# TECHNICAL BULLETIN

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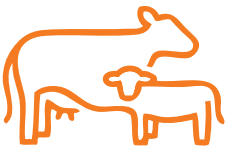

## INFORCE™ 3: Aids in the prevention of disease caused by bovine parainfluenza$_3$ virus.

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**SUMMARY**

- A vaccination/challenge efficacy study was conducted to assess the ability of INFORCE™ 3 to help protect cattle from respiratory disease caused by bovine parainfluenza$_3$ ($PI_3$) virus when administered as a single intranasal dose.[1]
- Compared with nonvaccinated control calves, calves vaccinated with INFORCE 3:
  - Shed significantly less virus on Days 1 through 6 post-challenge ($P \leq 0.0225$)
  - Stopped shedding virus significantly sooner than the controls ($P \leq 0.0019$)
  - Shed 99.9% less virus post-challenge as measured by the least squares mean area under the curve (AUC)
- Based on the data demonstrated in this study, the USDA granted INFORCE 3 the label claim "aids in the prevention of respiratory disease caused by bovine parainfluenza3 virus."

Bovine parainfluenza$_3$ ($PI_3$) virus was first isolated in the United States in 1959 from the nasal discharge of cattle with shipping fever by workers at the USDA.

Bovine parainfluenza$_3$ virus is in the genus *Respirovirus* of the subfamily *Paramyxovirinae*, order *Mononegavirales*, of the family *Paramyxoviridae*. The *Respirovirus* genus also includes the genetically and antigenically related human parainfluenza viruses types 1 and 3.

Like other respiratory viruses, $PI_3$ virus is spread primarily animal to animal by the aerosol route. A variety of clinical signs of variable severity have been reported in field cases of $PI_3$ virus-associated respiratory disease; however, the frequent involvement of other pathogens in bovine respiratory disease (BRD) makes it difficult to ascribe signs attributable to $PI_3$ virus alone.

The vaccine strains in INFORCE 3 were selected based on their demonstrated record of safety and efficacy, and their suitability for intranasal vaccination. The titer (amount of virus) of each vaccine strain was evaluated to ensure the optimal balance of protection and safety.

Reported herein is the pivotal challenge of immunity study that was used to support the label claim aids in the prevention of respiratory disease caused by bovine parainfluenza$_3$ virus.

## Overview of the INFORCE 3 USDA efficacy study

### *Animals*

Healthy calves were selected for this trial. The calves were 8 months of age at the time of vaccination and were sero-negative (serum neutralizing [SN] antibody titer of <1:2) for PI3 virus and were not persistently infected (PI) with bovine viral diarrhea virus (BVDV).

## Study Design

This study was conducted in a completely randomized design and divided into two phases: the vaccination phase and the challenge phase as described in Table 1. All animals were vaccinated on Day 0, challenged on Day 28 and monitored for virus isolation, rectal temperatures and clinical signs of disease for 14 days post-challenge.

### *Test vaccine*

The placebo control calves received a vaccine that contained IBR, BVD1, BVD2 and BRSV at levels above the minimum immunizing dose (MID) for each fraction.

The calves vaccinated with INFORCE 3 were administered a vaccine that had the MID level of the PI$_3$ virus and the IBR, BVD1, BVD2 and BRSV fractions at or above their respective MIDs.

*Minimum immunizing dose (MID) levels are established prior to licensing. Expiration levels of an MLV product are set at 0.7 log higher than MID, therefore, by using MID levels for vaccine antigens, investigators put the vaccine at its maximum potential disadvantage at the time of challenge. When a vaccine withstands challenge under these circumstances, it will be at least as effective when the antigen level is at its release level.*

### *Vaccination phase*

On Days -1 through two days before challenge, all animals were observed once daily for general health.

On Day 0, calves were vaccinated intranasally with 2 mL (1 mL per naris), with the appropriate vaccine as described in Table 1.

Animals were observed for any local and/or systemic reactions associated with vaccination (depression, trembling, tachypnea) once within four hours following vaccination. No reactions were noted due to vaccinations.

Animals were allocated to treatment groups per a completely randomized design, and treatment groups were randomly assigned to rooms during the vaccination phase (separate room for each treatment group). During the challenge phase, animals were commingled in a single room.

### *Samples and assay of specimens*

Blood samples were collected from all calves on Days 0 (pre-vaccination), 7, 21, 28 (pre-challenge), 35 and 42 (post-challenge). The serum was tested for SN antibodies to PI$_3$. Nasal secretions were collected using swabs on Days 0, 27 and 29 through 42 from each animal for virus isolation (VI). All serologic and VI assays were completed at Zoetis Laboratory Sciences in Kalamazoo, Mich.

**Table 1 – Study design**

| Vaccine | N | Vaccination Phase | | | Challenge Phase (Days) | |
|---|---|---|---|---|---|---|
| | | Day | Dose[1] | Route | Challenge | Clinical Observations and Virus Shedding |
| **Placebo Control (IBR-BVD1-BVD2-BRSV)** | 12 | 0 | 2 mL | IN | 28 | 29 - 42 |
| **INFORCE 3 (IBR-BVD1-BVD2-PI$_3$-BRSV)** | 24 | 0 | 2 mL | IN | 28 | 29 - 42 |

[1]1 mL per naris

### *Challenge phase (procedure and observations)*

Animals were challenged intranasally with a virulent $PI_3$ virus.

The day prior to challenge and for 14 days post-challenge, all calves were monitored for clinical signs of $PI_3$ disease. Daily rectal temperatures were recorded one day before challenge, day of challenge and for 14 days post-challenge.

### *Masking*

All post-challenge clinical monitoring, clinical sampling and laboratory testing were conducted and recorded without the knowledge of treatment group assignment.

## Results

### *Serology*

On Days 0, 7, 21, 28 and 35 all placebo control calves were sero-negative for PI3 SN antibodies (<1:2) (Table 2). Vaccination with INFORCE 3 induced detectable PI3 SN antibody responses (≥1:4) in 17 of 22 (70.8%) vaccinates on Day 28 (pre-challenge). Following challenge all animals seroconverted. There were significant differences between Days 28, 35 and 42. SN titers of vaccinates versus controls ($P \leq 0.0001$). Following challenge exposure there was an anamnestic response in the vaccinates, indicating that INFORCE 3 effectively primed the animals.

**Table 2 – Descriptive geometric mean (Days 0, 7, 21) and geometric least squares mean (LSM) (Days 28, 35 and 42) $PI_3$-specific SN antibody titers (percent seroconversion† rate)**

| Study Day | Treatment Group | |
|---|---|---|
| | Placebo Control | INFORCE 3 |
| 0 | 1 | 1 |
| 7 | 1 | 1 |
| 21 | 1 (0) | 3 (33.3) |
| 28 | 1[a] (0) | 6[b] (70.8) |
| 35 | 1[a] (0) | 470[b] (100) |
| 42 | 235[a] (100) | 925[b] (100) |

†Percent seroconversion = frequency of animals with $PI_3$ SN antibody titers ≥ 1:4.
Means within a row with different superscripts are significantly different ($P \leq 0.0001$)
Titers reported as <2 were analyzed as 1.

### *Rectal temperatures and clinical disease (morbidity)*

Daily rectal temperatures and clinical disease observations were recorded for 14 days post-challenge; however, as typical for studies using the USDA recommended $PI_3$ challenge virus, mild to non-existent fever and clinical signs of $PI_3$ respiratory disease were observed, which did not allow a clinically relevant comparison between vaccinates and controls. Instead, viral shedding using this challenge virus and model are consistent and predictable for determination of vaccine efficacy.

### *Virus Shedding*

Virus isolation results are summarized in Table 3 and Figure 1. None of the animals shed virus prior to PI3 challenge (on Study Days 0 and 27). Following challenge,

**Table 3 – Descriptive geometric means (Days 0 and 27) and geometric least squares mean (Days 29 to 42) of virus isolation titers[†]**

| Study Day | Treatment Group | |
|---|---|---|
| | Placebo Control | INFORCE 3 |
| 0 | 0.8 | 0.8 |
| 27 | 0.8 | 0.8 |
| 29 | 2.0[a] | 1.5[b] |
| 30 | 3.4[a] | 2.0[b] |
| 31 | 4.8[a] | 2.1[b] |
| 32 | 5.4[a] | 2.5[b] |
| 33 | 5.4[a] | 1.2[b] |
| 34 | 4.3[a] | 1.0[b] |
| 35 | 1.1[a] | 0.8[a] |
| 36 | 0.9[a] | 0.8[a] |
| 37 | 0.8[a] | 0.8[a] |
| 38 | 1.0[a] | 0.9[a] |
| 39 | 1.0[a] | 0.8[a] |
| 40 | 0.8[a] | 0.8[a] |
| 41 | 0.8[a] | 0.8[a] |
| 42 | 0.8[a] | 0.8[a] |

[†]The lower limit of quantitation for the virus isolation assay was 1.1 $\log_{10}$ $TCID_{50}$/mL, negative samples (≤1.1 $\log_{10}$ $TCID_{50}$/mL) were assigned a value twofold lower (0.8 $\log_{10}$ $TCID_{50}$/mL) prior to analysis.
Means within a row with different superscripts are significantly different ($P \leq 0.0225$)

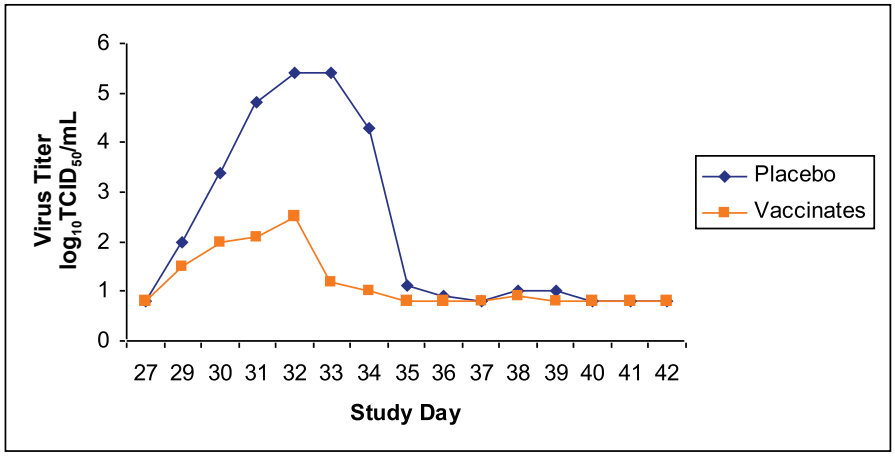


**Figure 1:** $PI_3$ virus isolation titers before and after challenge

placebo controls shed significantly ($P \leq 0.0225$) more virus than vaccinates on Days 29 through 34. Table 4 demonstrates the virus shedding frequencies of vaccinates and controls, and highlights the fact that a higher number of controls shed virus on Days 30 through 39.

The median days to normal for virus shedding (the day of study an animal stops shedding for two consecutive days and stays normal) is shown in Table 5 and Figure 2. There was a significant difference ($P \leq 0.0019$) between the duration of virus shedding of the placebo controls compared with the vaccinates. INFORCE 3 vaccinates shed 99.9% less virus post-challenge compared with placebo control calves as measured by the least squares mean AUC ($P \leq 0.0001$).

**Table 4 – Percentage of animals shedding $PI_3$ virus by study day**

| Treatment | Study Day | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 27 | 29 | 30 | 31 | 32 | 33 | 34 | 35 | 36 | 37 | 38 | 39 |
| **Placebo Control** | 0 | 83.3 | 100 | 100 | 100 | 100 | 100 | 41.7 | 16.7 | 8.3 | 16.7 | 16.7 |
| **INFORCE 3** | 0 | 83.3 | 83.3 | 87.5 | 83.3 | 54.2 | 20.8 | 0 | 4.2 | 4.2 | 8.3 | 0 |

All animals stopped virus shedding from Study Day 40 to end of study

**Table 5 – Median days to normal ($PI_3$ virus shedding stopped) and geometric least squares mean area under the curve (AUC)**

| Treatment | Median, Days to Normal† | Geometric Least Squares Mean AUC ($Log_{10}$ $TCID_{50}$/mL x day)†† |
|---|---|---|
| **Placebo Control** | 36.5[a] | 14.0 ± 0.31[a] |
| **INFORCE 3** | 34.3[b] | 7.5 ± 0.41[b] |

† Data within this column with different superscripts are significantly different ($P \leq 0.0019$)
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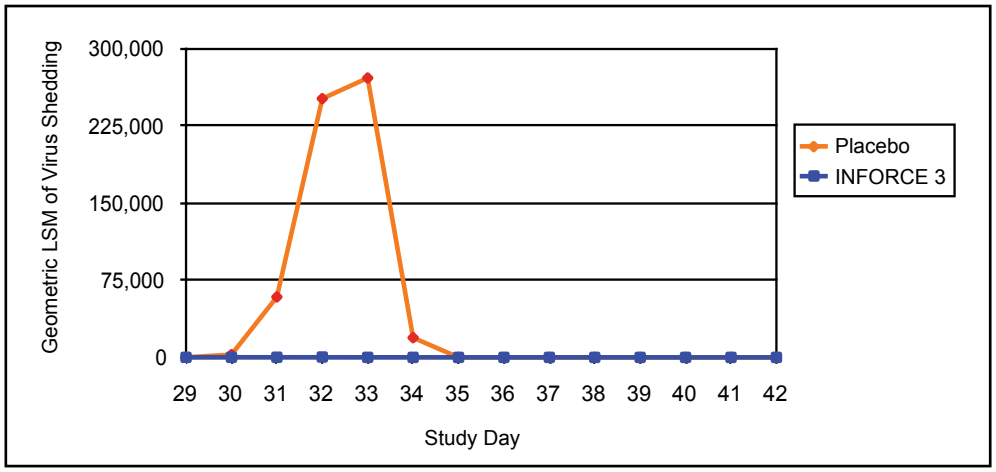


**Figure 2:** $PI_3$ virus shedding — geometric LSM area under curve

## Discussion

The objective of this study was to demonstrate the $PI_3$ efficacy of INFORCE 3 in naïve calves. The study was valid because all placebo control calves remained sero-negative for PI3 virus SN antibodies (<1:2) until challenge while 70.8% vaccinates seroconverted (≥1:4) prior to challenge. The rate of seroconversion was lower than seen with parenterally administered $PI_3$ virus vaccines and may be due to the mucosal route of administration used in this study. It was clear that all vaccinates were primed to $PI_3$ virus even if they did not have measurable SN antibodies at the time of challenge, since all animals had anamnestic responses seven days later (Day 35 titers) as indicated by greatly increased $PI_3$ virus SN antibody titers. Although frequency of clinical disease was greater in placebo controls than vaccinates, the overall low occurrence of symptoms and low incidence of fever is consistent with that observed in previous studies using the USDA-provided challenge virus (data not shown). Efficacy in this challenge model was defined by the ability of INFORCE 3 to reduce the level and duration of $PI_3$ virus shedding post-challenge. INFORCE 3 vaccinates were protected against $PI_3$ virus challenge because these calves shed significantly ($P \leq 0.0225$) lower levels of virus on Days 1 through 6 post-challenge and were able to eliminate shedding significantly ($P \leq 0.0019$) sooner than placebo control calves. INFORCE 3 vaccinates shed 99.9% less virus post-challenge compared with placebo control calves as measured by the least squares mean AUC ($P \leq 0.0001$).

## Conclusions

Bovine parainfluenza$_3$ virus is a longrecognized, currently underappreciated endemic infection in dairy and beef cattle populations. Clinical disease is most common in calves with poor passive transfer or decayed maternal antibodies. It is usually mild, consisting of fever, nasal discharge and dry cough. At least in part due to local immunosuppressive effects, $PI_3$ virus infection is often complicated by co-infection with other respiratory viruses and bacteria, and is therefore an important component of enzootic pneumonia in calves and BRD in feedlot cattle.

The requirement for licensure of a cattle vaccine containing bovine parainfluenza$_3$ virus is described in 9CFR. § 113.309 Bovine Parainfluenza$_3$ Vaccine. As described in the document:

> "Satisfactory resistance to challenge by vaccinates shall be determined by a significant difference between virus isolation rates from vaccinates and controls. The virus neutralization titers of post-challenge serums and respiratory symptoms and temperatures from all animals shall be considered in the evaluation of the test validity."

Based on the significant reduction in virus shedding post-challenge, the USDA granted INFORCE 3 the label claim "aids in the prevention of respiratory disease caused by bovine parainfluenza$_3$ virus."

## REFERENCES

1. Data on file, Study Report No. 3131R-60-08-568, Zoetis, Inc.