%, respectively.

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## Scientific Abstract

The study objectives were to compare the duration of immunity of commercially available, one and two dose, killed porcine circovirus type 2 (PCV2) vaccines. Sixty, 3.5-week-old pigs were randomly divided into 6 treatment groups: one dose vaccines (FDAH-1, BIVI-1), two dose vaccines (Intervet-2, FDAH-2), and non-vaccinated negative and positive controls. Tissue homogenate challenge was conducted 63 (two doses) or 84 (one dose) days post vaccination. Viremia was reduced by 78.5% in pigs vaccinated with one dose and by 97.1% in pigs vaccinated with two dose products and overall microscopic lymphoid lesions were reduced by 78.7% and 81.8%, respectively.

## Introduction

Porcine circovirus type 2 (PCV2) is a small, non-enveloped, single stranded DNA virus that is ubiquitous in the swine population [1]. As of January 1, 2008, ten years after its initial recognition, PCV2 is known as one of the most important viruses in the global pig industry. The acronym PCVAD (porcine circovirus associated disease) was introduced to summarize the different manifestations of PCV2-associated disease in fetal, growing and mature pigs. Most commonly, PCVAD manifests as systemic disease characterized by growth retardation [2], as respiratory disease [3], as diarrhea [4] or as a combination of all of these clinical presentations in grow-finish pigs. The manifestations vary in severity from mild lesions to severe PCV2-associated lesions and disease. Depending on the individual farm situation and influenced by concurrent infections [5], management [6], and genetics [7], PCVAD can affect a varying percentage of a population (1-50%). Coinfections have been shown to enhance subclinical PCV2 infection to manifest as clinical disease in the field studies [5;8] and in experimental trials [9-16]. Today, coinfections especially with porcine parvovirus (PPV) [9;10;12;13] and porcine reproductive and respiratory disease syndrome virus (PRRSV) [14-16] are widely used in controlled experimental PCV2 challenge studies to enhance clinical disease and PCV2 replication; however, the mechanism by which these pathogens interact with PCV2 is still not fully understood.

PCVAD has rapidly become one of the most devastating and economically important challenges for pork producers in North American; however, vaccination has been shown to be effective in combating PCVAD. Several field investigations have clearly demonstrated the efficacy of the current commercial PCV2 vaccines. In a field study using 1,542 pigs in a herd suffering from PRDC, PCV2 vaccination reduced the mean PCV2 viral load by 55-83% and the mean duration of viremia by 50% [17]. In addition, in the last 8 weeks prior to marketing, it was found that vaccination improved the average daily gain and reduced the time to market [17]. In another study using 485 commercial pigs from a herd with a history of PCVAD, PCV2 vaccination reduced the overall mortality by 50% and increased the daily gain during the finishing period by 9.3% [18]. In a small controlled experimental study, it was found that vaccinated pigs had significantly decreased nasal and fecal shedding of PCV2 after the pigs were challenged with 4 different PCV2 isolates of different genotype and geographic origin [19]. In another controlled experimental trial, PCV2 vaccination was effective in inducing a neutralizing antibody response and in reducing PCV2 associated lesions and viremia in pigs concurrently infected with PCV2 and PRRSV [20].

In the United States, PCV2 vaccines first became available in 2006 and to date three commercial inactivated products are available in the U.S. The currently available products differ in antigen type [open reading frame (ORF) 2 protein expressed in the baculovirus system versus a chimeric PCV1-2 virus], adjuvant type, and recommended administration dosage (one or two doses) [21]. It is common practice to administer PCV2 vaccines as early as possible and it is recommended to vaccinate at least 3-4 weeks ahead of anticipated exposure. Later administration may coincide with circulation of field PCV2 strains and reportedly has not been as successful (Wagner M. Porcine circovirus associated disease: A practitioner's perspective on clinical observations and control with vaccination. Proc Annual Swine Disease Conf, Ames, Iowa, 15:56-59, 2007).

Although initial indications from the field suggested that commercial PCV2 vaccines were highly efficacious, there have been increasing reports of apparent vaccine failure manifest in late finisher pigs. This

has generated concerns about duration of PCV2 vaccine-induced immunity. The objectives of this study were to compare the duration of immunity of commercially available PCV2 vaccines and to determine whether one dose and two dose PCV2 vaccine products are equivalent in inducing protective immunity.

## **Objectives**

- 1) Determine and compare the duration of immunity of commercially available PCV2 vaccines (FDAH, BIVI, and Intervet).
- 2) Determine whether one dose and two dose PCV2 vaccine products provide equivalent long-term protection in growing pigs.
- 3) Compare neutralizing antibody titers amongst commercially available PCV2 vaccines.

## **Materials and Methods**

### **Experimental design**

The experimental protocol was approved by the Iowa State University Institutional Animal Care and Use Committee. Sixty, conventional, PCV2-naïve piglets were randomly divided into 6 groups according to Table 1. At 3.5 and 6.5 weeks of age a portion of the pigs were vaccinated with different commercially available PCV2 vaccines (Table 1). At 15.5 weeks of age, pigs in the FDAH-1, BIVI-1, Intervet-2, FDAH-2, and positive control groups were challenged intranasally with a tissue homogenate containing PCV2. Twenty-one days after challenge, all pigs were necropsied and macroscopic and microscopic lesions were compared across groups. The amount of PCV2 antigen in lymphoid tissues was determined by immunohistochemistry (IHC) [22]. Serum samples were collected at arrival, periodically throughout the growing phase, at the day of challenge, and weekly thereafter. Serum samples were tested for presence of PCV2 antibodies and PCV2 DNA by ELISA [23] and quantitative real-time PCR [24] respectively.

### **Animals and housing**

Colostrum-fed, crossbred, specific-pathogen-free (SPF) conventional pigs were purchased from a herd that is routinely tested for major swine pathogens and known to be free of PCV2, porcine reproductive and respiratory syndrome virus (PRRSV), and swine influenza virus (SIV). The pigs were weaned at two weeks of age and transported to the Livestock Infectious Disease Isolation Facility at Iowa State University, Ames, Iowa. All pigs were comingled and randomly assigned to one of two rooms each containing six 2.5 × 3.6 m raised wire decks equipped with one nipple drinker and one self-feeder. At the time of challenge, the negative control group was moved into a separate room of similar design. The pigs were fed a phased diet for growing pigs free of animal proteins and antibiotics.

### **Vaccination**

Three different commercially available vaccine products were used in this study. At 3.5 weeks of age, four groups of 10 randomly selected pigs were each vaccinated with either 2ml Suvaxyn® PCV2 (Fort Dodge Animal Health Inc., serial number 1861161A), 1ml Ingelvac® CircoFLEX™ (Boehringer Ingelheim Vetmedica Inc.; serial number 309-024; BIVI-1), 2ml Cicumvent™ PCV2 (Intervet Inc., Millsboro, Denver, USA; serial number 02137920), or 1ml Suvaxyn® PCV2 (Fort Dodge Animal Health Inc.; serial number 1861161A). Twenty pigs remained non-vaccinated and served as negative and positive control groups, respectively (Table 1). Three weeks post initial vaccination, at 6.5 weeks of age, pigs in groups Intervet-2 and FDAH-2 were re-vaccinated with either 2ml Cicumvent® PCV2 or 1ml Suvaxyn® PCV2, respectively (Table 1). Vaccination was done intramuscularly into the right neck for all pigs.

### **Inoculation**

Inoculation was conducted at 15.5 weeks of age which was 84 days post vaccination in groups FDAH-1 and BIVI-1 and 63 days post booster vaccination in groups FDAH-2 and Intervet-2. Each pig received 6 ml of a tissue homogenate intranasally. Briefly, lungs and lymphoid tissues were processed as follows: Approximately one gram of tissue was minced, placed into 50ml of Earle's balanced salt solution and processed on high speed

for 120 seconds in a stomacher (Stomacher ® 80 biomaster; Seward Laboratory Systems Inc. Bohemia, NY). The homogenized sample was centrifuged at  $4200 \times g$  for 30 minutes at  $4^{\circ}\text{C}$ . The supernatant was removed and stored at  $4^{\circ}\text{C}$  overnight until use. The tissue homogenate originated from a case of PCVAD in a grow finish pig (abundant PCV2 staining in lungs and lymphoid tissues) and contained 734,428,417.19 PCV2b DNA copies/ml (virus isolation for PCV2 was negative) and swine influenza virus (SIV) H1N1 at a dose of  $10^{2.7}$  50% egg culture infectious dose ( $\text{ECID}_{50}$ ). North American PRRSV was detected in the homogenate by PCR (248,743 RNA copies/ml) but virus isolation attempts for PRRSV were negative. PPV was not detected in the homogenate by PCR and virus isolation attempts for PPV were negative.

### **Serology**

Blood samples were collected at the arrival of the pigs to the research facility and weekly thereafter until necropsy. The serum was tested by the ORF2-PCV2 IgG ELISA [23]. Samples were considered positive if the calculated S/P ratio was 0.2 or greater. A fluorescence focus neutralization (FFN) assay was done on the day of challenge in order to determine the presence of neutralizing antibodies against PCV2 according to the standard Iowa State University (ISU) Veterinary Diagnostic Laboratory operating protocol [25]. PCV2 isolate ISU-98-15237 was used in this assay.

In addition, serum samples from all pigs at 21 days post challenge (dpc) were tested for the presence of anti PRRSV antibodies by ELISA (HerdChek PRRS virus antibody test kit 2XR, IDEXX Laboratories, Inc. Westbrook, Massachusetts, USA), for the presence of SIV antibodies by an in house nucleoprotein NS1 ELISA [26], and for the presence of antibodies to PPV by hemagglutination inhibition (HI) assay [27].

### **Clinical evaluation**

Following PCV2-inoculation, the pigs were monitored daily for clinical signs including sneezing, lethargy, and coughing. Observers were blinded to vaccination status.

### **PCV2 DNA quantification**

DNA-extraction on serum samples collected on the day of challenge and on dpc 7, 14, and 21 was performed using the QIAamp® DNA Mini Kit (Qiagen, Valencia, CA). DNA extracts were used for quantification of PCV2 genomic DNA copy numbers by real-time PCR as described previously [24].

### **PRRSV PCR**

RNA extraction on serum samples collected on 21 dpc and real-time RT-PCR for PRRSV was performed as described [28].

### **Necropsy**

All pigs were humanely euthanized by pentobarbital overdose and necropsied on dpc 21. The total amount of macroscopic lung lesions (ranging from 0 to 100%) was estimated and scored by a pathologist blinded to treatment [29]. Additionally, the size of lymph nodes ranging from 0 (normal) to 3 (four times the normal size) were estimated and recorded. Sections of lymph nodes (superficial inguinal, mediastinal, tracheobronchial, mesenteric), tonsil, thymus, ileum, kidney, colon, spleen, and liver were collected at necropsy and fixed in 10% neutral-buffered formalin and routinely processed for histological examination.

### **Histopathology**

Microscopic lesions were evaluated by a pathologist blinded to the group designation of animal tissues. Sections were scored for the presence and severity of interstitial pneumonia ranging from 0 (normal) to 6 (severe diffuse) [11]. Sections of heart, liver, kidney, ileum, and colon were evaluated for the presence of lymphohistiocytic inflammation and scored from 0 (none) to 3 (severe). Lymphoid tissues including lymph nodes (tracheobronchial, mesenteric, mediastinal, superficial inguinal, and external iliac), tonsil, and spleen were evaluated for the presence of lymphoid depletion ranging from 0 (normal) to 3 (severe) and histiocytic inflammation and replacement of follicles ranging from 0 (normal) to 3 (severe) [11]. The overall microscopic

lymphoid lesions score which accounts for lymphoid depletion, histiocytic inflammation and PCV2-antigen present in lymphoid tissues was calculated as previously described and ranged from 0=normal to 9=severe [11].

### **Immunohistochemistry**

IHC for detection of PCV2-specific antigen was performed on selected formalin-fixed and paraffin-embedded sections of lymph nodes (superficial inguinal, mediastinal, tracheobronchial, and mesenteric), tonsil, spleen, and thymus using a rabbit polyclonal antiserum [22]. PCV2-antigen scoring was done by a pathologists blinded to animal group designation. Scores ranged from 0 (no signal) to 3 (more than 50% of the lymphoid follicles contain cells with PCV2-antigen staining). The mean group score was determined for each tissue and compared among groups [11]. In addition, IHC for detection of SIV antigen was performed on lung sections using a previously described protocol [30].

### **Statistical analysis**

Summary statistics were calculated for continuous variables (S/P ratios, Ct values) from all groups to assess the overall quality of the data. For repeated data, multivariate analysis of variance (ANOVA) was used to detect significant differences over time. If the multivariate ANOVA was significant ( $P < 0.05$ ), a non-parametric Kruskal-Wallis one-way ANOVA was used to analyze data at each time point. If significant differences were noted ( $P < 0.05$ ), pair-wise comparisons using Wilcoxin rank-sum were performed. For non-repeated measures of histopathological and neutralizing antibody data, a non-parametric Kruskal-Wallis one-way ANOVA followed by pair-wise comparisons using Wilcoxin rank-sum was utilized. Statistical analysis was performed using JMP 7.0.2 (SAS Institute, Cary, NC). Percent reduction for PCR data was measured as follows:  $100 - [(100 * \text{mean log}_{10} \text{ genomic copies/ml in the vaccinated group}) \div (\text{mean log}_{10} \text{ genomic copies/ml in positive control animals})]$ . Percent reduction for histopathological data was calculated similarly.

## **Results**

### **Clinical presentation**

After challenge, the majority of the pigs in the challenged groups were lethargic, had mild respiratory disease characterized by sneezing, coughing, and clear nasal discharge, and had markedly decreased appetite. Wasting was not observed in any of the pigs. There were no significant differences between treatment groups.

### **Macroscopic lesions**

Gross lesions were limited to enlarged lymph nodes and mild cranioventral purple consolidation of lungs and failure of the lungs to collapse. Individual vaccinated and non-vaccinated pigs had slightly enlarged mediastinal lymph nodes.

### **Anti-PCV2-IgG antibody levels**

At arrival in the research facility, all pigs were seronegative for PCV2. As expected, vaccinated animals developed anti-PCV2 IgG antibodies after vaccination. All animals in all vaccinated groups were seropositive for PCV2 starting on the day of the second vaccination. The PCV2 antibody response after challenge is summarized in Fig. 1. There was a significant ( $P > 0.05$ ) difference in group mean-S/P ratios between one dose and two dose vaccine administration one week after booster vaccination at 7.5 weeks of age but not thereafter (Fig. 2).

### **Neutralizing antibodies**

The log transformed group mean neutralizing antibody titers at the day of challenge 84 (one dose) or 63 (two doses) days post vaccination for all groups are summarized in Table 2. All vaccine treatments were effective at inducing a neutralizing antibody response in comparison to non-vaccinated groups although significant differences ( $P < 0.05$ ) among products were noted. The log transformed group mean neutralizing antibody titers at the day of challenge for one dose or two vaccine administration are summarized in Table 3. No significant differences between one dose and two dose vaccine administration were noted.

### **Anti-PRRSV, anti-SIV, and anti-PPV-antibodies in serum samples**

Forty-eight of fifty pigs exposed to the tissue homogenate had seroconverted to PRRSV by 21 dpc. The two pigs that had not seroconverted to PRRSV belonged both to the INTERVET-2 group. There were no significant differences in group mean PRRSV S/P ratios among groups. Forty-two of fifty pigs exposed to the tissue homogenate had seroconverted to SIV by 21 dpc. The eight pigs that had not seroconverted to SIV belonged to the following groups: INTERVET-2 (2 pigs), BIVI-1 (1 pig), FDAH-2 (1 pig), and non-vaccinated positive controls (4 pigs). The positive control group had significantly lower SIV S/P ratios compared to all vaccinated groups ( $P=0.03$ ) and compared to one-dose groups ( $P=0.02$ ). All pigs were seronegative to PPV by 21 dpc.

### **Prevalence and amount of PCV2 DNA in serum**

PCV2 DNA was not detected in any of the serum samples on the day of challenge. The log transformed group mean PCV2 DNA amounts for all groups are summarized in Table 4. At 21 dpc, the reduction of PCV2 compared to the non-vaccinated POS pigs was 100% for Intervet-2 and FDAH-1, 94.3% for FDAH-2, and 56.9% for BIVI-1. The log transformed group mean PCV2 DNA amounts for one and two dose vaccinations are summarized in Table 5. At 21 dpc, the reduction of PCV2 compared to the non-vaccinated positive control pigs was 97.1% for two dose products and 78.5% for one dose products.

### **Prevalence of PRRSV in serum**

Forty-five of fifty challenged pigs were PRRSV viremic by 21 dpc. The pigs that were negative for PRRSV RNA on serum were distributed as follows: Each one pig belonged to the BIVI-1, FDAH-1, and FDAH-2 groups and two pigs belonged to the non-vaccinated positive control group.

### **Microscopic lesions**

In general, microscopic lesions were mild and limited to lymphoid tissues (mild depletion of follicles and mild histiocytic lymphadenitis). As evident from the overall lymphoid lesion score, all vaccine treatments were equally effective in reducing PCV2-associated microscopic lesions as compared to non-vaccinated control pigs (Tables 2 and 3). The mean percentage of reduction of lesions compared to the non-vaccinated positive control group was 91.2% for FDAH-1, 83.4% for FDAH-2, 74.0% for Intervet-2, and 72.4% for BIVI-1.

### **Prevalence of SIV antigen in lung tissues**

All pigs were negative for SIV specific antigen in formalin-fixed, paraffin-embedded lung tissue sections collected on 21 dpc.

## **Discussion**

To our knowledge, the current study represents the first randomized, controlled experimental trial which directly compares product efficacy of several PCV2 vaccine products. To determine if there are differences in duration of vaccine-induced immunity we conducted a head-to-head comparison in which pigs were challenged at 84 days post vaccination for one dose products or at 63 days post booster vaccination for two dose products. The interval between the time of challenge and vaccination is different for one and two dose products and was based on manufacturer's recommendations and common vaccination age in the field. When comparing results between one and two dose products it needs to be considered that vaccine efficacy may be influenced by the interval between vaccination and challenge.

Although, studies investigating the duration of immunity have been performed individually for licensure of each product, the current study represents a direct comparison of all products using a challenge at 15.5 weeks of age. Other controlled PCV2 vaccine efficacy studies were based one vaccine product and used vaccination-challenge intervals of 15 [19] and 28 [20] days with a mean age of the pigs at challenge of 8 weeks [19;20]. Similar to the previous studies, all vaccines tested induced a neutralizing antibody response which was still

detectable at the time of challenge. Although the pathogenesis of PCV2 infections remains unclear, it has been hypothesized that serum neutralizing antibodies may be an important deterrent for virus replication [31]. In our study, we found significant differences between products when we compared the log transformed neutralizing antibody titers at the 15.5 weeks of age. Similar to our findings with the anti-IgG response, the difference was detectable only in direct comparison of products; both FDAH groups (FDAH-1 and FDAH-2) had significantly ( $P < 0.05$ ) higher neutralizing antibody titers compared to all other groups. Since the neutralizing antibody assay is independent of baculovirus, interference with anti-baculovirus antibody and potential artificial decrease of titers can be ruled out.

As expected, vaccination reduced PCV2 DNA load in serum samples and PCV2 antigen in tissues sections compared to non-vaccinated positive controls. At 21 dpc, the reduction of PCV2 DNA in serum was 78.5% and 97.1% for one and two dose products, respectively. The difference in one and two dose products was mainly influenced by one of the products as FDAH-1 and FDAH-2 performed similar and were not different from each other. Most importantly, the overall microscopic lymphoid lesions were reduced by 78.7% and 81.8% in pigs vaccinated with one and two doses, respectively, and there was no difference between one or two dose products.

Field reports of apparent vaccine failures are increasingly common particularly in cases when exposure to PCV2 occurs in the mid to late finishing phase in pigs vaccinated at an early age. Potential reasons for vaccine failures include farm-associated factors such as compliance with proper vaccine protocols, failure to use the recommended dosage, vaccination of sick and immunocompromised pigs, or the presence of interfering passively-acquired antibodies. Under experimental conditions, evidence suggests that maternal antibodies do not influence vaccine efficacy [19;32] and field experience (more than 90% of the pigs that enter the market in the US are currently vaccinated against PCV2; Edgar Diaz: personal communication) further supports this. Possible vaccine-associated factors for vaccine failures include antigenic differences between the vaccine strain and field strain, adjuvant type, amount of PCV2 antigen in the vaccine preparation, and administration regimen.

Products approved for single dose administration are often more popular because of decreased cost associated with labor and perception of decrease spread of other diseases associated with vaccination. A major disadvantage of not giving a booster dose is the lack of generating a larger number of memory B cells resulting in a longer lag period after encounter with the antigen. In this study, besides higher anti-PCV2-antibody levels one week after booster vaccination in the two dose groups, we found no other difference in humoral response to vaccination between one and two dose products. When vaccines were compared individually; however, there were significant differences between products in amount of anti-PCV2-antibodies (Fig. 1) which was not related to dose as the FDAH product performed very similar in the one and the two dose versions. The S/P ratio in the Intervet-2 group may have been artificially reduced as it has been shown that the anti-baculovirus response induced by this particular vaccine cross-reacts with the negative control well in the ELISA used [33].

Although it may limit the reproducibility of the current study, we chose to use a tissue homogenate for challenge to more closely mimic the field situation where coinfections with known and unknown pathogens are common. Singular PCV2 infection doesn't consistently result in clinical disease in singular infected pigs [24;34]. It was anticipated, that a challenge material containing several pathogens can be more easily extrapolated to field situations where PCV2 is rarely found by itself [5]. The tissue homogenate in this case was obtained from a field case of an apparent PCV2 vaccine failure. Serology and PCR assays confirmed that the pigs in this study became infected with H1N1 SIV, North American PRRSV, and PCV2b. Although the pigs were clinically affected and showed reduced feed intake and lethargy, none of the pigs developed clinical PCVAD. In addition, there were no significant differences in incidence or serological response to PRRSV. However, vaccinated pigs and particularly pigs vaccinated with the one dose products had a better anti-SIV response compared to non-vaccinated pigs. It could be argued that the challenge material used was not strong enough; however, 8 of 10 non-vaccinated positive controls had PCV2 antigen present in lymphoid tissues at necropsy which is similar to challenge studies using cell culture propagated PCV2 [20].

In summary, one and two dose PCV2 vaccine administration in naïve pigs significantly reduced PCV2 viremia and overall microscopic lymphoid lesions following challenge at 63 and 84 days post vaccination

compared to non-vaccinated positive controls. Future studies need to evaluate the effect of maternal antibodies on the long term efficacy of one and two dose PCV2 vaccines.

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**Table 1:** Experimental design.

<i>Group</i>	<i>N</i>	<i>Vaccine</i>	<i>Timing of vaccination</i>		<i>CHALLENGE</i>
			<b>3.5*</b>	<b>6.5*</b>	<b>15.5*</b>
<b>FDAH-1</b>	n=10	Suvaxyn® PCV2	2ml	-	YES
<b>BIVI-1</b>	n=10	Ingelvac® CircoFLEX™	1ml	-	YES
<b>Intervet-2</b>	n=10	Circumvent® PCV2	2ml	2ml	YES
<b>FDAH-2</b>	n=10	Suvaxyn® PCV2	1ml	1ml	YES
<b>POSITIVE</b>	n=10	-	-	-	YES
<b>NEGATIVE</b>	n=10	-	-	-	NO

\* Weeks of age

**Table 2:** Log<sub>10</sub> group mean ( $\pm$  standard error) neutralizing antibody titers on the day of challenge by group, overall PCV2-associated lymphoid lesion score, and prevalence of PCV2 antigen in lymphoid tissue sections.

<i>Group</i>	<i>Neutralizing antibodies</i>	<i>Overall lymphoid Score</i>	<i>Prevalence of PCV2 antigen</i>
<b>FDAH-1</b>	3.8 $\pm$ 0.1 <sup>A*</sup>	0.2 $\pm$ 0.1 <sup>A</sup>	1/10
<b>BIVI-1</b>	2.9 $\pm$ 0.2 <sup>B</sup>	0.5 $\pm$ 0.2 <sup>A</sup>	2/10
<b>Intervet-2</b>	3.4 $\pm$ 0.1 <sup>C</sup>	0.5 $\pm$ 0.1 <sup>A</sup>	1/10
<b>FDAH-2</b>	3.7 $\pm$ 0.1 <sup>A</sup>	0.3 $\pm$ 0.1 <sup>A</sup>	1/10
<b>POSITIVE</b>	1.8 $\pm$ 0.1 <sup>D</sup>	1.8 $\pm$ 0.6 <sup>B</sup>	8/10
<b>NEGATIVE</b>	1.7 $\pm$ 0.3 <sup>D</sup>	0.0 $\pm$ 0.0 <sup>A</sup>	0/10

\*Different superscripts (A,B,C,D) indicate significantly ( $P < 0.05$ ) different group means within the same column.

**Table 3:** Comparison of one dose and two dose PCV2 vaccine administration on log<sub>10</sub> group mean ( $\pm$  standard error) neutralizing antibody titer, overall PCV2-associated lymphoid lesion score, and prevalence of PCV2 antigen in lymphoid tissue sections.

<i>Vaccination</i>	<i>Neutralizing antibodies</i>	<i>Overall lymphoid Score</i>	<i>Prevalence of PCV2 antigen</i>
<b>One dose</b>	3.3 $\pm$ 0.1 <sup>A*</sup>	0.3 $\pm$ 0.1	3/20
<b>Two doses</b>	3.6 $\pm$ 0.1 <sup>A</sup>	0.4 $\pm$ 0.1	2/20
<b>None (POSITIVE)</b>	1.8 $\pm$ 0.1 <sup>B</sup>	1.8 $\pm$ 0.6	8/10

\*Different superscripts (A,B) indicate significant ( $P < 0.05$ ) different group means within the same column.

**Table 4:** Prevalence and group mean log<sub>10</sub> of PCV2 genomic copies at different days post challenge (dpc). Data presented as number of pigs that were PCR positive/total number of pigs (group mean  $\pm$  standard error).

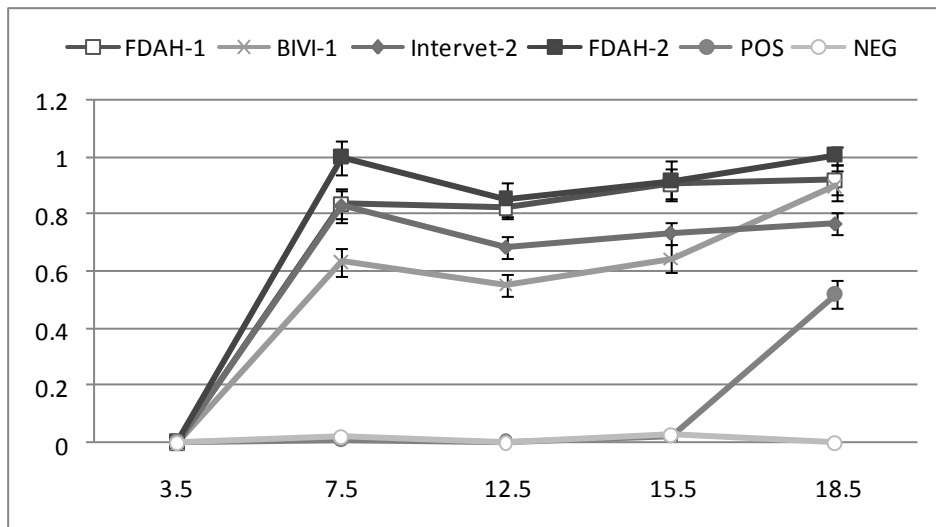
<i>Group</i>	<i>dpc 7</i>	<i>dpc 14</i>	<i>dpc 21</i>
<b>FDAH-1</b>	2/10 (0.6 $\pm$ 0.4) <sup>A*</sup>	7/10 (1.9 $\pm$ 0.4) <sup>A</sup>	0/10 (0.0 $\pm$ 0.0) <sup>A</sup>
<b>BIVI-1</b>	3/10 (0.9 $\pm$ 0.5) <sup>A</sup>	10/10 (3.1 $\pm$ 0.1) <sup>B</sup>	7/10 (2.6 $\pm$ 0.6) <sup>B</sup>
<b>Intervet-2</b>	1/10 (0.3 $\pm$ 0.3) <sup>A</sup>	4/10 (1.0 $\pm$ 0.4) <sup>A</sup>	0/10 (0.0 $\pm$ 0.0) <sup>A</sup>
<b>FDAH-2</b>	1/10 (0.3 $\pm$ 0.3) <sup>A</sup>	6/10 (1.6 $\pm$ 0.4) <sup>A</sup>	1/10 (0.3 $\pm$ 0.3) <sup>A</sup>
<b>POSITIVE</b>	7/10 (2.7 $\pm$ 0.6) <sup>B</sup>	10/10 (6.0 $\pm$ 0.2) <sup>C</sup>	10/10 (6.0 $\pm$ 0.3) <sup>C</sup>
<b>NEGATIVE</b>	0/10 (0.0 $\pm$ 0.0) <sup>A</sup>	0/10 (0.0 $\pm$ 0.0) <sup>D</sup>	0/10 (0.0 $\pm$ 0.0) <sup>A</sup>

\*Different superscripts (A,B,C,D) indicate significantly ( $P < 0.05$ ) different group means within the same column.

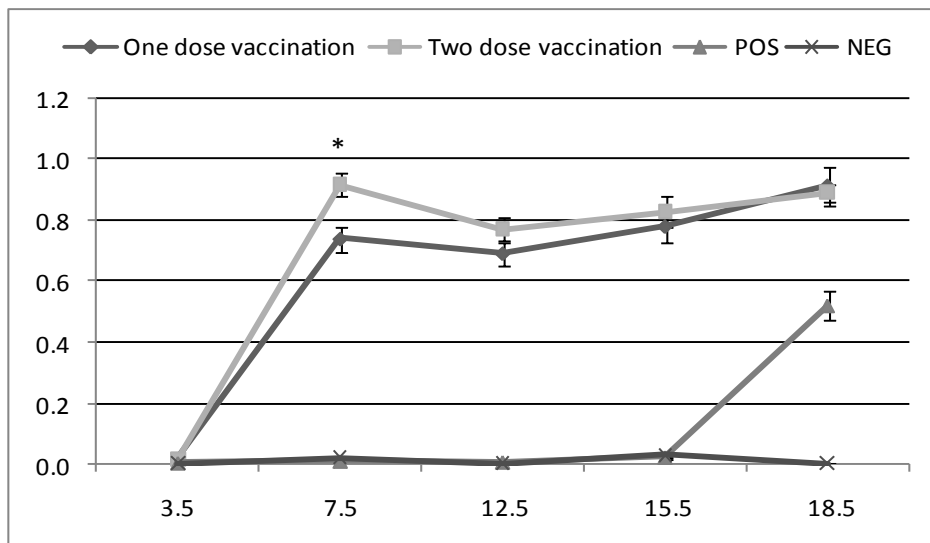
**Table 5:** Comparison of one dose and two dose PCV2 vaccine administration on prevalence and group mean amounts of PCV2 genomic copies in serum samples at days post challenge (dpc) 7, 14, and 21. Data presented as number of pigs that were PCR positive/total number of pigs (group mean  $\pm$  standard error).

<i>Number of Doses</i>	<i>dpc 7</i>	<i>dpc 14</i>	<i>dpc 21</i>
<b>One dose</b>	2/20 (0.8 $\pm$ 0.3) <sup>A*</sup>	10/20 (2.5 $\pm$ 0.3) <sup>A</sup>	1/20 (1.3 $\pm$ 0.4) <sup>A</sup>
<b>Two doses</b>	5/20 (0.3 $\pm$ 0.2) <sup>A</sup>	17/20 (1.3 $\pm$ 0.3) <sup>B</sup>	7/20 (0.2 $\pm$ 0.2) <sup>B</sup>
<b>POSITIVE</b>	7/10 (2.7 $\pm$ 0.6) <sup>B</sup>	10/10 (6.0 $\pm$ 0.2) <sup>C</sup>	10/10 (6.0 $\pm$ 0.3) <sup>C</sup>

\*Different superscripts (A,B,C) indicate significantly ( $P < 0.05$ ) different group means within the same column.



**FIG. 1.** Group mean optical density ratios and standard errors for anti-PCV2-IgG antibody response at different weeks of age (3.5 weeks of age=prior to vaccination; 7.5 weeks of age=1 week after 2<sup>nd</sup> vaccination in 2 dose groups; 15.5 week of age=day of challenge; 18.5 week of age=21 days post challenge).



**FIG. 2.** Group mean optical density ratios and standard errors for anti-PCV2-IgG antibody response at different weeks of age (3.5 weeks of age=prior to vaccination; 7.5 weeks of age=1 week after 2<sup>nd</sup> vaccination in 2 dose groups; 15.5 week of age=day of challenge; 18.5 week of age=21 days post challenge). \* indicates a significant difference between one and two dose vaccine administration.