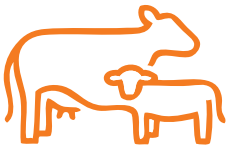


TECHNICAL BULLETIN

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INFORCE™ 3: **Aids in the prevention of respiratory disease caused by infectious bovine rhinotracheitis.**

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SUMMARY

- Two vaccination/challenge efficacy studies were conducted to assess the ability of INFORCE™ 3 to help protect cattle from respiratory disease caused by infectious bovine rhinotracheitis (IBR) virus when administered as a single intranasal dose.^{1,2}
- Animals in both studies were challenged intranasally by instillation of a 2 mL dose containing the virulent IBR virus (Cooper strain).
- In Study 1, using 7- to 9-month-old calves, INFORCE 3 vaccinates had:
 - Significantly lower incidence of clinical disease (morbidity* = 5% vs. 100%) ($P \leq 0.0001$)
 - Significantly less duration of disease (percent days ill = 0.07% vs. 31.97%) ($P \leq 0.0001$)
 - Significantly lower rectal temperatures 1 day and 3 to 9 days following challenge ($P \leq 0.0452$)
 - Significantly less virus shedding on Days 5 through 11 post-challenge ($P \leq 0.0203$)
- In Study 2, using 3- to 8-day-old calves, INFORCE 3 vaccinates had:
 - Significantly lower incidence of clinical disease (morbidity* = 5.6% vs. 100%) ($P \leq 0.0001$)
 - Significantly less duration of disease (percent days ill = 0.02% vs. 29.42%) ($P \leq 0.0001$)
 - Significantly less virus shedding on Days 2 through 13 post-challenge ($P \leq 0.0027$)
- Based on the significant protection demonstrated in Study 1, the USDA granted INFORCE 3 the label claim “aids in the prevention of respiratory disease caused by IBR.”

*Morbidity was defined as whether an animal ever exhibited acute clinical IBR disease (positive morbidity score) following challenge.

INFORCE 3 aids in the prevention of respiratory disease caused by IBR and PI₃, and prevents BRSV respiratory disease. In addition, extensive safety

studies were conducted with INFORCE 3, demonstrating safety in all ages and classes of animals, including newborn calves and high-stress stockers.

Overview of the IBR respiratory efficacy studies

Study 1 animals

Healthy weaned calves were selected for this trial. The calves were 7 to 9 months of age at the time of vaccination and were sero-negative (serum neutralizing [SN] antibody titer of <1:2) for IBR virus and not persistently infected (PI) with BVDV.

Study 2 animals

Healthy colostrum-deprived bull calves were selected for this trial. The calves were 3 to 8 days of age at the time of vaccination and were seronegative for IBR virus and not PI with BVDV.

Study Design

Both studies were divided into two phases: the vaccination phase and the challenge phase as described in Tables 1 and 2. For both studies, all animals were vaccinated on Day 0, challenged on Day 28 and monitored for virus isolation, rectal temperatures and clinical signs of IBR disease for 14 days post-challenge.

Test vaccine

For both studies, the placebo control calves did not receive IBR. The placebo control animals received either a vaccine that contained BVD, PI₃ and BRSV (Study 1) or PI₃ and BRSV (Study 2) at levels above the minimum immunizing dose (MID) for each fraction.

The calves vaccinated with INFORCE™ 3 (T02) were administered a vaccine that had the MID level of the IBR virus and the remaining fractions (BVD, PI₃ and BRSV for Study 1) or (PI₃ and BRSV for Study 2) at or above their respective MIDs.

Vaccination phase

For both studies, on Days 0 through to 2 days before challenge, all animals were observed once daily for general health.

For both studies, on Day 0, calves were vaccinated intranasally with 2 mL (1 mL per naris), with the appropriate vaccine as described in Tables 1 and 2. Animals were observed for any local and/or systemic reactions associated with vaccination (depression, trembling, tachypnea) once within four hours following vaccination. No

Minimum Immunizing Dose

Minimum immunizing dose (MID) levels are established prior to licensing of a vaccine. 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.

Table 1 – Study 1 design

Vaccine	N	Vaccination Phase			Challenge Phase (Days)	
		Day	Dose	Route ¹	Challenge	Clinical Observations
Placebo Control (BVD1-BVD2-PI ₃ -BRSV)	10	0	2 mL	IN	28	29 - 42
INFORCE 3 (IBR-BVD1-BVD2-PI ₃ -BRSV)	20	0	2 mL	IN	28	29 - 42

¹1 mL per naris

Table 2 – Study 2 design

Vaccine	N	Vaccination Phase			Challenge Phase (Days)	
		Day	Dose	Route [†]	Challenge	Clinical Observations
Placebo Control (PI ₃ -BRSV)	18 ^{††}	0	2 mL	IN	28	29 - 42
INFORCE 3 (IBR-PI ₃ -BRSV)	18 ^{†††}	0	2 mL	IN	28	29 - 42

[†]1 mL per naris

^{††}One animal removed from study on Day 28 prior to challenge due to injury

^{†††}One animal removed from study on Day 40 post-challenge due to illness not related to IBR disease

reactions were noted due to vaccinations. For Study 1: Animals were randomly assigned by treatment group to three rooms for the vaccination phase. During the challenge phase, animals were commingled and randomly assigned into three identical isolation rooms. Two of the three rooms contained three controls and six vaccinates, and the third room contained four controls and eight vaccinates.

For Study 2: Animals were housed in a biosafety level-2 facility for the duration of the study. During the vaccination phase, animals were housed in identical individual pens contained in two separate but identical rooms by treatment group. During the challenge phase, animals were randomly assigned to individual pens in two rooms so that both treatment groups were represented in each room.

Samples and assay of specimens

For Study 1, blood samples were collected from all calves on Days 0 (pre-vaccination), 14, 28 (pre-challenge) and 42. The serum was tested for SN antibodies to IBR. Nasal secretions were collected using swabs on Days 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.

For Study 2, blood samples were collected from all calves on Days 0 (pre-vaccination), 27 (pre-challenge) and 42. The serum was tested for SN antibodies to IBR. Nasal secretions were collected using swabs on Days 27 through 42 (excluding Day 28) from each animal for VI. All

serologic and VI assays were completed at Zoetis Laboratory Sciences in Kalamazoo, Mich.

Challenge phase (procedure and observations)

For both studies, animals were challenged intranasally by instillation of a 4 mL dose (approximately 2 mL per nostril) of virulent IBR (Cooper strain) using a compressed air nebulizer.

From three days prior to challenge through 14 days post-challenge, all calves were monitored for clinical signs of IBR respiratory disease and daily rectal temperatures were recorded.

Challenge virus

For Study 1, the IBR challenge virus was prepared at the Zoetis facility in Lincoln, Neb., as per United States Department of Agriculture, Animal and Plant Health Inspection Services, Center for Veterinary Biologics (USDA-APHIS-CVB) instructions.

For Study 2, the IBR challenge virus was prepared at Zoetis Veterinary Medicine Research and Development (VMRD), Kalamazoo, Mich., as per USDA-APHIS-CVB instructions.

Masking

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

Results – Study 1

Serology

On Days 0, 14 and 28, all placebo control calves were sero-negative for IBR SN

**Since animals were housed separately by treatment group during the Day 0 and 14 time-points of this study, only descriptive geometric means can be calculated for treatment groups during that time period. (No statistical analysis is appropriate since treatment is completely confounded with housing.)*

Once animals from the treatment groups were commingled (Day 28 and Day 42), analysis was possible and therefore we could calculate geometric LSMs (An LSM (Least Squares Mean) indicates that these are estimates from a statistical model).

Table 3 – Geometric means and geometric least squares means (LSMs)* of IBR-specific SN antibody titers (percent seroconversion rate)

Treatment Group	Treatment	Study Days [†]			
		0	14	28	42
T01	Placebo Control	1 (0)	1 (0)	1.0 ^a (0)	44.5 ^a (100.0)
T02	INFORCE 3	1 (0)	1.1 (5.0)	1.3 ^a (10)	62.4 ^a (100.0)

[†]Days 0 and 14 are geometric means and Days 28 and 42 are geometric LSMs*
 Different superscripts within a column represent significant differences (P ≤ 0.05).

antibodies (<1:2). Vaccine induced detectable IBR SN antibody responses ($\geq 1:2$) were observed in two of 20 (10%) vaccinates on Day 28. Following challenge, all animals in both groups seroconverted. Although the vaccine induced limited seroconversion, it primed immunity as demonstrated by development of strong anamnestic SN antibody responses post-challenge (Table 3). Intranasal vaccination does not induce an appreciable systemic antibody response to IBR vaccine and hence SN antibodies should not be used as a method of determining response or exposure to vaccination.

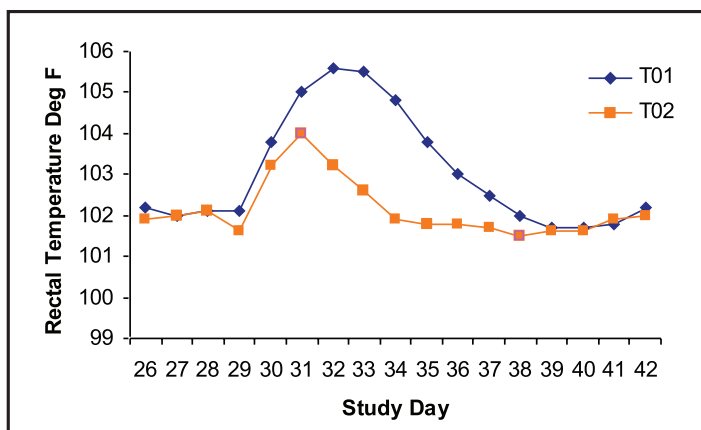


Figure 1: LSM rectal temperatures before and after IBR challenge[†]

[†]Rectal temperatures on Days 26, 27 and 28 are expressed as geometric means

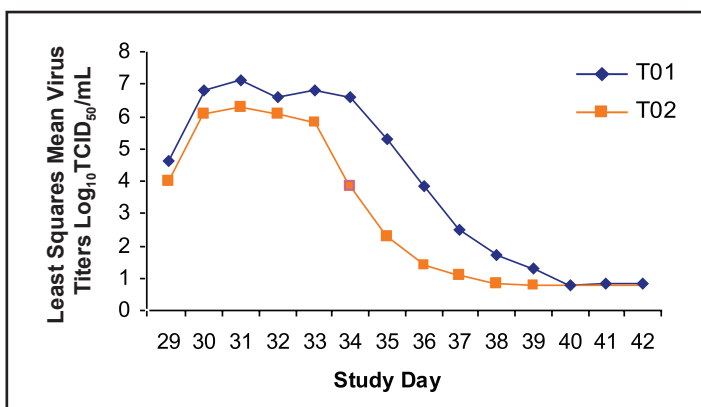


Figure 2: IBR virus isolation titers after challenge

Rectal temperatures

The post-challenge LSM rectal temperature profiles of vaccinates and controls are shown in Figure 1. The LSM rectal temperatures of vaccinates were

significantly lower ($P \leq 0.0452$) than those of the controls on Days 29 and 31 through 37.

Clinical disease

The virulent IBR challenge induced acute clinical disease in all control animals (Table 4). In comparison, only one of 20 (5%) vaccinated animals developed acute IBR clinical disease. The LSM percent days (see box for definition) with disease for the placebo controls was 31.97 compared with 0.07 for the vaccinates ($P \leq 0.0001$).

Virus shedding

Virus isolation results are summarized in Figure 2. The control animals shed significantly more virus than vaccinates on Study Days 33 to 39 ($P \leq 0.0203$).

Discussion – Study 1

The objective of this study was to demonstrate the IBR efficacy of INFORCE™ 3 in naïve calves. The study was valid because all control animals were sero-negative (serum neutralizing [SN] antibody titer of <1:2) for IBR specific antibodies prior to the start of the study and developed severe disease consisting of fever and acute IBR clinical disease following challenge. Rectal temperatures in vaccinates were significantly lower ($P \leq 0.0452$) than those in the control animals on Days 1 and 3 through 9 following challenge. While all control calves exhibited acute IBR disease following challenge, only one vaccinated calf was clinically ill. The level of IBR in nasal secretions of vaccinates was significantly lower ($P \leq 0.0203$) from 5 days through 11 following challenge compared with controls, indicating a clinically relevant reduction in virus shedding. Vaccination using tsIBR by the intranasal route of administration did not give a detectable systemic antibody response and cannot be used as a method of determining response to vaccination. No attempt was made to measure local (upper respiratory) immunity or cell mediated immunity; however, given the level of efficacy observed following challenge, it

is reasonable to assume that INFORCE 3 induced a protective immune response even though systemic neutralizing antibody was not measurable in the majority of the vaccinates at the time of challenge and the protection induced against challenge.

Results – Study 2

Serology

On Days 0 and 27, all placebo control calves were sero-negative for IBR SN antibodies (<1:2). Vaccine induced detectable IBR SN antibody responses ($\geq 1:2$) in eight of 18 (44.4%) vaccinates on Day 27 (pre-challenge). Following challenge, all animals seroconverted (Table 5). Although the IBR systemic neutralizing antibody response to vaccination was poor, upon exposure to the disease, a strong anamnestic response developed. Intranasal vaccination will not give an appreciable systemic antibody response in all animals and therefore SN antibodies should not be used as a method of determining response to vaccination.

Rectal temperatures

The post-challenge LSM rectal temperature profiles of vaccinates and controls are shown in Figure 3. The LSM rectal temperatures of vaccinates were significantly lower ($P \leq 0.0302$) than those of the controls on Days 31 through 37.

Clinical disease

The virulent IBR challenge induced acute clinical disease (morbidity) in all control animals (17/17). These calves were morbid for one or more days during the challenge phase of the study (Table 6). In contrast, only one of 18 (5.6%) IBR-vaccinated

animals developed acute IBR clinical disease for one day. The LSM percent days with disease for the placebo controls was 29.42 compared with 0.02 for the vaccinates ($P \leq 0.0001$).

Table 4 – Frequency of clinical disease following challenge

Study Day	Percentage of Animals that Developed Clinical Disease (Morbidity)	
	Placebo Control	INFORCE 3
27	0	0
28	0	0
29	0	0
30	0	0
31	0	0
32	10	0
33	90	0
34	100	5
35	100	5
36	80	5
37	50	0
38	20	0
39	0	0
40	0	0
41	0	0
42	0	0
Disease if ever present	10 of 10 (100%)^a	1 of 20 (5%)^b
LSM percent Positive Days Morbid	31.97^a	0.07^b

Different superscripts within a row represent significant differences ($P \leq 0.0001$)

Virus shedding

Virus isolation results are summarized in Figure 4. The control animals shed significantly more virus than vaccinates on Study Days 30 to 41 ($P \leq 0.0027$).

Table 5 – Individual geometric LSM IBR serum neutralizing antibody titers and seroconversion rate (%)

Treatment Group	Treatment	Study Days		
		0	27	42
T01	Placebo Control	1 (0)	1.0 (0) ^a	13.4 (100.0) ^a
T02	INFORCE 3	1 (0)	1.7 (44.4) ^b	53.7 (100.0) ^b

Different superscripts within a column represent significant differences ($P \leq 0.0030$).

Clinical assessments of respiratory disease were made according to the following criteria:

No = Normal animal.

Yes = Acute clinical IBR present: Clinical signs may include depression (animal tends to lie down frequently, moves and responds to stimuli reluctantly, holds head low, staggers and shows little interest in surroundings), mucopurulent nasal discharge and increased respiratory effort (rapid abdominal breathing, and labored and audible grunts).

Understanding Least Squares Mean Percent Days

The term “Least Squares (LS) Mean Percent Days” is used by Zoetis scientists to help characterize the extent that a particular disease parameter was observed in groups of test animals following challenge. LS mean percent days factors in the time intervals that animals are affected as well as the percentage of animals affected. In brief, LS mean percent days is the average percent of days animals in a treatment group were afflicted with a given condition.

Table 6 – Frequency of clinical disease following challenge

Study Day	Percentage of Animals that Developed Clinical Disease (Morbidity)	
	Placebo Control	INFORCE 3
26	0	0
27	0	0
28	0	0
29	0	0
30	0	0
31	0	5.6
32	23.5	0
33	82.4	0
34	82.4	0
35	88.2	0
36	52.9	0
37	35.3	0
38	35.3	0
39	23.5	0
40	0	0
41	0	0
42	0	0
Disease if ever present	17 of 17[†] (100%)^a	1 of 18 (5.6%)^b
LSM percent Positive Days Morbid^{††}	29.42^a	0.02^b

[†]One animal was removed from study prior to challenge due to injury
^{††}Different superscripts within a row represent significant differences (P ≤ 0.0001)

Discussion – Study 2

The objective of this study was to demonstrate the IBR efficacy of INFORCE™ 3 in naïve 3- to 8-day-old calves. The study was valid because all control calves (18) remained sero-negative until challenge and all (17) had IBR disease following challenge with virulent IBR virus. The LSM rectal temperatures of vaccinates were significantly lower (P ≤ 0.0302) than those of the controls on Days 31 through 37. While all control calves exhibited acute IBR disease following challenge, only one vaccinated calf was clinically ill. The level of IBR in nasal secretions from the control animals was significantly higher than vaccinated animals on 12 days post-challenge (P ≤ 0.0027). Consistent with Study 1, vaccination using tsIBR by the intranasal route of administration did not give a detectable and appreciable systemic antibody response in all animals and therefore SN antibodies should not be used as a method of determining response to vaccination. No attempt was made to measure local (upper respiratory) immunity or cell mediated immunity; however, given the level of efficacy observed following challenge, it is reasonable to assume that INFORCE 3 induced a strong protective immune response even though systemic neutralizing antibody was not measurable in the majority of the vaccinates at the time of challenge. The vaccinates did appear to be primed to IBR by the strong anamnestic SN antibody response seen following challenge.

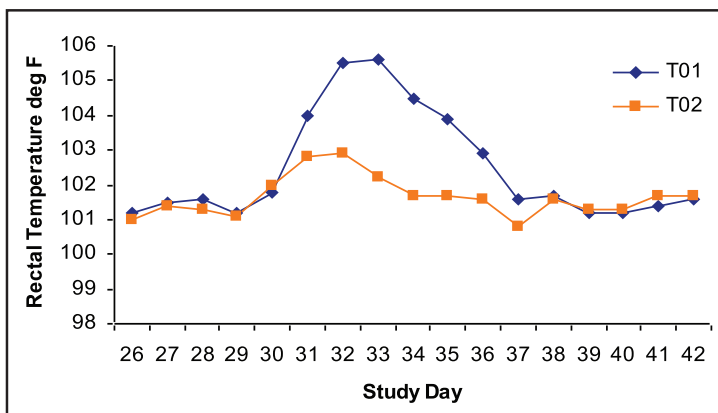


Figure 3: Least squares mean rectal temperatures before and after IBR challenge

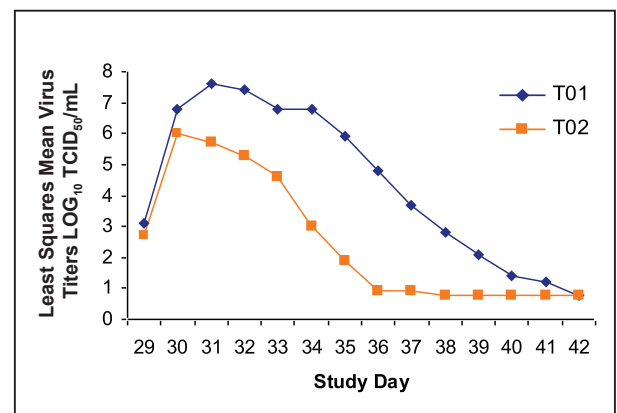


Figure 4: IBR virus isolation titers after challenge

Conclusion – Study 1 and Study 2

Based on the significant protection demonstrated in Study 1, the USDA granted INFORCE™ 3 the label claim “aids in the prevention of respiratory disease caused by IBR disease.” Study 2 data demonstrates that INFORCE 3 is effective when used in 3- to 8-day-old calves, which is important because the immune system of neonatal calves is significantly different from that of older cattle.



REFERENCES

1. Data on file, Study Report No. 3131R-60-08-548, Zoetis, Inc.
2. Data on file, Study Report No. 3131R-60-09-720, Zoetis, Inc.

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