Boehringer Ingelheim

KNOW THE FACTS

PCV2 CROSS-PROTECTION

FLEX

WHERE WE ARE TODAY

Currently, there are three major PCV2 genotypes in circulation: PCV2a, b and d.[1] Since it was identified in 2010, PCV2d has become the predominant genotype circulating in the United States.[2,3]

While evidence indicates that PCV2a-based vaccines can provide cross-protection against new field strains,[3–7,8–10] the discussion continues about whether a homologous PCV2d vaccine could provide better protection against today's most prevalent genotype.

KNOW THE FACTS: PCV2 CROSS-PROTECTION

DNA viruses like porcine circovirus Type 2 (PCV2), by their nature, mutate more slowly than RNA viruses.[11] Still, relative to other DNA viruses, the mutation rate of PCV2 is one of the highest,[12] giving rise to the shifting strains the swine industry has been grappling with in recent years.

As PCV2 strains evolve, researchers at Boehringer Ingelheim (BI) continue to test Ingelvac CircoFLEX®, a PCV2a-based vaccine, under numerous conditions to ensure that vaccinated pigs receive full protection from all strains of the pathogen.

BUILDING KNOWLEDGE

- **A STARTING POINT**
  An initial exploratory study that focused on identifying PCV2 variants on certain farms using INGELVAC CIRCOFLEX successfully provided the first indication that the vaccine could cross-protect against evolving PCV2 strains.[4]

- **LOOKING CLOSER**
  Next, in a field-exposure study, INGELVAC CIRCOFLEX was shown to provide cross-protection against PCV2d.[5]

- **MIMICKING REAL-WORLD CONDITIONS**
  To take the previous findings one step further, in an experimental, highly-controlled study using commercial pigs, 3FLEX®, which includes INGELVAC CIRCOFLEX, was also shown to provide cross-protection against PCV2d in the face of a severe porcine respiratory disease complex (PRDC) challenge.[6]

- **GOING FURTHER**
  Finally, in a peer-reviewed, experimental challenge study, INGELVAC CIRCOFLEX provided similar protection as a PCV2d construct vaccine against a clinically severe, mixed PCV2a/PCV2d challenge.[7]

A STARTING POINT

As a first step, an exploratory study was conducted on 48 farms in the Midwestern United States.[1] Participants included farms that met the following criteria: 1) pigs were being vaccinated with INGLEVAC CIRCOFLEX at weaning age; 2) both the pig owner and veterinarian were satisfied with the vaccine; 3) pig performance was meeting the systems' expectations; and 4) no clinical health issues suggestive of Porcine circovirus associated disease (PCVAD) were present.[1]

Polymerase chain reaction (PCR) testing was performed on pig serum, oral fluids and lung tissues. Out of 48 farms, 27 were PCR-positive for PCV2, with nearly half of those being classified as PCV2d and the rest PCV2a or PCV2b.[4] These findings aligned with previous studies that described the rising prevalence of PCV2d in the United States.[1]

LESSONS LEARNED

The fact that vaccinated pigs showed no signs of porcine circovirus associated disease (PCVAD), despite evidence of exposure to a wide range of modern PCV2 strains, suggested INGELVAC CIRCOFLEX could provide cross-protection against PCV2d, and that additional side-by-side studies were needed.

For more information see:
Payne B, Jacobs, Dvorak C, et al. PCV2 vaccine cross-protection: Identification of sequences in successfully vaccinated field cases. In Proceedings. AASV Annual Meeting 2016;202–206.

LOOKING CLOSER

To take the initial findings one step further, BI conducted a PCV2d field-exposure study to test the impact of vaccination with FLEXcombo® (which includes INGELVAC CIRCOFLEX) on average daily gain (ADG) and mortality. Weaning-age pigs were separated into three groups, and administered either FLEXCOMBO, a competitor's split-dose PCV2 and *Mycoplasma hyopneumoniae* (*Mhp*) vaccine, or no vaccine.[5]

When PCV2 viremia was detected in non-vaccinated pigs, molecular testing indicated it was a PCV2d infection. There was also evidence of a porcine reproductive and respiratory syndrome (PRRS) challenge late in the study.[5]

LESSONS LEARNED

The study found that under PCV2d field-exposure conditions, pigs vaccinated with FLEXCOMBO demonstrated no clinical signs of PCVAD.[5] One dose of FLEXCOMBO was just as efficacious against a PCV2d challenge as the competitor's split-dose vaccine, while reducing pig handling and stress, labor and time (Tables 1 and 2).[5]

TABLE 1: STUDY RESULTS - AVERAGE DAILY GAIN[5]

Treatment	INGELVAC CIRCOFLEX	Competitor Vaccine	Barrows	Gilts
Days 0–71	1.36	1.35	1.35	1.35
Days 72–153	2.07	2.06	2.21[a]	1.93[b]
Days 0–153	1.74	1.73	1.81[a]	1.66[b]

[a,b] Means with different superscripts indicate difference at $P \leq 0.05$ (Student's t).

TABLE 2: STUDY RESULTS - % MORTALITY[5]

Treatment	% Mortality (INGELVAC CIRCOFLEX)	% Mortality (Competitor Vaccine)
Days 0–71	3.02	2.40
Days 72–153	2.18	2.78
Days 0–153	5.14	5.11

[a,b] Means with different superscripts indicate difference at $P \leq 0.05$.

For more information see:
Fano E, Schaefer N, Schmaling E, et al. Comparison of efficacy between two PCV2 vaccination protocols under PCV2d field exposure. In Proceedings. AASV Annual Meeting 2017;95–97.

MIMICKING REAL-WORLD CONDITIONS

Because pigs in the field are often co-infected with multiple pathogens, BI sought to build on the previous findings by testing 3FLEX®, which includes INGELVAC CIRCOFLEX, in an experimental, severe challenge study with numerous pathogens, using commercial pigs.[6]

Pigs in this study were split into two groups of 20: non-vaccinated challenged controls and pigs vaccinated with 3FLEX. Both groups were simultaneously inoculated with a contemporary virulent PCV2d field isolate (intranasal and intramuscular), *Mhp* strain 232 (intratracheal) and PRRSV strain SDSU-73 (intranasal and intramuscular).[6]

LESSONS LEARNED

Significant differences ($P < 0.05$) in PCV2 parameters, including lymphoid depletion, immunohistochemistry (IHC) and viremia, were observed between the two groups (Table 3).[6] Vaccinated pigs also had higher ADG than the non-vaccinated control group.[6]

These findings demonstrate the ability of 3FLEX to provide protection against PCV2d in the face of a severe PRDC challenge, similar to what pigs would experience in the field.[6]

TABLE 3: STUDY RESULTS - % MORTALITY[6]

Variable	Reduction compared to challenge control	P-Value
LN Depletion	Yes	0.038
LN IHC	Yes	0.001
Lung IHC	Yes	0.01
Viremia %	Yes	0.01
Viremia CT	Yes	0.00001

For more information see:
Philips R, Fano E, Schmaling E, Edler R. A severe PRDC challenge and the effect of a trivalent PRDC vaccine for PCV2, Mhp and PRRS. Boehringer Ingelheim Vetmedica, Inc., Health Management Center (HMC), Field Research Services. 2018.

GOING FURTHER

While previous studies show that PCV2a-based vaccines in the face of a PCV2d challenge can offer cross-protection against PCV2b,[3,8] reduce viremia, increase antibody titers and enhance average daily gain,[9,10] discussion continues about whether a homologous PCV2d vaccine could provide better protection against today's most prevalent genotype.

To address this question, the next step was to test INGELVAC CIRCOFLEX against a PCV2d construct vaccine in an experimental challenge study.

The study compared four groups of pigs challenged with PCV2d: pigs vaccinated with INGELVAC CIRCOFLEX, pigs vaccinated with a PCV2d experimental vaccine, and two groups of non-vaccinated controls. In addition, at the time of challenge, it was determined that the pigs in this study had been naturally exposed to PCV2a following vaccination.[7]

LESSONS LEARNED

The study found that both vaccines prevented clinical signs and mortality, while control pigs were severely affected (Figure 1).[7] The median lymphoid tissue lesion scores for both control groups were 4.75 and 5.50, respectively, compared with 0.00 for both PCV2a and PCV2d vaccine groups ($P < 0.0001$) (Figure 2).[7]

In addition, both vaccine groups showed significantly reduced viremia ($P < 0.0001$), and average daily gain was significantly higher compared with pigs in the control groups (Table 4).[7]

The results of this study demonstrate the ability of INGELVAC CIRCOFLEX to provide protection against a clinically severe, mixed PCV2a/PCV2d challenge.[7]

For more information see:
Friedrich R, Patterson AR, Johnson W, et al. Efficacy of porcine circovirus Type 2a– and 2d–based vaccines following PCV2 challenge. Journal of Vaccines and Vaccination 2019;10(2):1–5.

Figure 1: Morbidity and mortality among treatment groups[7]

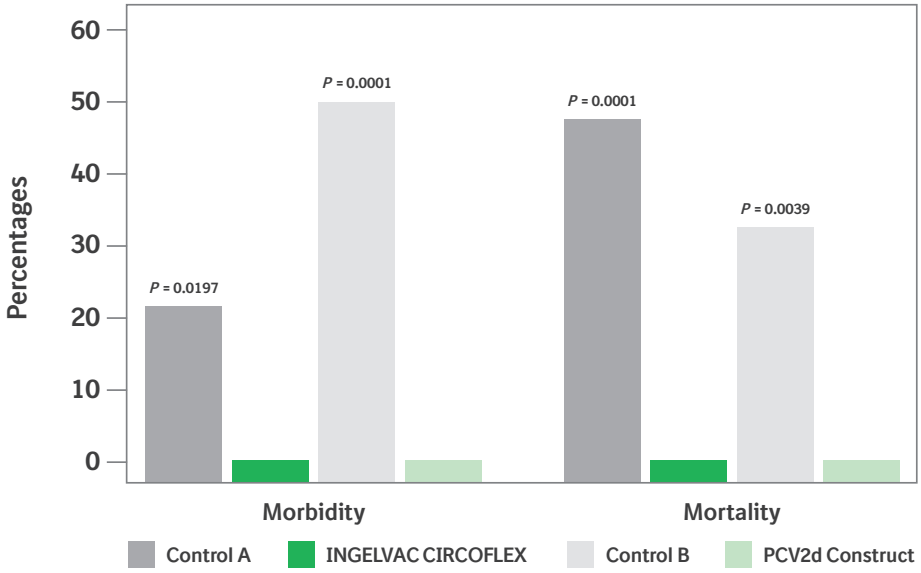

Figure 2: Lymphoid tissue lesion scores by group, mean and median, represented by the diamond and horizontal line, respectively[7]

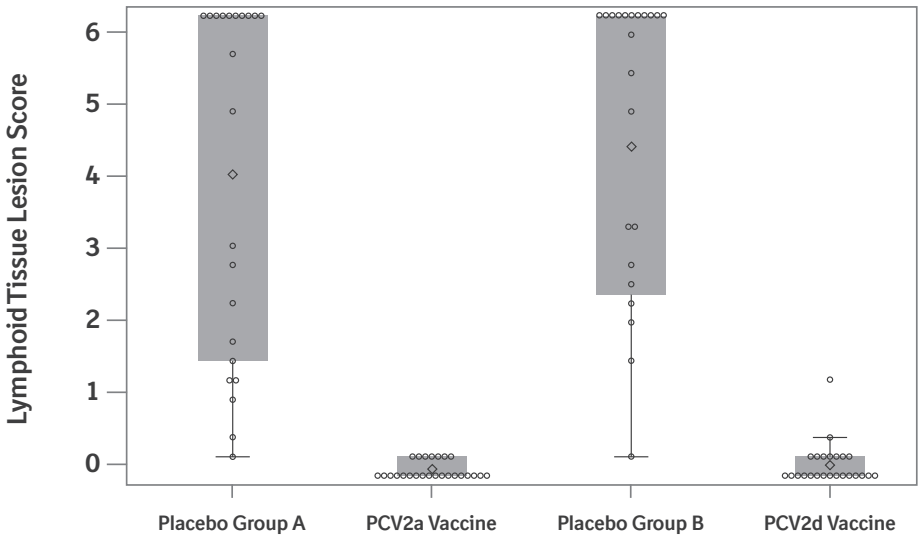

TABLE 4: LEAST-SQUARE ESTIMATE OF ADG BY GROUP[7]

Group	ADG (lbs.)	95% Confidence Interval (CI)	P-Value
Control A	0.97	0.82, 1.12	< 0.001
INGELVAC CIRCOFLEX	1.39	1.23, 1.52	
Control B	1.01	0.86, 1.17	< 0.001
PCV2d Construct	1.46	1.34, 1.57	

SUMMARY

When viewed both individually and as a whole, this body of research clearly demonstrates that INGELVAC CIRCOFLEX, a PCV2a-based vaccine, can provide cross-protection against PCV2d,[4–7] the predominant genotype circulating in the United States.[1–2]

But the same way PCV2d emerged and pathogens like PRRS continue to evolve, researchers at Boehringer Ingelheim know the next challenge is just around the corner. As your partner in swine health, we continue to conduct research and new investigations to ensure that producers and veterinarians are able to provide the best possible protection for their animals.

NOTES

[1] Payne B, Jacobs, Dvorak C, et al. PCV2 vaccine cross-protection: Identification of sequences in successfully vaccinated field cases, in Proceedings. AASV Annual Meeting 2016;202–206.

[2] Fano E, Schaefer N, Schmaling E, et al. Comparison of efficacy between two PCV2 vaccination protocols under PCV2d field exposure, in Proceedings. AASV Annu Meet 2017;95–97.

[3] Philips R, Fano E, Schmaling E, Edler R. A severe PRDC challenge and the effect of a trivalent PRDC vaccine for PCV2, Mhp and PRRS.Boehringer Ingelheim Vetmedica, Inc., Health Management Center (HMC), Field Research Services. 2018.

[4] Friedrich R, Patterson AR, Johnson W, et al. Efficacy of porcine circovirus Type 2a- and 2d-based vaccines following PCV2 challenge. J Vaccines Vaccination 2019;10(2):1-5.

[5] Xiao CT, Halbur PG, Opriessnig T. Global molecular genetic analysis of porcine circovirus Type 2 (PCV2) sequences confirms the presence of four main PCV2 genotypes and reveals a rapid increase of PCV2d. J Gen Virol 2015;96(Pt 7):1830-1841.

[6] Franzo G, Cortey M, Segalés J, et al. Phylodynamic analysis of porcine circovirus Type 2 reveals global waves of emerging genotypes and the circulation of recombinant forms. Mol Phylogenet Evol 2016;100:269-280.

[7] Afghah Z, Webb B, Meng XJ, Ramamoorthy S. Ten years of PCV2 vaccines and vaccination: Is eradication a possibility? Vet Microbiol 2017;206:21-28.

[8] Li J, Yu T, Zhang F, et al. Inactivated chimeric porcine circovirus (PCV) 1-2 vaccines based on genotypes 2b and 2d exhibit similar immunological effectiveness in protecting pigs against challenge with PCV2b strain 0233. Arch Virol 2017;162(1):235-246.

[9] Jeong J, Park C, Choi K, Chae C. Comparison of three commercial one-dose porcine circovirus Type 2 (PCV2) vaccines in a herd with concurrent circulation of PCV2b and mutant PCV2b. Vet Microbiol 2015;177(1-2):43-52.

[10] Opriessnig T, Chao-Ting X, Halbur PG, et al. A commercial porcine circovirus (PCV) Type 2a-based vaccine reduces PCV2d viremia and shedding, and prevents PCV2d transmission to naïve pigs under experimental conditions. Vaccine 2017;35(2):248-254.

[11] Sanjuan, R, Domingo-Calap P. (2016). Mechanisms of viral mutation. Cell. Mol. Life Sci., 73, 4433-4448. doi:10.1007/s00018-016-2299-6.

[12] Karuppmannan A, Opriessnig T. Porcine Circovirus Type 2 (PCV2) Vaccines in the Context of Current Molecular Epidemiology. Viruses. 2017;60-74.

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