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Technical Update

Fostera™ PCV MH: Efficacy of Single-Dose Vaccination in Swine Subsequently Challenged with PCV2 and/or *Mycoplasma hyopneumoniae*

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Mycoplasmal (or enzootic) pneumonia is a major cause of economic losses to the swine industry due to reduced growth rate, poor feed efficiency, and extended time to market. *Mycoplasma hyopneumoniae* (*M. hyo*) bacteria primarily infect and damage epithelial cilia and cells on the surface of the trachea, bronchi, and bronchioles, resulting in gross lung lesions characterized by purple/gray areas of consolidation. As a major contributor in porcine respiratory disease complex (PRDC), *M. hyo* is often identified in mixed infections with viral pathogens such as porcine respiratory and reproductive syndrome virus (PRRSV) and porcine circovirus Type 2 (PCV2).

Regarding the latter of these important viral pathogens, porcine circovirus-associated disease (PCVAD) encompasses several distinct clinical syndromes that can include respiratory disease, enteritis, reproductive failure, porcine dermatitis and nephropathy syndrome, and systemic infection typified by unthriftiness and wasting. Viremia and protracted viral shedding are major features of PCVAD, with subclinical infection characterized by lymphocytic depletion in lymph nodes often accompanied by histiocytic infiltration. PCVAD has become one of the most economically important swine diseases

due to progressive weight loss, high rates of mortality, and other clinical impacts.

Because unmitigated *M. hyo* and PCV2 can pose major financial threats to virtually any pork production unit, vaccination against these pathogens has become a critical and fundamental component of most health programs. Herd protection has required separate vaccinations for each pathogen, but the prospect of combining

Combining PCV and *M. hyo* antigens in one bottle for one-shot dosing helps reduce labor and animal stress.

these antigens into a single vaccine would help reduce the labor and animal stress involved in vaccination protocols. Development of a combination vaccine involves multiple technical factors that can impact efficacy, safety, and ease-of-use (e.g., field mixing, multiple doses, etc.), and some products have emerged. Zoetis has devoted significant research and develop-

ment to enable an innovative manufacturing process that yields a highly effective and user-friendly combination PCV/*M. hyo* vaccine.

Fostera™ PCV MH

Fostera™ PCV MH, from Zoetis, is the first and only combination, **one-bottle** vaccine that helps to protect swine from **both** PCVAD and enzootic mycoplasma pneumonia after administration of a

single dose (other combination vaccines require mixing in the field or multiple doses). Foster PCV MH was uniquely developed to ensure proper antigen/adjuvant balance, helping deliver safe and effective protection against both PCVAD and enzootic mycoplasma pneumonia.

A single 2-mL intramuscular (IM) dose of Foster PCV MH is licensed for the vaccination of healthy pigs at 3 weeks of age or older as an aid in preventing viremia, lymphoid depletion, and colonization of lymphoid tissue caused by PCV2; and as an aid in reducing PCV2 virus shedding and enzootic pneumonia caused by *M. hyo*. Thus, Foster PCV MH helps provide disease protection afforded by other monovalent Zoetis products: protection from *M. hyo* similar to RespiSure-ONE,[®] and protection against PCVAD similar to Foster PCV, all in a convenient one-bottle, one-dose formulation.

As part of the body of research conducted for licensure of Foster PCV MH, several challenge studies assessed the efficacy of the PCV and *M. hyo* components of Foster PCV MH.¹⁻³

PCV – Experiment Design

A 6-week study investigating the efficacy of the PCV component of Foster PCV MH involved 48 weaned, clinically healthy cross-bred piglets averaging 3 weeks of age (19-23 days) that were PCV2 seronegative and viremia free (data presented are a subset of treatments from a larger study).¹ On study day 0, piglets were randomized within litter for assignment to treatment groups (24/group) and administered a single dose of either of the following vaccines:

- Control: no PCV antigens, only *M. hyo* antigens;
- Foster PCV MH: both PCV antigens and *M. hyo* antigens.

Vaccine treatments were administered as a 2-mL IM injection in the right neck, thus initiating the 3-week ‘vaccination phase’ of the study (3 to 6 weeks of age; Figure 1).

Three weeks after vaccination, the ‘challenge phase’ of the study (6 to 9 weeks of age) involved dosing each pig on study day 21 with 3 mL of an inoculum containing PCV2 via the IM (1 mL) and intranasal (IN) routes (2 mL).

Pigs were observed daily for general health and clinical signs of respiratory distress, lethargy, etc. Feed and water were offered ad libitum, and 1 control pig was euthanized due to chronic arthritis. All pigs were necropsied 3 weeks after challenge for tissue collection/analysis. Prior to necropsy, serum samples and challenge-phase fecal swabs were collected weekly and analyzed for PCV2 using a quantitative polymerase chain reaction (qPCR) for detection of PCV2 viremia and fecal shedding, and an enzyme-linked immunosorbent assay (PCV2 ELISA) for detection of circulating antibodies. Tissue samples collected at necropsy (tracheobronchial, mesenteric, inguinal lymph nodes, tonsil) were evaluated for PCV histopathology (histiocytic replacement and lymphoid depletion) and immunohistochemistry (IHC; for detection of PCV in tissue). Clinical assessments and data collections/evaluations were conducted by personnel without knowledge of treatment group assignments.

Data were statistically analyzed by appropriate methods using the pig as the experimental unit. Least squares (LS) means (back-transformed where appropriate) and 95% confidence intervals (CI) or ranges were calculated for each treatment, with differences assessed at the 5% level of significance ($P \leq 0.05$). The impact of vaccination

Figure 1. PCV study design and time flow.

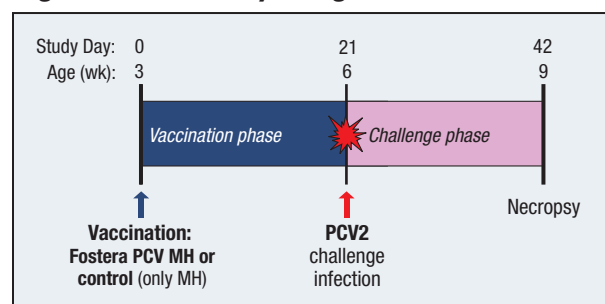


Figure 2. PCV2 viremia (geometric LS means, qPCR).

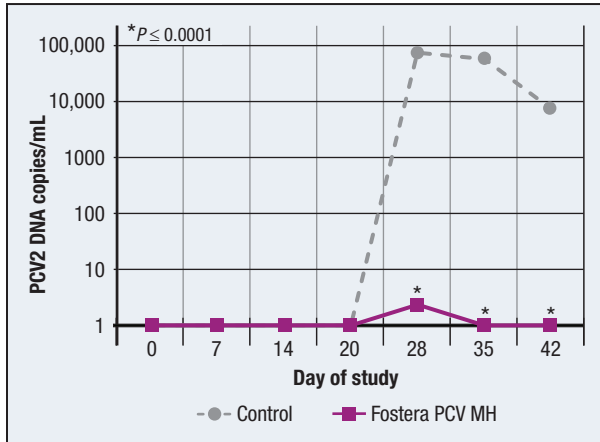
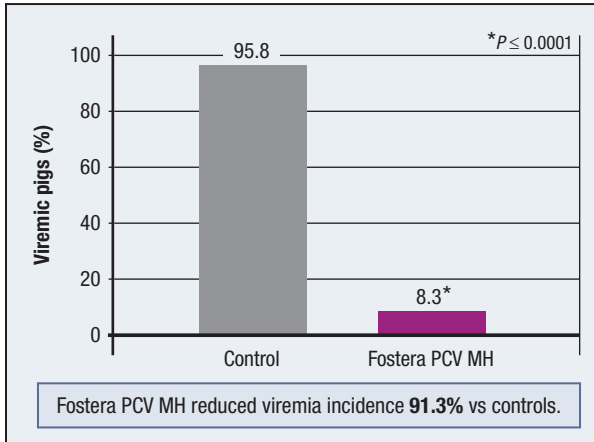


Figure 3. Pigs PCV2 viremic at any time during study.



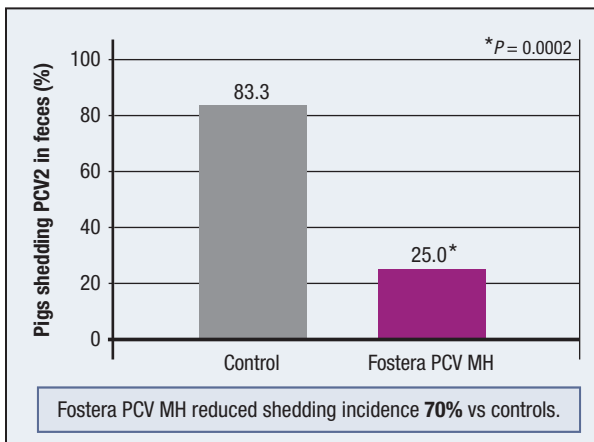
on PCV2 viremia was the primary variable of interest, with secondary outcomes including PCV2 fecal shedding, lymphoid depletion, histiocytic replacement, and PCV2 colonization based on IHC. The study was conducted in accordance with the Zoetis Institutional Animal Care and Use Committee.

PCV – Results

Viremia: Figure 2 summarizes PCV2 viremia (DNA copies) as determined by qPCR analysis. No viremia was detected before challenge but viremia quickly developed in control pigs after challenge. In contrast, pigs vaccinated with Fostera PCV MH experienced little or no viremia during the 3 weeks following challenge ($P \leq 0.0001$ vs controls).

The percentage of pigs positive at any time for PCV2 viremia was significantly reduced ($P \leq 0.0001$) in pigs vaccinated with Fostera PCV MH compared to controls (Figure 3). Throughout the study, over 91% of pigs in the Fostera PCV MH group remained negative for PCV2 viremia while almost all control pigs (95.8%) were positive at some point. Thus, Fostera PCV MH vaccination significantly reduced the incidence of viremic pigs by 91.3% ($P \leq 0.0001$) compared to controls. The PCV component of Fostera PCV MH was deemed effective as an aid in preventing PCV2 viremia.

Figure 4. Pigs shedding PCV2 in feces at any time during study.



Fecal Shedding: Analysis of fecal swabs collected post-challenge revealed that 83.3% of control pigs shed PCV2 in feces compared to only 25% of Fostera PCV MH-vaccinated pigs (Figure 4). PCV shedding was significantly reduced by 70.0% ($P = 0.0002$) in Fostera PCV MH vaccinates compared to controls. The PCV component of Fostera PCV MH was clearly effective as an aid in reducing fecal shedding of PCV2.



Figure 5. PCV2 antibody titers (LS means of S/P ratios, ELISA).

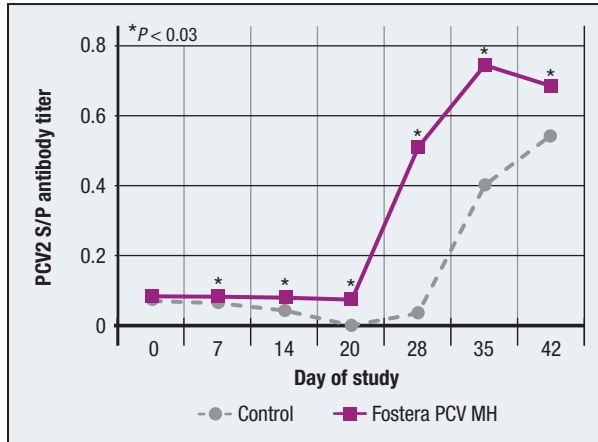
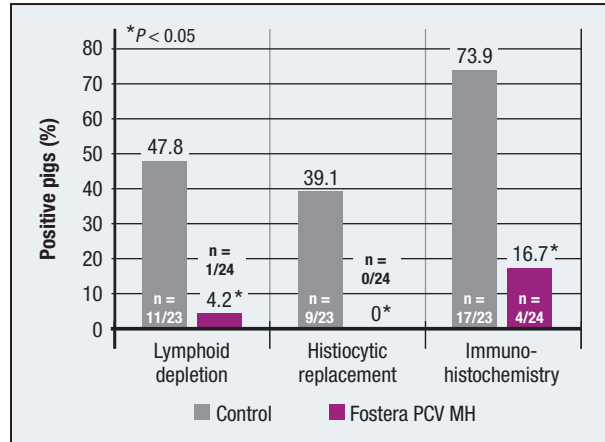


Figure 6. PCV2 tissue analyses (microscopic lesions and IHC scores).



Serology: While all pigs were PCV2 seronegative prior to vaccination, control pigs remained seronegative after vaccination and prior to challenge. Pre-challenge PCV2 antibody titers of pigs vaccinated with Fostera PCV MH did not substantively decay and were maintained at levels statistically elevated ($P < 0.03$) relative to controls (Figure 5). Fostera PCV MH vaccinates generated post-challenge antibody titers significantly higher ($P \leq 0.0056$) than controls on all sample dates. These serologic outcomes indicate that Fostera PCV MH induced active antibody responses to PCV2 after vaccination, and anamnestic responses to PCV2 challenge.

Lymphoid Lesions and Colonization: Figure 6 shows the percent and number of pigs in each treatment group showing lymphoid depletion, histiocytic replacement, and/or lymphoid tissues positive for PCV2 (by IHC). Fostera PCV MH vaccinates demonstrated significant ($P < 0.05$) improvements relative to controls for all parameters:

- lymphoid depletion reduced 91.2%;
- histiocytic replacement reduced 100%;
- amount of PCV2 antigen detected in tissues reduced 77.4%.

The PCV component of Fostera PCV MH was deemed effective as an aid in preventing lymphoid depletion and colonization of lymphoid tissue caused by PCV2.

PCV – Conclusions

Study results confirm that the PCV component of Fostera PCV MH:

- aids in the prevention of post-challenge PCV2 viremia;
- aids in the reduction of PCV2 fecal shedding;
- generates active antibody responses to PCV2 following vaccination, and anamnestic responses to PCV2 challenge;
- aids in the prevention of microscopic PCV2 lesions (lymphoid depletion);
- aids in the prevention of PCV2 colonization of lymphoid tissues.

A single dose of Fostera PCV MH helps provide effective protection against PCV2, and the presence of a *M. hyo* component in the formulation does not appear to interfere with PCV2 efficacy.

M. hyo – Experiment Design

A 7-week study investigating the efficacy of the *M. hyo* component of Foster PCV MH involved 60 weaned, clinically healthy cross-bred piglets approximately 3 weeks of age and seronegative for *M. hyo* (data presented are a subset of treatments from a larger study).² On study day 0, piglets were randomized within litter for assignment to treatment groups (30/group) and administered a single dose of either of the following vaccines:

- Control: no *M. hyo* antigens, only PCV antigens;
- Foster PCV MH: both *M. hyo* and PCV antigens.

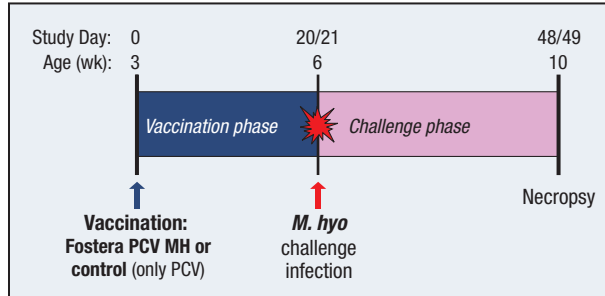
Vaccine treatments were administered as a 2-mL IM injection in the left neck, thus initiating the 3-week ‘vaccination phase’ of the study (3 to 6 weeks of age; Figure 7).

Three weeks after vaccination, the ‘challenge phase’ of the study (6 to 10 weeks of age) involved intratracheal dosing of each pig with 10 mL of a live, virulent *M. hyo* lung homogenate on 2 consecutive days.

Pigs were observed daily for general health and clinical signs of respiratory distress, lethargy, etc. Feed and water were offered ad libitum. All pigs were necropsied 4 weeks after challenge (study days 48/49) for scoring of lung lesions and collection of lung swabs and tissue samples for detection of *M. hyo* by qPCR and IHC. Serum samples were collected for serological analysis (*M. hyo* ELISA, IDEXX S/P ratio) prior to vaccination, prior to challenge, and at necropsy. Clinical assessments and data collections/evaluations were conducted by personnel without knowledge of treatment group assignments.

Data were analyzed in a manner similar to the previous study. The impact of vaccination on the severity of post-challenge *M. hyo* lung lesions was the primary variable of interest. The study was conducted in accordance with the Zoetis Institutional Animal Care and Use Committee.

Figure 7. *M. hyo* study design and time flow.



M. hyo – Results

The percent of lungs with *M. hyo* lesions for the two treatment groups are summarized in Figure 8. The severity of lung lesions was significantly reduced ($P \leq 0.0001$) in pigs vaccinated with Foster PCV MH (1.66%) compared to controls (7.01%), representing a relative risk reduction of 76.3% compared to controls.

Serological outcomes (Figure 9) revealed that all pigs were negative for *M. hyo* antibody prior to vaccination (day 0). However, Foster PCV MH vaccinates demonstrated an anamnestic response after *M. hyo* challenge (day 48/49, $P \leq 0.0001$ vs controls).

Figure 8. Percent of lungs with *M. hyo* lesions (LS means).

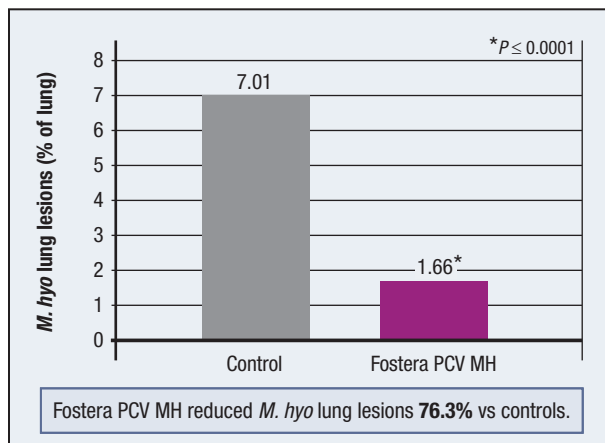
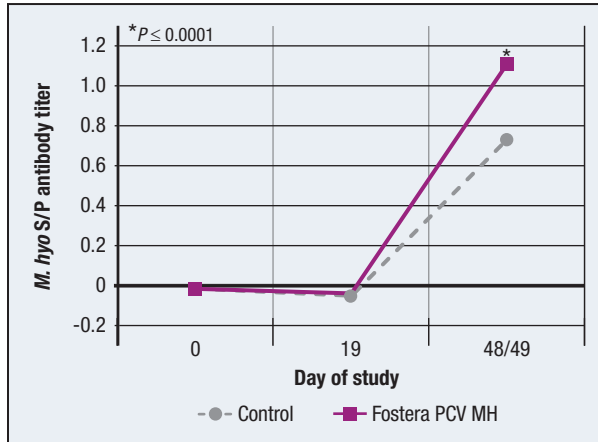


Figure 9. *M. hyo* antibody titers (geometric LS means of S/P ratios, IDEXX ELISA).



***M. hyo* – Conclusions**

Study results confirmed that the *M. hyo* component of Foster PCV MH:

- significantly reduced the severity of *M. hyo* lung lesions;
- generated a significant anamnestic response to *M. hyo* challenge.

A single dose of Foster PCV MH helps provide effective protection against *M. hyo*, and the presence of a PCV component in the formulation does not appear to interfere with *M. hyo* efficacy.

Dual-Challenge Comparative Trial – Experiment Design

A study compared vaccination with Foster PCV MH and other vaccines/regimens in their ability to limit PCV2 viremia and sustain favorable growth performance in swine challenged with **both** *M. hyo* and virulent PCV2b.³ The study involved 334 baby piglets (barrows and gilts) negative for PRRS and *M. hyo* that were farrowed at a high-health herd. At processing (3 to 7 days of age) piglets were ear-tagged, weighed, and randomly assigned to 4 treatment groups by blocks based on body weight and gender. The

‘vaccination phase’ of the study (0 to 6 weeks of age) involved administration of the following products:

- Ingelvac® CircoFLEX-MycoFLEX® (1 dose mixed into a single bottle before vaccination), Boehringer Ingelheim Vetmedica;
- RespiSure-ONE® (1 dose) and Foster PCV (1 dose), Zoetis;
- Foster PCV MH (1 dose), Zoetis.

With the inclusion of a saline-injected control group, 4 treatment groups were thus evaluated as summarized in Table 1, with all IM vaccinations administered according to label directions. Pigs were weaned at 3 weeks of age and moved to a wean-to-finish barn.

The ‘challenge phase’ of the study (8 to 10 weeks of age) involved 2 separate events:

- ***M. hyo* challenge:** at approximately 8 weeks of age (16 days after the vaccination phase), each pig was challenged intratracheally;
- **PCV2b challenge:** at approximately 10 weeks of age, each pig was challenged via both the IM and intranasal (IN) routes.

Pigs were observed daily for general health and clinical signs of respiratory distress, lethargy, wasting, etc. Individual serum samples were collected at approximately 1, 3, 6, 8, 10, 14, 17, and 21 weeks of age and analyzed by PCR for detection of PCV2 viremia as well as serological assessments and screenings (e.g., to confirm absence of PRRSV or SIV co-infections). To allow computation of average daily gain (ADG), pigs were weighed at multiple time points throughout the study until marketing at approximately 260 lb (151 days of age, ~22 weeks).

Data were statistically analyzed by appropriate methods using each pig as an experimental unit, with statistical significance recognized at $P \leq 0.05$. Viremia and ADG results were analyzed as least squares (LS) means. Clinical observations, data collections, and assays were conducted by personnel without knowledge of treatment group assignments. The study was conducted in

Table 1 – Study design, vaccination phase.

Treatment group	No. pigs	Age of pigs	
		<1 week (processing)	3 weeks (1 day pre-weaning)
Control (saline)	84	—	2 mL
Ingelvac CircoFLEX-MycoFLEX	80	—	2 mL
RespiSure-ONE and Fostera PCV	85	2 mL RespiSure-ONE	2 mL Fostera PCV
Fostera PCV MH	85	—	2 mL

accordance with the Zoetis Institutional Animal Care and Use Committee.

Dual-Challenge Comparative – Results

The virulence of the PCV2 challenge was evidenced by 3 deaths in the control group due to PCVAD (PCV₂-positive by PCR and IHC, plus lymphoid depletion and weight loss). No co-infections with PRRSV were detected, but some pigs were positive for SIV (NP ELISA) during the last month of the study. The *M. hyo* challenge (serologically confirmed) appeared to be successfully moderated by *M. hyo* vaccination which was based on reduction of lung lesions in vaccinates (data not shown).

The intensity of PCV2 viremia experienced by the various treatment groups is summarized in Figure 10. On each post-challenge sample day,

all vaccinated groups demonstrated viremia that was several orders of magnitude lower than the control group ($P \leq 0.05$). These outcomes suggest that each PCV2 vaccine helped reduce PCV2 viremia. The Fostera PCV MH group demonstrated excellent viremia control all the way to market weight, confirming that viremia protection was afforded by the single-dose combination vaccine.

ADG outcomes for the study are summarized in Table 2. During the immediate 9 weeks following the vaccination phase of the study (6 to 15 weeks of age, encompassing 5 weeks of post-challenge performance), all vaccinated groups generated sizeable ADG improvements (7.0%-8.5%) relative to controls ($P \leq 0.05$). No differences in ADG were detected between vaccinated groups for this time period ($P > 0.05$).

Figure 10. PCV2 viremia (geometric means, assessed by PCR).

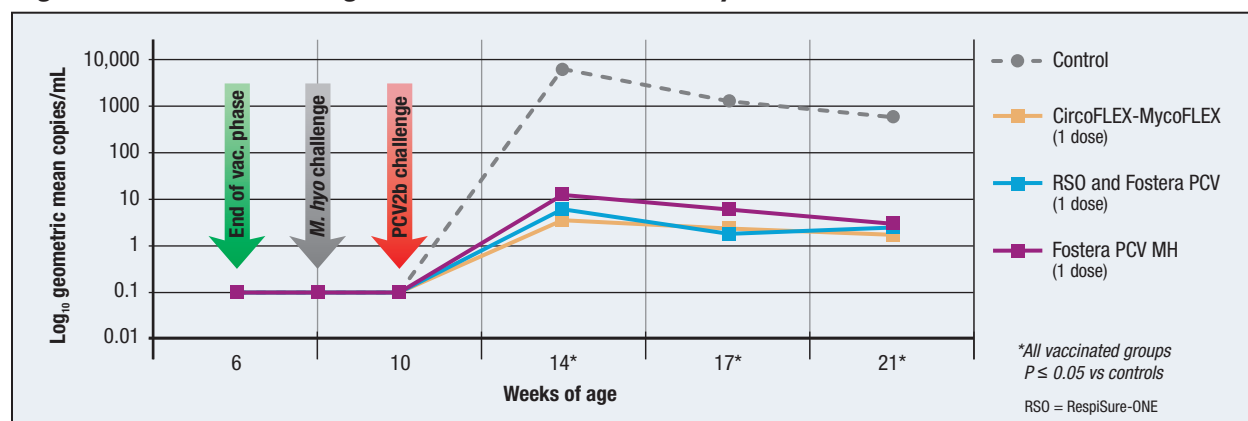


Table 2 – Average daily gain (LS means) from 6 to 15 weeks of age and for entire study.

Treatment	Weeks 6-15	Weeks 1-22
Control	1.29 ^a	1.56 ^a
Ingelvac CircoFLEX-MycoFLEX	1.40 ^b	1.61 ^b
RSO and Fosterer PCV	1.38 ^b	1.61 ^b
Fosterer PCV MH	1.40 ^b	1.63 ^b

^{a,b} Means in columns with different superscripts are significantly different ($P \leq 0.05$). RSO= RespiSure-ONE

Overall ADG results (piglet processing to market, weeks 1 to 22) also revealed significant ($P \leq 0.05$) improvement for vaccinates compared to controls (up to 4.5% for the Fosterer PCV MH group, 1.63 vs 1.56). Again, growth performance outcomes between vaccinated groups were similar ($P > 0.05$). No differences in feed efficiency were detected between any treatment groups over the course of the study ($P > 0.05$; data not shown).

Dual-Challenge Comparative – Conclusions

This dual-challenge study demonstrated that Fosterer PCV MH effectively helped control PCVAD and helped reduce PCV2 viremia, which helped vaccinated pigs sustain favorable growth performance. No significant differences ($P > 0.05$) in viremia or ADG outcomes were detected between the Fosterer PCV MH group and other vaccines/regimens. In addition, the *M. hyo* challenge was successfully controlled by Fosterer PCV MH vaccination.

Fosterer PCV MH is an effective combination vaccine that helps provide excellent control of PCV2 viremia, a critical feature for helping reduce fecal shedding (exposure risk for other pigs) and for sustaining profitable growth performance.

Overall Conclusions

These 3 research studies demonstrated that a single dose of Fosterer PCV MH helps provide effective protection from both PCVAD and mycoplasmal pneumonia. The novel one-bottle/one-dose formulation helps deliver excellent PCV and *M. hyo* efficacy while optimizing convenience for swine managers and reducing handling stress for animals. The innovative, cost-effective vaccine offers the same reliable protection as other Zoetis monovalent *M. hyo* and PCV vaccines, RespiSure-ONE and Fosterer PCV.

References

1. Data on file, Study Report No. B822R-US-12-046, Zoetis Inc.
2. Data on file, Study Report No. B823R-US-13-136, Zoetis Inc.
3. Data on file, Study Report No. 12ORBIOPORK01, Zoetis Inc.

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