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# Technical Update

## ***Efficacy and Safety of Foster<sup>™</sup> PCV MH When Administered Using the Flexible 2-Dose Option***

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Porcine circovirus Type 2 (PCV2) and *Mycoplasma hyopneumoniae* (*M. hyo*) are common swine pathogens capable of imposing significant financial harm to unprotected herds. Most progressive pork producers, therefore, have adopted health programs that include vaccination against these pathogens in an effort to moderate losses due to porcine circovirus-associated disease (PCVAD) and enzootic pneumonia caused by *M. hyo*.

Separate vaccinations for each pathogen have been historically required, but the recent advent of combination vaccines containing both antigens offers to help reduce the labor and animal stress involved in vaccination protocols and optimize convenience for producers. Zoetis has further enhanced these benefits by devoting significant research and development to deliver a highly effective and user-friendly *single-dose* PCV2/*M. hyo* combination vaccine. However, because some veterinarians and producers prefer to employ protocols involving 2 doses instead of a single dose, Zoetis has conducted additional efficacy and safety research to provide flexible dosing options for users of a PCV2/*M. hyo* combination vaccine.

***Foster<sup>™</sup> PCV MH is the only PCV2/*M. hyo* combination vaccine that offers flexible dosing options.***

### ***Foster<sup>™</sup> PCV MH***

Foster<sup>™</sup> 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 either a **single dose** or a **2-dose** regimen (other combination vaccines *require* two doses or mixing in the field). Foster<sup>™</sup> PCV MH was uniquely developed to ensure proper antigen/adjuvant balance, helping deliver safe and effective protection against both PCVAD and enzootic pneumonia.

Foster<sup>™</sup> 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*. The one-bottle combination product can be **flexibly** administered in either of two ways:

- a single 2-mL intramuscular (IM) dose;
- two 1-mL IM doses spaced 2 weeks apart.

Either way, Foster<sup>™</sup> PCV MH helps provide disease protection afforded by other monovalent Zoetis products: protection from *M. hyo* similar to RespiSure-ONE,<sup>®</sup> and protection against PCVAD

similar to Foster PCV, all in a convenient one-bottle formulation.

As part of the body of research conducted for licensure of Foster PCV MH, two challenge studies assessed the efficacy of the PCV and *M. hyo* components of Foster PCV MH when administered using the flexible 2-dose option.<sup>1,2</sup>

### **PCV – Experiment Design**

An 8-week study investigating the efficacy of the PCV component of Foster PCV MH involved 60 weaned, clinically healthy cross-bred piglets averaging 3 weeks of age (18-24 days) that were PCV2 seronegative and viremia free.<sup>1</sup> On study day 0, piglets were randomized within litter for assignment to treatment groups (30/group) and administered 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 1-mL IM injection in the right neck, thus initiating the 5-week ‘vaccination phase’ of the study (3 to 8 weeks of age; Figure 1). A second 1-mL IM vaccination was administered identically 2 weeks later (day 14) in the left neck.

Three weeks after the second vaccination, the ‘challenge phase’ of the study (8 to 11 weeks of age) involved dosing each pig on study day 35 with 3 mL of an inoculum containing PCV2 via the IM (1 mL) and intranasal 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. Three animals (2 controls, 1 vaccinee) were removed from the study due to unrelated health conditions. 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

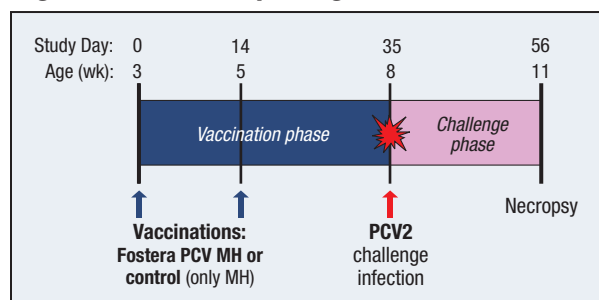
and fecal shedding, and an enzyme-linked immunosorbent assay (PCV2 ELISA) was used for detection of circulating antibodies. Tissue samples collected at necropsy (tracheobronchial, mesenteric, inguinal lymph nodes, tonsil) were evaluated for PCV histopathology (lymphoid depletion and histiocytic replacement) 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 or ranges were calculated for each treatment, with differences assessed at the 5% level of significance ( $P \leq 0.05$ ). The impact of vaccination 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.

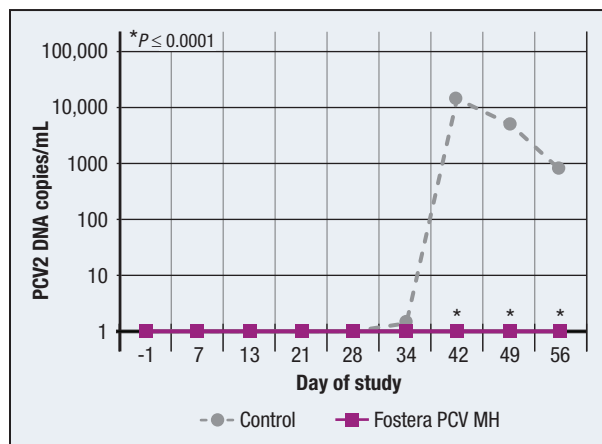
### **PCV – Results**

**Viremia:** Outcomes for PCV2 viremia (DNA copies) as determined by qPCR analysis are summarized in Figure 2. No viremia was detected before challenge (with the exception of 1 control pig on day 34), but viremia quickly developed in control pigs after challenge. In contrast, pigs vaccinated with 2 doses of Foster PCV MH

**Figure 1. PCV study design and time flow.**



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



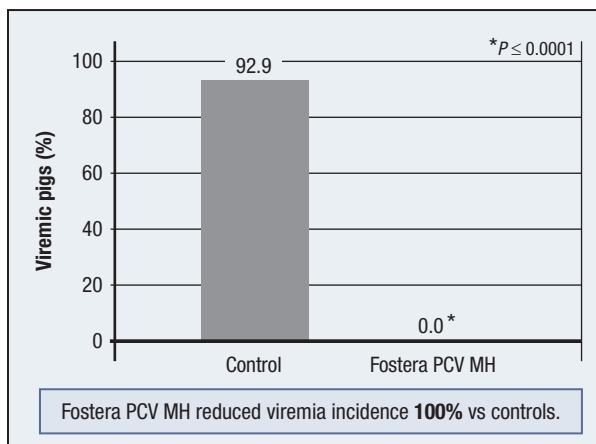
experienced no viremia during the 3 weeks following challenge ( $P \leq 0.0001$  vs controls).

The percentage of pigs positive at any time for PCV2 viremia was significantly reduced ( $P \leq 0.0001$ ) in pigs vaccinated with Foster PCV MH compared to controls (Figure 3). Throughout the study, all pigs in the Foster PCV MH group remained negative for PCV2 viremia while almost all control pigs (92.9%) were positive at some point. Thus, 2-dose Foster PCV MH vaccination significantly reduced the incidence of viremic pigs by 100% ( $P \leq 0.0001$ ) compared to controls. The PCV component of Foster PCV MH was deemed effective as an aid in preventing PCV2 viremia.

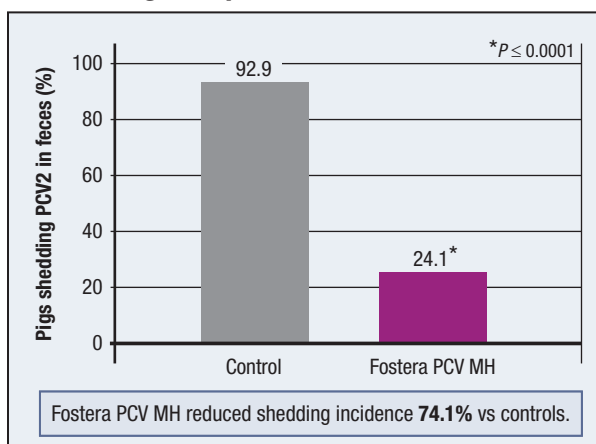
**Fecal Shedding:** Analysis of fecal swabs collected post-challenge revealed that 92.9% of control pigs shed PCV2 in feces compared to only 24.1% of Foster PCV MH-vaccinated pigs (Figure 4). PCV shedding was significantly reduced by 74.1% ( $P \leq 0.0001$ ) in Foster PCV MH vaccinates compared to controls. The PCV component of Foster PCV MH was clearly effective as an aid in reducing fecal shedding of PCV2.

**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 Foster PCV MH increased after the

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

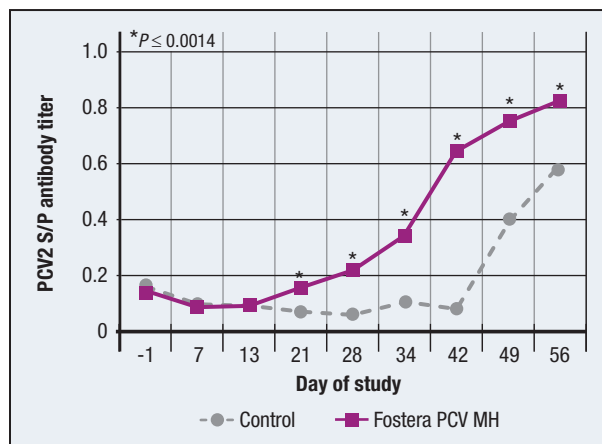


**Figure 4. Pigs shedding PCV2 in feces at any time during study.**

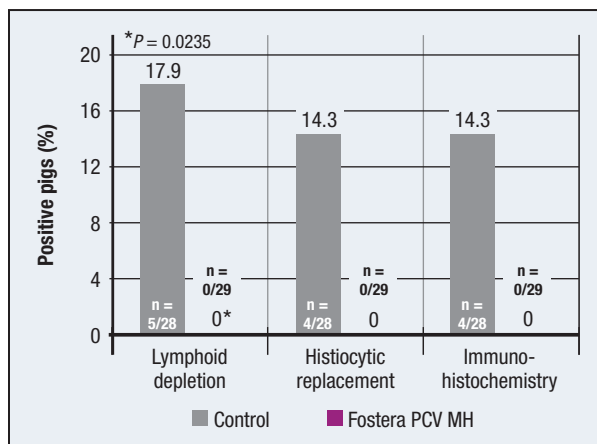


second vaccination (administered on day 14) and were statistically elevated ( $P \leq 0.0014$ ) relative to controls on days 21, 28, and 34 (Figure 5). Foster PCV MH vaccinates generated post-challenge antibody titers significantly higher ( $P \leq 0.0001$ ) than controls on all sample dates. These serologic outcomes indicate that Foster PCV MH induced active antibody responses to PCV2 after vaccination, and anamnestic responses to PCV2 following challenge.

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



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



**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. The level of microscopic lesions and lymphoid tissue colonization was low among controls even though viremia was markedly elevated. The underlying cause of this relatively low colonization in the lymphoid tissues of control animals was not known. Still, Fostera PCV MH vaccinates demonstrated 100% reduction relative to controls for lymphoid depletion ( $P = 0.0235$ ), histiocytic replacement, and PCV2 antigen in tissues.

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 following PCV2 challenge;

- aids in the prevention of microscopic PCV2 lesions (lymphoid depletion);
- aids in the prevention of PCV2 colonization of lymphoid tissues.

A 2-dose regimen 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 9-week study investigating the efficacy of the *M. hyo* component of Fostera PCV MH involved 63 weaned, clinically healthy cross-bred piglets approximately 3 weeks of age and seronegative for *M. hyo*.<sup>2</sup> On study day 0, piglets were randomized within litter for assignment to treatment groups and administered either of the following vaccines:

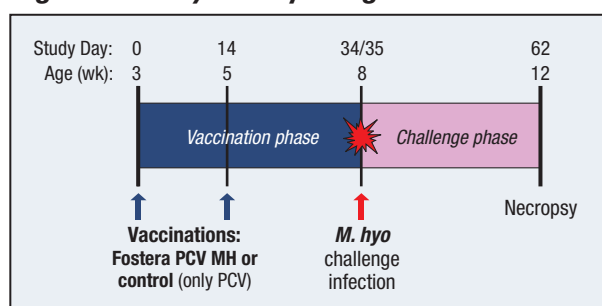
- Control: no *M. hyo* antigens, only PCV antigens (n=31);
- Fostera PCV MH: both *M. hyo* and PCV antigens (n=32).

Vaccine treatments were administered as a 1-mL IM injection in the left neck, thus initiating the 5-week ‘vaccination phase’ of the study (3 to 8 weeks of age; Figure 7). A second 1-mL IM vaccination was administered identically 2 weeks later (day 14) in the right neck.

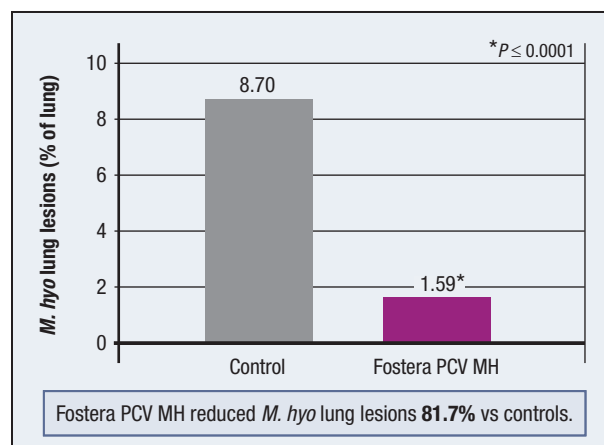
Three weeks after the second vaccination, the ‘challenge phase’ of the study (8 to 12 weeks of age) involved intratracheal dosing of each pig on days 34 and 35 with 10 mL of a live, virulent *M. hyo* lung homogenate.

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 day 62) for scoring of lung lesions. 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.

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



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



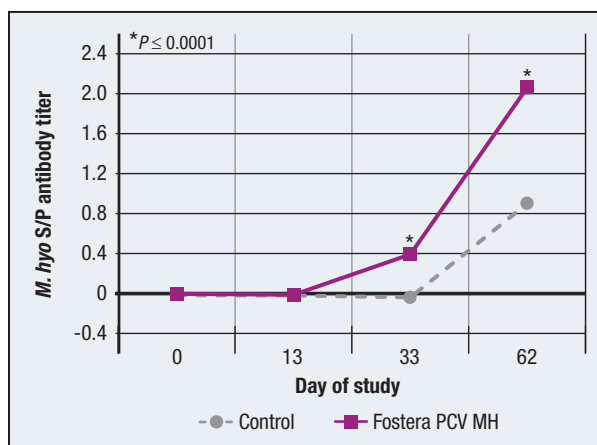
Data were analyzed in a manner similar to the previous study. The impact of vaccination on the severity of post-challenge *M. hyo* lung lesions was the primary variable of interest. The study was conducted in accordance with the Zoetis Institutional Animal Care and Use Committee.

### ***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 (1.59%) compared to controls (8.70%), representing a relative risk reduction of 81.7% compared to controls.

Serological outcomes (Figure 9) revealed that all pigs were negative for *M. hyo* antibody prior to vaccination (day 0) and on day 13 (prior to the second vaccination). Pre-challenge *M. hyo* antibody titers of pigs vaccinated with Foster PCV MH were statistically elevated ( $P \leq 0.0001$ ) relative to controls (day 33). Foster PCV MH vaccinates demonstrated an anamnestic response after *M. hyo* challenge (day 62,  $P \leq 0.0001$  vs controls).

**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 increased *M. hyo* IDEXX ELISA S/P ratios prior to challenge;
- significantly reduced the severity of *M. hyo* lung lesions;
- generated a significant anamnestic response to *M. hyo* challenge.

A 2-dose regimen 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.

### **Safety**

Additional research conducted for licensure of Foster PCV MH included a multi-site field-safety study that evaluated local injection site reactions and systemic events after administration of Foster PCV MH using the flexible 2-dose option.<sup>3</sup> The study involved 750 weaned pigs, 17 to 26 days of age, that were enrolled at 3 separate commercial production sites in Wisconsin (n=250), Kentucky (n=250), and Minnesota (n= 250). Healthy pigs at each site were randomly assigned to 2 treatment groups (approximately 4:1 ratio). On study day 0, each pig received a 1-mL IM injection in the right neck with either:

- Foster PCV MH (n=602);
- Saline (non-vaccinated control, n=148).

Each pig received a second 1-mL IM injection in the left neck on day 14.

All animals were observed for adverse reactions at 1 and 6 hours of each vaccination, and clinical observations were conducted on days 1, 3 or 4, 7 or 8, 15, 17, 21, 28 and 35 (study conclusion). All injection sites were observed and palpated for adverse reactions at 1, 3 or 4, 7 or 8, and 14 or 15 days after vaccination, and on day 35. Any reactions were rechecked daily until resolution. Animals were also observed daily for

any clinical disease or adverse events requiring treatment. All cases were determined whether to be related to vaccine or saline administration, and any pigs that died were necropsied and a diagnosis determined when possible. Personnel performing clinical observations, injection site reaction measurements, post-mortem examinations, or laboratory measurements were masked as to treatment group assignments.

The non-vaccinated control group provided baseline information regarding the current herd health conditions at each site. Thus, the incidence and severity of health events in the 2-dose Foster PCV MH group were compared to those occurring in the control group. Data were analyzed by appropriate statistical methods using the pig as the experimental unit. The protocol was reviewed and approved by the Zoetis Kalamazoo Ethical Review Board. A Zoetis Animal Welfare Risk Assessment was completed for each site prior to initiation of the study.

### **Results**

No confounding disease factors were detected during the study which affected evaluation of vaccine safety. No clinical signs of PCV2 or *M. hyo* pneumonia were observed, but other clinical signs/diagnoses of disease were occasionally noted in some animals (e.g., mulberry heart, inappetence, lethargy, bacterial infection, rotavirus enteritis). One pig died at the WI site (mulberry heart and secondary bacterial pathogens), 8 pigs died at the KY site (inappetence, bacterial pathogens, lameness due to injury), and 9 mortalities were recorded at the MN site (bacterial pathogens, mulberry heart, rotavirus enteritis).

No pigs demonstrated any abnormal post-vaccination clinical observations at 1 and 6 hours after the first vaccination on day 0. At approximately 1 hour after the second vaccination on day 14, 0.7% of control pigs and 1.0% of Foster PCV MH vaccinates demonstrated abnormal post-vaccination clinical symptoms (depression, lameness, etc.). At 6 hours post-vaccination, the rate

was 1.4% for controls and 1.3% for vaccinates. Thus, the rate of abnormal post-vaccination observations was approximately the same in Foster PCV MH vaccinates as in controls, suggesting no acute abnormalities attributable to use of 2 doses of the combination vaccine.

Injection sites were categorized as 'normal' for 99.7% and 98.3% of Foster PCV MH vaccinates at 1 day after the first and second vaccinations, respectively (not more than a visible injection site with < 0.5 cm diameter zone of swelling only associated with the injection site, and no evidence of irritation). Reactions in the other 0.3% (first vaccination) to 1.7% (second vaccination) of Foster PCV MH vaccinates (Table 1) were 'mild' (0.5-1.5 cm diameter swelling, possible evidence of irritation such as occasional rubbing) and injection sites resolved by 4 days post-vaccination.

Also noted in Table 1 is the complete absence of anaphylactic reactions in study animals. Rates of all other abnormal health events in Foster PCV MH vaccinates were similar to rates that occurred in control pigs. These events, along with necropsy diagnoses and diagnostic laboratory results, were consistent with herd health observations and not related to administration of 2 doses of Foster PCV MH.

### **Safety Conclusions**

Results of this multi-site clinical field study confirmed the safety of the flexible 2-dose Foster PCV MH regimen in young pigs. The incidence of abnormal health events was similar between animals vaccinated with Foster PCV MH and controls, and only a low incidence of mild, quick-resolving injection site reactions was observed.

### **Overall Conclusions**

Results of 2 challenge studies confirmed that the flexible 2-dose Foster PCV MH regimen helps provide effective protection from both PCVAD and mycoplasmal pneumonia. The innovative one-bottle/flexible-dose formulation helps deliver excellent PCV and *M. hyo* efficacy while offering swine managers the convenience and choice of administration as a single dose or 2 doses, for optimal compatibility with existing vaccination protocols. In addition, the excellent safety profile demonstrated by Foster PCV MH allows pork producers and veterinarians to deploy the flexible-dose combination vaccine in their herds with the confidence that safety will not be compromised.

**Table 1 – Incidence of injection site reactions and anaphylactic reactions (summary of 3 sites).**

Treatment	No. pigs <sup>a</sup>	Injection site reactions (days after vaccination)			Anaphylactic reactions
		Day 1	Day 3	Day 7	
<b><i>First Dose<sup>b</sup></i></b>					
Control	148	0%	0%	0%	0%
Fostera PCV MH	602	0.3%	0%	0%	0%
<b><i>Second Dose</i></b>					
Control	146	0%	0%	0%	0%
Fostera PCV MH	598	1.7%	0.3%	0%	0%

<sup>a</sup> Number of pigs on day 1 post-vaccination.

<sup>b</sup> Injection sites assessed on days 4 and 8 after vaccination for the KY site.



### ***References***

1. Data on file, Study Report No. B822R-US-12-111, Zoetis Inc.
2. Data on file, Study Report No. B822R-US-12-112, Zoetis Inc.
2. Data on file, Study Report No. B921R-US-12-113, Zoetis Inc.

### ***Acknowledgements***

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