

REPORT ON THE IMMUNOGENICITY OF SUVAXYN® PCV2 ONE DOSE

Fort Dodge Animal Health's Single-Dose Porcine Circovirus Vaccine Type 1-Type 2 Chimera (Killed Virus)

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KEY POINTS

- Vaccinating healthy, commercial 4-week-old pigs with Suvaxyn PCV2 One Dose provided significant efficacy compared with unvaccinated controls.
- Compared to controls, the vaccinated group had:
 - A significantly lower percentage of viremic pigs;
 - A significantly lower percentage of pigs with moderate to severe lymphoid depletion;
 - A significantly lower percentage of pigs with moderate to severe histiocytic replacement; and
 - A significantly lower percentage of pigs with medium to high levels of immunohistochemistry (IHC) staining.

EXPERIMENTAL DESIGN AND PROTOCOL

Forty-four healthy, mixed-breed, 4-week-old male and female commercial pigs were obtained from a single-source herd. The pigs were housed communally in isolation facilities at Fort Dodge Animal Health facilities in Fort Dodge, Iowa.

The pigs were selected based on serological results for PCV2 antibody titer, then randomized into groups in 4 rooms.

GROUP	TREATMENT	NO. PIGS
Vaccinates	Suvaxyn PCV2 One Dose (2 mL, IM)	22
Controls	Placebo injection (2 mL, IM)	21

The study was blinded for observations, sampling, necropsies and laboratory sample evaluations. All samples and records were labeled using eartag numbers not associated with group identification.

Pigs were tested on Day 0 post-vaccination to ensure no PCV2 infection was present in test animals prior to vaccination. The results indicated the pigs' lack of exposure to environmental PCV2 infection at the time of vaccination.

Vaccinates and controls were commingled throughout the study. Just prior to vaccination on Day 0, 1 control pig was removed from the study for reasons not related to the study (swollen joint), leaving a total of 22 vaccinates and 21 controls.

Six weeks (42 days) post-vaccination, each pig was challenged with a wild-type pathogenic PCV2 (U.S. strain).

Postchallenge clinical observations were recorded, including rectal temperature, poor appetite, lethargy, depression, sneezing, coughing, nasal or ocular discharge, and difficulty breathing. Nasal swabs were collected on Day 0 post-vaccination. Serum samples for serology were taken on Days 0, 14, 21, 28 and 35 post-vaccination and on Days -1, 7, 14 and 21/22 post-challenge. Serum samples for PCV2 viremia testing were taken on Days -1, 3, 7, 11, 14 and 21/22 post-challenge. All pigs were necropsied at Day 21/22 post-challenge. Tissue samples from 3 lymph nodes (iliac, inguinal and tracheobronchial), the spleen and tonsils were examined for histopathology and PCV2 immunohistochemistry (IHC).

A single occurrence of PCV2 viremia at any time following challenge resulted in classifying that pig as positive for PCV2 viremia.

Histopathology and IHC lesions were evaluated by a pathologist at Iowa State University. Each pig was scored from 0 (normal/no lesions) to 3 (severe lesions) to gauge lymphocyte depletion in the spleen, tonsils and lymph nodes.

RESULTS AND DISCUSSION

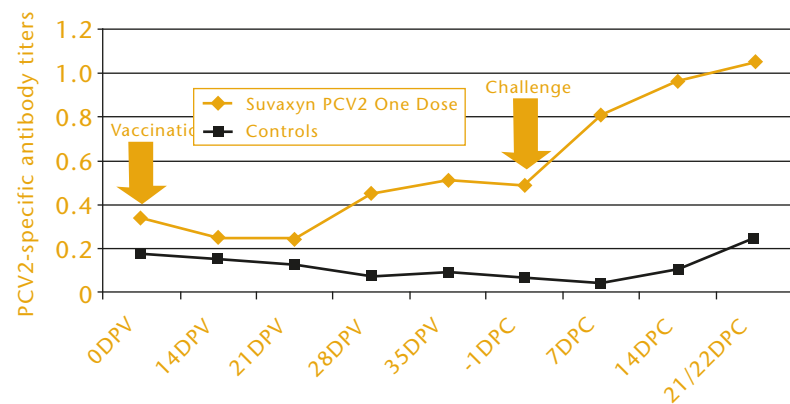
Post-challenge Clinical Observations

Daily rectal temperatures were measured from Days -2 to 21/22 post-challenge. No significant differences were found between vaccinated and control pigs.

Only 1 pig in the control group showed evidence of clinical signs (poor appetite, lethargy, depression, sneezing, coughing, nasal or ocular discharge, or difficulty breathing) during the observation period (Days -2 to 21/22 days post-challenge). The affected control pig began to show signs of paddling on Day 15 post-challenge and was removed from the trial that day. Upon testing, the pig had high levels of PCV2 viremia, severe lymphoid depletion, histiocytic replacement and high levels of IHC staining for PCV2 antigen in tissues. A tentative diagnosis of porcine dermatitis and nephropathy syndrome (PDNS) was made.

No pigs in the vaccinated group exhibited any clinical signs throughout the observation period.

FIGURE 1: SEROCONVERSION TO PCV2 IN SERA OF VACCINATED AND CONTROL PIGS



Differences post-vaccination (DPV) and post-challenge (DPC) are statistically significant ($p < 0.0001$).

Serology

The results of PCV2 testing are shown in Figure 1. Significant seroconversion to PCV2-specific antibody ($p < 0.0001$) was observed in the vaccinated group following vaccination, while all controls remained negative prior to challenge. PCV2-specific antibody titers of vaccinates were further boosted by PCV2 challenge and remained significantly different from controls ($p < 0.0001$) starting at Day 7 post-challenge.

PCV2 Viremia

Data regarding detection of PCV2 viremia in sera of vaccinates and controls are shown in Figure 2.

On Day-1 (prior to challenge), all pigs were demonstrated as negative for PCV2 viremia in all serum samples. PCV2 viremia-negative results on Day-1 indicated that pigs had not been exposed to environmental PCV2 infection.

PCV2 viremia testing results demonstrated that 4/22 vaccinates (18%) had a single occurrence of positive virus detection, while 21/21 (100%) of controls were reported with single and/or multiple occurrence(s) of positive virus detection. This finding proved statistically significant prevention of PCV2 viremia ($p < 0.0001$), with vaccine efficacy for prevention of viremia of 82% (95% CI 60, 95).

It must be noted that of the 4 positive vaccinates, 2 were identified as “marginally positive or inconclusive” with a single occurrence and probably were the result of accidental cross-contamination between the samples at the time of bleeding. However, we have included them as positive for PCV2 viremia.

MICROSCOPIC LESION EVALUATION

Lymphoid Depletion

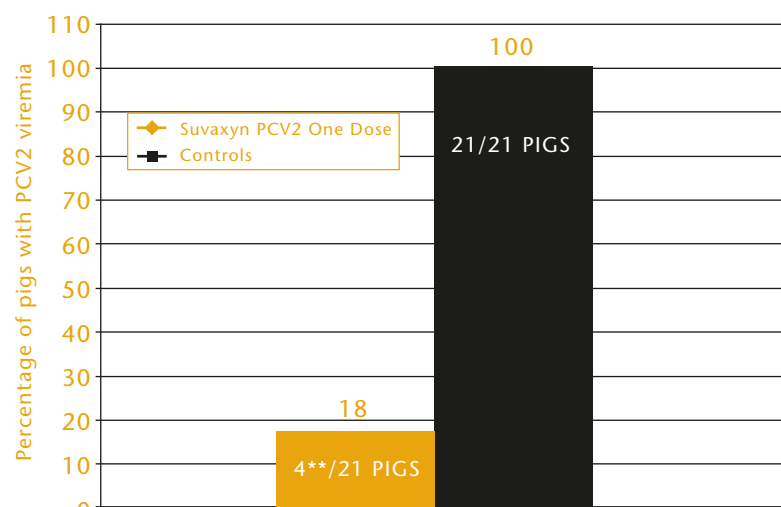
Tissues of lymph nodes, spleen and tonsil were examined microscopically for lymphoid depletion and a cumulative histologic score was assigned. Among vaccinates, normal or mild lymphoid depletion was observed in most tissues, with average total scores of 3.8 (out of a possible maximum score of 15). In the placebo-vaccinated control group, moderate to severe lymphoid depletion was observed in most tissues, with an average total score of 7.3.

Microscopic lymphoid depletion results are shown in Figure 3. The results of the histopathology evaluation of 3 lymph nodes, spleen and tonsil demonstrated that 5/22 vaccinates (22.7%) showed moderate to severe microscopic lymphoid depletion, while 17/21 controls (81.0%) showed moderate to severe microscopic lymphoid depletion. Severity scores for lymphoid depletion were significantly lower in vaccinated pigs compared to control pigs ($p = 0.0006$). The preventable fraction was 71.9% (95% CI 40.7, 89.9) for vaccinates compared to controls.

Histiocytic Replacement

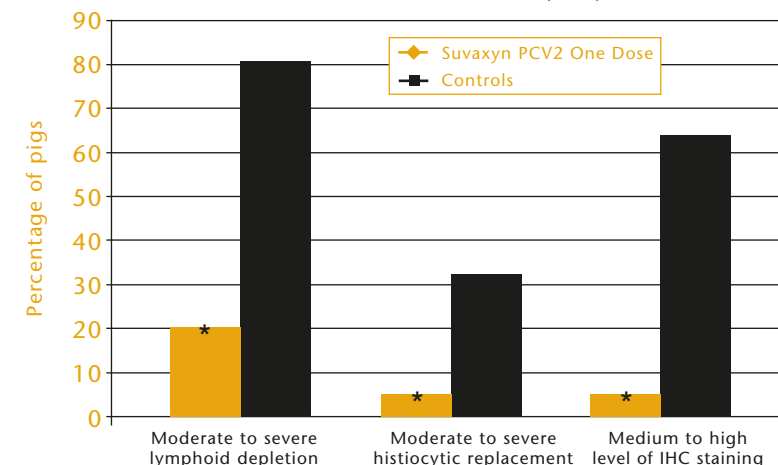
Tissues of lymph nodes, spleen and tonsil were examined microscopically for histiocytic-to-granulomatous inflammation with replacement follicles. In vaccinated pigs, normal or mild histiocytic replacement was observed in most tissues, with an average total score of 1.9. In control pigs,

FIGURE 2: DETECTION OF PCV2 VIREMIA IN SERA



Preventable fraction of PCV2 viremia: 82% (95% CI 60, 95) for vaccinate group. **Includes 2 pigs with inconclusive results at 21/22 days post-challenge (suspected sample cross-contamination).

FIGURE 3: HISTOPATHOLOGICAL LESIONS AND IMMUNOHISTOCHEMISTRY (IHC) STAINING



*Differences between groups are statistically significant ($p < 0.05$).

moderate to severe lymphoid depletion was observed in most tissues, with an average total score of 5.3.

Microscopic histiocytic replacement results are shown in Figure 3. The results of the histopathology evaluation of 3 lymph nodes, spleen and tonsil demonstrated that 1/22 vaccinates (4.5%) showed at least 1 tissue with moderate to severe histiocytic replacement, while 7/21 controls (33.3%) showed at least 1 tissue with moderate to severe histiocytic replacement. Severity scores for histiocytic replacement were significantly lower in vaccinated pigs compared to control pigs ($p = 0.0317$). The preventable fraction for lymphoid depletion was 86%.

Immunohistochemistry

The results of detection of amount of PCV2 antigen by IHC staining in lymph nodes, spleen and tonsil are summarized in Figure 3. Among vaccinates, negative or low-level IHC staining was observed in most tissues, with an average total score of 1.5. Among controls, medium to high levels of IHC staining was observed in tissues, with an average total score of 7.6.

The cumulative IHC staining score for 3 lymph nodes, spleen and tonsil indicated that 1/22 (4.5%) vaccinates presented medium to high levels of IHC staining of PCV2 antigen, while 14/21 (66.7%) of controls showed medium to high levels of IHC staining. There were statistically significant differences in the amount of PCV2 antigen in lymphoid tissues between vaccinates and controls ($p < 0.0003$). The preventable fraction for IHC staining was 93%.

CONCLUSION

Suvaxyn PCV2 One Dose effectively prevents PCV2 viremia and reduces PCV2-associated lymphoid depletion compared to unvaccinated controls.