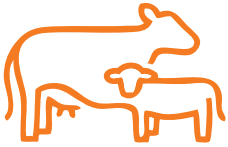


TECHNICAL BULLETIN

January 2016



INFORCE™ 3: Prevents respiratory disease caused by bovine respiratory syncytial virus.

Zoetis

100 Campus Drive
Florham Park, New Jersey 07932

SUMMARY

- Two vaccination/challenge efficacy studies were conducted to assess the ability of INFORCE™ 3 to protect cattle from respiratory disease caused by bovine respiratory syncytial virus (BRSV) when administered as a single intranasal dose.^{1,2}
- Animals in both studies were challenged with virulent BRSV (Asquith strain). Animals were placed in a sealed room and exposed to aerosolized BRSV; distributed using ultrasonic nebulizers.
- In Study 1, using 6- to 8-week-old calves, INFORCE 3 vaccinates had:
 - Significantly lower mortality (0% vs. 100%) ($P \leq 0.0001$)
 - Significantly less lung damage (5.8% vs. 57.6%) ($P \leq 0.0001$)
 - Significantly better lung function [PaO_2 (mm Hg) 83.24 vs. 42.47] ($P \leq 0.0001$)
 - Significantly less virus shedding on Days 4 and 5 post-challenge ($P \leq 0.0001$) and for a significantly shorter duration of shedding following challenge (1.3 days vs. 6.5 days) ($P \leq 0.0001$)
- In Study 2, using 3- to 9-day-old calves, INFORCE 3 vaccinates had:
 - Significantly lower mortality (0% vs. 90%) ($P \leq 0.0001$)
 - Significantly less lung damage (13.5% vs. 43.8%) ($P = 0.0003$)
 - Significantly better lung function [PaO_2 (mm Hg) 75.22 vs. 49.25] ($P \leq 0.0001$)
 - Significantly less virus shedding on Days 5 and 6 post-challenge ($P \leq 0.0055$) and for a significantly shorter duration of shedding following challenge (1.2 days vs. 3.8 days) ($P = 0.0011$)
- Study 1 demonstrated that INFORCE 3 stimulated both the local and systemic immune system to produce higher SN, IgG and IgA antibody responses following challenge.
- Based on the outstanding protection demonstrated in Study 1, the USDA granted INFORCE 3 the strongest label claim against BRSV of any vaccine on the market.

INFORCE 3 is the first and only intranasal vaccine to be granted a “prevents BRSV respiratory disease” claim. INFORCE 3 also aids in the prevention of respiratory disease caused by IBR and PI₃. In addition, an extensive safety study was conducted with INFORCE 3 demonstrating safety in all ages and classes of animals, including newborn calves and high-stress stockers.

BRSV disease

Respiratory disease is the most economically significant health problem in the beef industry, and after mastitis, the second most important health problem in the dairy industry. One of the main culprits of respiratory disease is bovine respiratory syncytial virus (BRSV), which is highly prevalent (41% to 70%)³ in the United States. BRSV infects the upper and lower respiratory tracts, including tonsillar epithelium. Severe disease caused by BRSV is most prevalent in young beef and dairy cattle.⁴ This is due to waning passive immunity and housing/management of calves in close confinement — enhancing transmission of the virus. BRSV can spread quickly in naïve cattle (three to 10 days)⁵ and is found in the nasal and tracheal mucosa in infected calves,

replicating and causing inflammation in these tissues. Clinical signs of BRSV can take two to four days to develop.⁵ BRSV infection is associated with high morbidity (60% to 80%), and fatality rates may be as high as 20%.⁴ BRSV can cause clinical disease in older heifers and adult cows, but generally older individuals will often have less severe or subclinical BRSV infection.

BRSV disease

Study 1 animals

Healthy dairy calves were selected for this trial. The calves were 6 to 8 weeks of age at the time of vaccination and were sero-negative (serum neutralizing [SN] antibody titer of ≤1:8) for BRSV. All calves were challenged except for one vaccinated animal that died of severe coccidiosis prior to challenge.

Study 2 animals

Healthy dairy calves were selected for this trial. The calves were 3 to 9 days of age at the time of vaccination and were sero-negative for BRSV, except for two vaccinated animals that were included in the study. The two initially seropositive calves in the study were not exposed to BRSV prior to enrollment,

Table 1 – Study 1 design

Vaccine	N	Vaccination Phase			Challenge Phase (Days)	
		Day	Dose	Route [†]	Challenge	Necropsy
Placebo control (IBR-BVD1-BVD2-PI ₃)	10	0	2 mL	IN	21	29
INFORCE™ 3 (IBR-BVD1-BVD2-PI ₃ -BRSV)	20	0	2 mL	IN	21	29

[†]1 mL per naris

Table 2 – Study 2 design

Vaccine	N	Vaccination Phase			Challenge Phase (Days)	
		Day	Dose	Route [†]	Challenge ^{††}	Necropsy ^{††}
Placebo control (IBR-PI ₃)	10	0	2 mL	IN	49 or 57	57 or 65
INFORCE 3 (IBR-PI ₃ -BRSV)	20	0	2 mL	IN	49 or 57	57 or 65

[†]2 mL (one naris)

^{††}Vaccinations were conducted on two separate dates in order to enroll adequate numbers of 3- to 9-day-old calves. Thirteen calves were vaccinated in the first group and 17 calves in the second group eight days later. Calves were challenged 49 days after vaccination (Day 49 or 57) and all surviving calves were humanely euthanized eight days following challenge (Day 57 or 65).

but had passively acquired a low level of colostral antibodies that had declined to sero-negative levels ($\leq 1:8$) by day of challenge. Two vaccinates also were diagnosed as persistently infected with BVDV and were removed following study initiation.

Study Design

The studies were divided into a vaccination phase and a challenge phase as described in Tables 1 and 2.

For Study 2, vaccinations were administered on two separate days due to lack of availability of calves on a single day. Vaccine was administered to 13 calves in Group 1 and to 17 calves in Group 2. For study design purposes, all animals were vaccinated on Day 0 and were challenged either 49 days post-vaccination (Group 1) or 57 days post-vaccination (Group 2) with necropsy eight days following challenge.

Test vaccines

For both studies, the placebo control calves did not receive BRSV. The placebo control animals received either a vaccine that contained IBR, BVD and PI_3 (Study 1) or IBR and PI_3 (Study 2) at levels above the minimum immunizing dose (MID) for each fraction.

The calves vaccinated with INFORCE 3 (TO2) were administered a vaccine that had the MID level of BRSV and the remaining fractions (IBR, BVD and PI_3 for Study 1) or (IBR and PI_3 for Study 2) at or above their respective MIDs.

Vaccination phase

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

For Study 1, on Day 0, calves were vaccinated intranasally with 2 mL (1 mL per naris), for Study 2, on Day 0, calves were vaccinated intranasally with 2 mL (single 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.

For Study 1: During the vaccination phase, animals were held individually in calf huts and the huts were grouped by treatment in separate airspaces. During the challenge phase, starting on Study Day 21, animals were commingled into one pen.

For Study 2: During the vaccination phase, animals were housed in individual pens and the pens were grouped by treatment. During the challenge phase, on Day 49 (Group 1 vaccinates) or Day 57 (Group 2 vaccinates) post-vaccination, all animals were commingled into one single room/pen.

Samples and assay of specimens

For Study 1, blood samples were collected from all calves on Day 0 (pre-vaccination), Day 21 (pre-challenge) and Day 29 (or on necropsy day). The serum was tested for SN antibodies and IgG to BRSV. Nasal secretions were collected using swabs on Days 20 and 23 through 29 (or day of necropsy) for virus isolation (VI). Secretions from Days 0, 20 and 29 (or day of necropsy) were tested for BRSV IgA levels. All serologic and VI assays were completed at the Western College of Veterinary Medicine Laboratories in Saskatoon, Canada.

For Study 2, blood samples were collected from all calves on Day 0 (pre-vaccination), Day 28, Day 48/56 (pre-challenge), and Day 57/65 (or on necropsy day). The serum was tested for SN antibodies and IgG to BRSV (except for the Day 28 serum samples). Nasal secretions were collected using swabs on Day 48/56 (pre-challenge) and Days 51/59 through 57/65 (or day of necropsy) for VI. Secretions from Day 0, Day 48/56 (pre-challenge) and Day 57/65 (or day of necropsy) were tested for BRSV IgA levels. All serologic and VI assays were completed at the Western College of Veterinary Medicine Laboratories in Saskatoon, Canada.

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.

Challenge phase (procedure and observations)

For both studies, animals were challenged with virulent BRSV (Asquith strain). Animals were placed in a sealed room and exposed to aerosolized BRSV for 20 to 30 minutes; distributed using three ultrasonic nebulizers.

Masking

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

Necropsy

For both studies, necropsy was performed specifically to evaluate lung lesions. The lungs were scored for percent pneumonic lesions eight days post-challenge or whenever animals died or were humanely euthanized prior to end of study.

Results – Study 1

Serology

All placebo control calves were sero-negative for BRSV SN antibodies ($\leq 1:8$) up to the day before challenge (Table 3). Vaccine induced detectable BRSV SN antibody responses ($>1:8$) in six of 19 (31.6%) vaccinates on Day 21 and 16 of 19 (84.2%) vaccinates on Day 29. The Day 21 and day of necropsy geometric least squares mean (LSM) SN titers of vaccinates were significantly greater than titers of controls ($P \leq 0.05$). The LS mean BRSV IgG titers and nasal IgA titers of vaccinates were significantly greater than titers of controls on day of necropsy ($P \leq 0.05$).

Lung lesion score, PaO₂ and mortality

There was significantly lower mortality (0% vs. 100%), significantly less lung damage (5.8% vs. 57.6%) and significantly better lung function as measured by PaO₂ levels (mm Hg) (83.24 vs. 42.47) in vaccinates

Table 3 – Summary of BRSV-specific SN, IgG and IgA titers

Treatment	Day 0 [†]	Day 20/21	Day of Necropsy
	Geometric Mean BRSV SN Titers		
T01 – Placebo control	4	4 ^a	4 ^a
T02 – INFORCE™ 3	4	6 ^b	93 ^b
Geometric Mean BRSV ELISA Serum IgG Titers			
T01 – Placebo control	6	7 ^a	8 ^a
T02 – INFORCE 3	4	10 ^a	51 ^b
Geometric Mean BRSV Nasal IgA Titers			
T01 – Placebo control	13	12 ^a	19 ^a
T02 – INFORCE 3	5	16 ^a	58 ^b

[†]Day 0 data are shown as descriptive means.
Different superscripts within a column represent significant differences ($P \leq 0.0019$).

Table 4 – Summary of lung lesions, PaO₂ and mortality

Treatment	Lung Lesion (%)		PaO ₂ (mm Hg)	Mortality
	Mean	Range		
T01 – Placebo control	57.6 ^a	33.53-84.39	42.47 ^{at}	10/10 ^a
T02 – INFORCE 3	5.8 ^b	0.26-35.55	83.24 ^b	0/19 ^b

^tOnly three controls were available for PaO₂ measurements.
Different superscripts within a column represent significant differences ($P \leq 0.0001$).

Arterial Partial Pressure of Oxygen (PaO₂)

Arterial partial pressure of oxygen (PaO₂) was measured from arterial blood samples drawn from the caudal thoracic aorta six days post-challenge. It reflects the amount of oxygen dissolved in the arterial blood. It primarily measures the effectiveness of the lungs in pulling oxygen into the bloodstream from the inhaled air. Decreased PaO₂ levels indicated pulmonary disease.

versus controls ($P \leq 0.0001$) (Table 4). As a result of severe BRSV respiratory disease, one control animal was found dead and six others were humanely euthanized on Day 27 as they met the criteria of severe respiratory disease. The remaining three control animals were euthanized on Day 28 with severe BRSV disease. Virus shedding, rectal temperatures, arterial blood partial pressure of oxygen and clinical scores were used as supportive efficacy data.

Table 5 – Summary of virus isolation								
Treatment	Study Day							
	-0	2	3	4	5	6	7	8
	Geometric Least Squares Mean Titer (PFU/mL)							
T01 – Placebo control	5 ^a	12 ^a	284.5 ^a	3769 ^a	5525 ^a	1069 ^a	216.7 ^a	-
T02 – INFORCE 3	5 ^a	5 ^a	34.0 ^a	265.8 ^b	97.6 ^b	6.1 ^a	5 ^a	5
Percent Positive Animals								
T01 – Placebo control	0	60	90	100	100	100	100	-
T02 – INFORCE 3	0	0	32	47	42	11	0	0

Different superscripts within a column represent significant differences ($P \leq 0.0001$).
A titer of ≤ 10 PFU/mL = negative

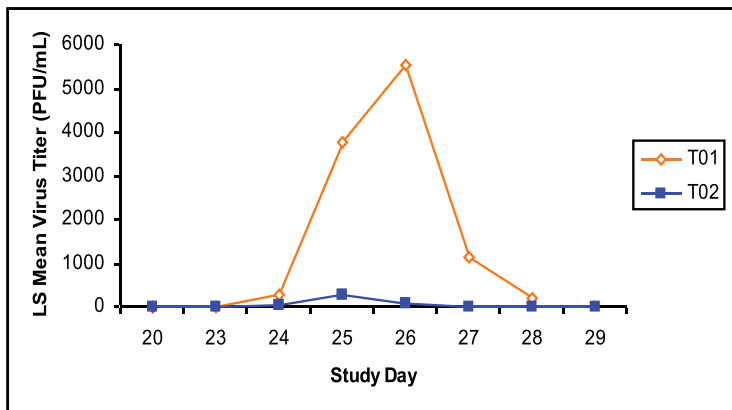


Figure 1 – Least squares mean virus shedding
Challenge was on Day 21

Virus shedding

All placebo control calves (T01) and 52.6% of vaccinates (T02) shed virus during the challenged phase of the study. Virus shedding was significantly greater ($P \leq 0.05$) in the control calves than vaccinates on Study Days 25 and 26 and was of longer duration (6.50 days vs. 1.34 days, Table 5 and Figure 1).

Discussion – Study 1

The objective of this study was to demonstrate the BRSV efficacy of INFORCE™ 3 in naïve calves. The study was valid because all animals were sero-negative (serum neutralizing [SN] antibody titer of <1:8) for BRSV prior to the start of the study and the controls remained sero-negative until the day of challenge. In addition, no BRSV was isolated from the nasal secretions of any animal on the day before challenge. All control animals developed severe BRSV disease and either died or had to be humanely euthanized prior to the end of the study, while none of the vaccinates died. In addition, vaccinates had significantly lower ($P \leq 0.0001$) lung lesion scores. The protective effect of the vaccine was further exemplified by clinically relevant differences in the observed virus shedding and PaO₂ levels

between controls and vaccinates. Overall, the vaccine primed the local and systemic immune response of vaccinates to produce higher SN, IgG and IgA antibody responses following challenge.

Results – Study 2

Serology

All control calves remained sero-negative for BRSV SN antibodies ($\leq 1:8$) throughout the study (Table 6). Following vaccination, there was little measurable response to BRSV in the circulating and local antibodies; however, following challenge exposure, there was a strong anamnestic response in all antibody types.

Lung lesion score, PaO₂ and mortality

There was significantly lower mortality (0% vs. 90%), significantly less lung damage (13.5% vs. 43.8%) and significantly better lung function as

Table 6 – Summary of BRSV-specific SN, IgG and IgA titers

Treatment	Day 0	Day 28	Challenge Day-1	Day of Necropsy
	Least Squares Mean BRSV SN Titers			
T01 – Placebo control	4 ^a	3 ^a	2 ^a	2 ^a
T02 – INFORCE 3	5 ^a	4 ^a	3 ^b	32 ^b
Least Squares Mean BRSV ELISA Serum IgG Titers				
T01 – Placebo control	11 ^a	NA	4 ^a	3 ^a
T02 – INFORCE 3	20 ^a	NA	13 ^b	81 ^b
Least Squares Mean BRSV Nasal IgA Titers				
T01 – Placebo control	7 ^a	NA	5 ^a	7 ^a
T02 – INFORCE 3	1 ^a	NA	9 ^a	37 ^b

Different superscripts within a column represent significant differences ($P \leq 0.0104$).
NA = Not available

Table 7 – Summary of lung lesions, PaO₂ and mortality

Treatment	Lung Lesion (%)		PaO ₂ (mm Hg)	Mortality
	Mean	Range		
T01 – Placebo control	43.8 ^a	10.9-86.25	49.25 ^a	9/10 ^a
T02 – INFORCE 3	13.5 ^b	0 to 28.6	75.22 ^b	0/18 ^b

Different superscripts within a column represent significant differences ($P \leq 0.0003$).

measured by PaO₂ levels (mm Hg) (75 vs. 49.3) in vaccinates versus control calves (P ≤ 0.0003) (Table 7). The BRSV challenge caused mortality in 90% of control calves and no mortalities in the vaccinated group.

Virus shedding

Eighty percent (80%) of placebo control calves and 55% of vaccinates shed virus during the challenged phase of the study (Table 8). Virus shedding was significantly greater (P ≤ 0.0055) in the control calves than vaccinates on Days 5 and 6 post-challenge. Vaccinates also shed for a

shorter duration post-challenge (1.2 days vs. 3.8 days; P = 0.002).

Discussion – Study 2

The objective of this study was to demonstrate the efficacy of INFORCE 3 following challenge with a virulent isolate of BRSV in 3- to 9-day old naïve calves. The study was valid because control calves remained sero-negative (serum neutralizing [SN] antibody titer of <1:8) until challenge and all calves were BRSV negative in pre-challenge nasal secretions.

Table 8 – Summary of virus isolation								
Treatment	Challenge Day							
	2	3	4	5	6	7	8	Ever
	Geometric Least Squares Mean Titer (PFU/mL)							
T01 – Placebo control	5 ^a	7 ^a	14 ^a	37 ^a	23 ^a	8 ^a	7 ^a	-
T02 – INFORCE 3	6 ^a	7 ^a	11 ^a	6 ^b	5 ^b	5 ^a	5 ^a	-
Percent Positive Animals								
T01 – Placebo control	0	20	50	75	50	0	0	80%
T02 – INFORCE 3	5.5	22.2	44.4	16.6	5.5	5.5	0	55.5%

Different superscripts within a column represent significant differences (P ≤ 0.0055).
A titer of ≤ 10 PFU/mL = negative

Nine of 10 control animals developed severe BRSV disease and either died or had to be humanely euthanized prior to the end of the study. In contrast, no vaccinated calves died from this challenge (0 of 18 calves).

A protective vaccine effect was observed when comparing lung lesion scores and mortality. The protective effect of the vaccine was further exemplified by clinically relevant differences in virus isolation and PaO₂ levels between controls and vaccinates.

Conclusions – Study 1 and Study 2

Based on the outstanding protection demonstrated in Study 1, the USDA granted INFORCE 3 the label claim “prevents respiratory disease caused by BRSV.” Study 2 data demonstrated that INFORCE 3 is effective when used in 3- to 9-day-old calves, which is critical because of the difference in the immune system of neonatal calves. Vaccine-induced protection from both studies seemed to correspond with the induction and boosting of higher levels of mucosal BRSV IgA and systemic SN and IgG antibody responses.



REFERENCES

- ¹ Data on file, Study Report No. 3131R-60-08-557, Zoetis, Inc.
- ² Data on file, Study Report No. 3131R-60-09-669, Zoetis, Inc.
- ³ Steven T, Grubbs ST, Kania SA, Potgieter LND. Prevalence of ovine and bovine respiratory syncytial virus infections in cattle determined with a synthetic peptide-based immunoassay. *J Vet Diagn Invest* 2001;1:128-132.
- ⁴ Valarcher JF, Taylor G. Bovine respiratory syncytial virus infection *Vet Res* 2007;38:153-180.
- ⁵ Belknap EB. Recognizing the clinical signs of BRSV infection. *Vet Med* 1993;88:886-887.