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Research Article

Efficacy of Porcine Circovirus Type 2a and 2d Based Vaccines Following PCV2 Challenge

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ABSTRACT

This study evaluated the efficacy of PCV2a and PCV2d vaccines against a PCV2 challenge. Three-week-old, cesarean-derived, colostrum-deprived pigs were blocked by litter and randomized to treatment group. Pigs received a single 2 mL intramuscular dose of either placebo (PLAC, n=50), PCV2a vaccine (PCV2aV, n=25), or PCV2d vaccine (PCV2dV, n=25) on D0 and were challenged with a PCV2d isolate on D28. Prior to challenge, a naturally occurring PCV2a infection was identified. Both vaccines similarly prevented lymphoid tissue lesions, mortality and clinical signs of PCVAD while PLAC pigs were severely affected. Viremia was significantly reduced 7, 14, 21, and 28 days post-challenge and average daily weight gain was significantly increased for both vaccine groups. The prevention of mortality and very minimal occurrence of lymphoid tissue lesions in both vaccine groups, provides clear evidence of the benefit of PCV2 vaccination in the face of a virulent mixed PCV2 challenge.

Keywords: Cross-protection; Vaccine efficacy; PCV2d; PCV2a; PCVAD

Abbreviations: PCV2: Porcine Circovirus Type 2; PCVAD: Porcine Circovirus Associated Disease; KLH ICFA: Keyhole Limpet Hemocyanin Emulsified in Incomplete Freund's Adjuvant; IHC: Immunohistochemistry; PRRSV: Porcine Reproductive and Respiratory Syndrome Virus; ADWG: Average Daily Weight Gain; ISU VDL: Iowa State University Veterinary Diagnostic Laboratory

INTRODUCTION

Porcine circovirus type 2 (PCV2) is a small, non-enveloped, circular, single-stranded DNA virus. Based on sequence analysis, there are three major global PCV2 genotypes currently circulating (PCV2a, b, and d) [1]. Retrospective phylogenetic analyses have indicated that prior to 2003, PCV2a was the predominant circulating genotype. During or prior to 2003, there was a global shift in the predominate genotype from PCV2a to PCV2b [2]. Since 2010, PCV2d has been identified worldwide and is becoming the predominate genotype [1,3].

Since the introduction of PCV2 vaccines into the market in 2006, the benefit of vaccination has been clearly demonstrated [4], yet protective immunity for PCV2 is not fully understood [5]. Due to reports of PCV2d identification in vaccinated herds [6], the level of protection provided by PCV2a-based vaccines against PCV2d genotypes has been questioned. Several previous studies have provided evidence that PCV2a vaccines can protect against PCV2b [5,7]. In addition, vaccination has been shown to reduce viremia, increase antibody titers, and increase average daily gain in field studies [8] and under experimental conditions [9] following challenge with PCV2d. Despite these previous reports of heterologous protection, continued evaluation of PCV2 vaccine performance is still warranted, especially in coinfection models with existing variant strains.

Information on the efficacy of 3FLEX® (Boehringer Ingelheim Vetmedica, Inc.) and an experimental inactivated, baculovirus-vectored PCV2d vaccine mixed with Ingelvac PRRS® MLV and Ingelvac MycoFLEX® against a clinically severe mixed PCV2 challenge has not been previously reported. The objective of this

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study was to assess protection of PCV2a and PCV2d vaccines against a virulent PCV2 challenge.

MATERIALS AND METHODS

Experimental design

Three-week-old, cesarean-derived, colostrum-deprived (CDCD) crossbred pigs were blocked by litter and randomized to treatment group. Pigs received a single 2 mL intramuscular dose of either placebo (PLAC, n=50), PCV2a vaccine (PCV2aV, n=25), or PCV2d vaccine (PCV2dV, n=25) on study day 0 (D0). Pigs were challenged with a PCV2d isolate on D28. Based on detection of PCV2a at the time of PCV2d challenge, pigs were naturally exposed to PCV2a following vaccination. KLH ICFA was administered on D24 and D31. Necropsies were conducted at the time of death or study completion (D56). All personnel involved in collecting data or performing laboratory assays were blinded to the allocation of pigs to treatment group.

Animals and Housing

The experimental protocol was approved by the VRI, Inc. Animal Care and Use Committee (Protocol #2017107 / 2017112) in compliance with the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications NO. 8023, revised 1978). Pigs had no detectable quantity of PCV2 DNA or Porcine reproductive and respiratory syndrome virus (PRRSV) RNA by PCR in serum prior to vaccination and were seronegative for PRRSV (PRRS X3 Ab Test, IDEXX Laboratories, Inc.; Westbrook MA) and Mycoplasma hyopneumoniae (M.hyo Ab Test, IDEXX Laboratories, Westbrook, MA). Animal welfare considerations were included in the study design by providing appropriate housing and diet and minimizing handling. Pigs were housed by litter in BSL-2 rooms throughout the study with appropriate pen and feeder space for their age and size. During the challenge phase, PCV2aV and PCV2dV pigs were housed in two separate rooms with PLAC pigs comingled in each room (n=25/room, designated PLACa, PLACd). Pigs were fed a proprietary milk replacer diet and a medicated feed ration. All pigs were treated with Baytril® (Enrofloxacin, Bayer, Shawnee Mission, KS) on D15. Health observations were recorded at least once daily throughout the study. Three PLAC pigs and one PCV2dV pig died prior to challenge (D14 or 15) due to failure to thrive post-weaning and were excluded from analysis. The study design did not include procedures requiring anesthesia. All study personnel were trained and experienced in animal care and humane animal handling.

Vaccines

The PCV2a vaccine, 3FLEX® was used as indicated on the label. The PCV2d vaccine was an experimental inactivated, baculovirus-vectored PCV2d vaccine mixed with Ingelvac PRRS® MLV and Ingelvac MycoFLEX®. The placebo vaccine was 0.1% carbopol saline solution mixed with Ingelvac® PRRS MLV and Ingelvac® MycoFLEX. All vaccines were produced by Boehringer (St. Joseph, MO).

Challenge material

The PCV2d challenge material was isolated at Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL) from tissues collected from an approximately 60 day old pig exhibiting respiratory signs. The material was expanded and passaged on VIDO-RI cells and confirmed as PCV2d by sequencing. Pigs received a total dose of 3.81 log10TCID50; 1.0 mL intramuscularly and 1.0 mL intranasally.

Clinical observations and mortality

Pigs were observed for clinical signs of PCVAD (abnormal respiratory signs, neurologic signs, body condition, and diarrhea) daily from D27 to D56. A pig was defined as clinically affected if it had at least one abnormal clinical sign on at least two days post-challenge. Humane endpoints were established because PCV2 infection can lead to multiple clinical signs which can be debilitating. In addition, unexpected death without clinical signs is known to occur in experimental PCV2d infection. Pigs displaying clinical signs of severe dyspnea, watery diarrhea, emaciation with anorexia, tremors, recumbency, and/or seizures would have qualified for analgesia and/or humane euthanasia via intravenous barbiturate injection: however, no pigs demonstrated these severe clinical signs prior to being found dead. All mortalities were confirmed as PCV2 mortalities by gross lesions and histological lesions consistent with PCVAD.

Histopathology and Immunohistochemistry

Tonsil, tracheobronchial lymph node, iliac lymph node, and mesenteric lymph node were collected from each animal at necropsy. All tissue samples were processed by ISU-VDL in accordance with standard procedures for histology and PCV2 immunohistochemistry (IHC). Each histology and IHC slide was scored by a pathologist blinded to treatment group for the presence and severity of lymphoid depletion and PCV2 colonization as previously described [9].

Viremia

Venous blood samples were collected from all pigs prior to study initiation (D -1), prior to challenge on D28, and 7, 14, 21, and 28 days post-challenge. All post-challenge samples were tested by qPCR at ISU-VDL to quantify the amount of PCV2 DNA present. Results are expressed as log10GE/mL.

Average Daily Weight Gain (ADWG)

Body weights were collected on D0, D28, and at study removal; ADWG was summarized by treatment group.

Statistics Methods

Data analysis was conducted using SAS version 9.4 (SAS, Cary, North Carolina/USA, SAS Institute, Inc.). Analyses were conducted by room with a p-value<0.05 indicated statistical significance. Mortality and clinical observation rates were compared between groups using Fisher's Exact Test. Challenge phase viremia and weight data were analyzed with a linear mixed

model utilizing group, study day and group by study day as fixed effects and random effect litter. Contrasts conducted include ADWG and daily comparisons between groups (viremia). To account for severity of lesions in analysis, the mean depletion score for all tissues and the mean IHC score for all tissues were calculated for each pig. These mean scores were summed to create a composite lymphoid tissue lesion score similar to previous publications [9,10]. Lymphoid tissue lesion scores were compared between groups using the Generalized Wilcoxon Test, stratifying on litter.

RESULTS

Mortality and clinical observations

Mortality due to PCV2 was 48% in PLACa (p=0.0001) and 33% PLACd (p=0.0039). No vaccinated pigs died post-challenge. Necropsy of PCV2 mortalities revealed gastric ulcers and lymphadenopathy consistent with PCVAD. Diagnostic testing for additional infectious agents beyond PCV2 was not pursued.

No vaccinated pigs were clinically affected while 22% of PLACa pigs (p=0.0197) and 50% of PLACd pigs (p=0.0001) demonstrated clinical signs.

Lymphoid tissue lesions

Both vaccinated groups were protected from lymphoid tissue lesions, while the PLAC pigs were severely affected. The severity of lesions was similarly reduced in both vaccinated groups compared to placebo controls (Figure 1), as demonstrated by group median lymphoid tissue lesion score of 4.75 for PLACa compared to 0.00 for PCV2aV (p<0.0001) and 5.50 for PLACd compared to 0.00 for PCV2dV (p<0.0001).

Viremia

Viremia was significantly reduced in both PCV2aV and PCV2dV pigs on 7, 14, 21, and 28 days post-challenge compared to PLAC pigs. (Table 1). (All p< 0.0001.)

Average daily weight gain

Both vaccinate groups had similar significant increases (p<0.0001) in ADWG at the end of the challenge period compared to PLAC pigs (0.19 kg/day and 0.20 kg/day respectively) (Table 2).

Figure 1

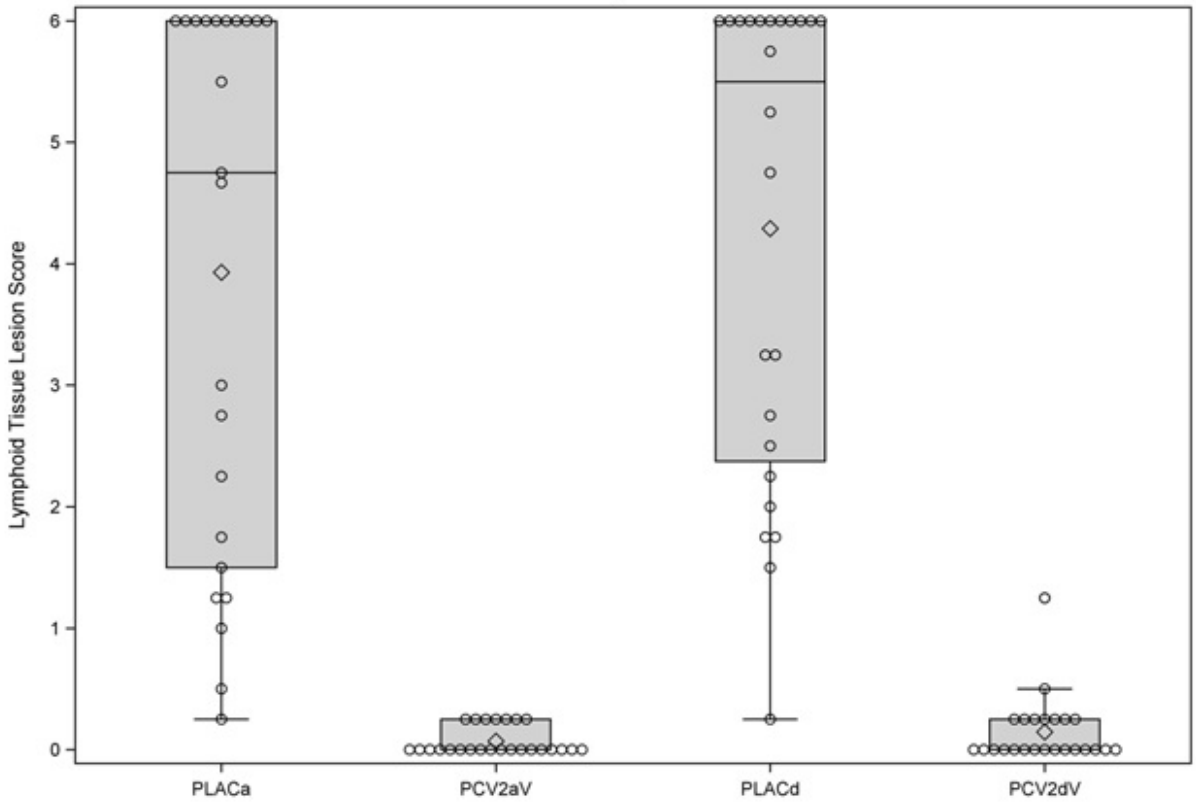

Figure 1: Box and whisker plot of lymphoid tissue lesion score by group. Mean and median represented by the diamond and horizontal line respectively.

Table 1: Least square mean magnitude of viremia (log10 GE/mL) by group and day.

PCV2aV	PCV2dV

Day Post-Challenge	Difference Estimate*	Std Error	95% CI	Difference Estimate*	Std Error	95% CI
7	2.975	0.405	2.158, 3.793	4.33	0.229	3.866, 4.794
14	4.115	0.263	3.583, 4.646	5.451	0.205	5.039, 5.863
21	4.116	0.369	3.371, 4.862	4.599	0.305	3.979, 5.220
28	4.108	0.39	3.320, 4.896	4.58	0.343	3.882, 5.278

Table 2: Least squares estimate of average daily weight gain by group.

Group	Average Daily Weight Gain (kg)	95% CI	Average Daily Weight Gain Difference* (95% CI)
PLAC	0.44	0.37, 0.51	0.19 (0.11, 0.26)
PCV2aV	0.63	0.56, 0.69	
PLAC	0.46	0.39, 0.53	0.20 (0.12, 0.29)
PCV2dV	0.66	0.61, 0.71	

* Vaccinate minus Placebo

DISCUSSION AND CONCLUSION

The observed increase of severe clinical cases following global shifts in the predominate genotype [2] has led to discussion over the pathogenicity of PCV2d, the ability of PCV2a-based vaccines to provide protection against emerging strains, and the role of vaccines in the genotype shifts. While it remains unclear why global genotype shifts of PCV2 have occurred, it is intriguing that the first global shift in genotype occurred prior to the introduction of PCV2a vaccines [11]. In addition, PCV2a vaccines remain one of the most highly utilized pig vaccines globally with multiple studies demonstrating PCV2a-based vaccine protection against PCV2b and PCV2d [7-10,12,13]. This study provides further evidence that homology of vaccine and challenge strains are not essential for protection: both PCV2a and PCV2d vaccines provided similar protection against a clinically severe mixed PCV2a/PCV2d challenge.

While the extrapolation of results from experimental studies to field situations is not straightforward, the severity of the mixed PCV2a/PCV2d infection (evidenced by greater than 30% PLAC mortality) allows this experimental study to more closely represent field situations than other experimental study designs, making it unique. The authors acknowledge that the contribution of each PCV2 challenge virus to the observed severity of infection cannot be determined. However, the result of this study clearly established that vaccination with the PCV2a-based vaccine was efficacious against virulent challenge including PCV2d and provides protection similar to that of the PCV2d-based vaccine.

AUTHOR DISCLOSURE STATEMENT

This study was funded by Boehringer Ingelheim Animal Health USA Inc.

CONTRIBUTORS

Rachel Friedrich and Wesley Johnson were the clinical leaders for the study. Brian Fergen reviewed the study protocol, designed the studies, and analysed the data. Luis Hernandez generated the prototype vaccines used in the study. Rachel Friedrich and Abby Patterson were involved with interpreting the study data and production of the study report. Joseph Hermann and Bernd Grosse Liesner reviewed the study protocol and design, and were involved in interpreting the study data. All authors reviewed the manuscript and approved it for submission.

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