# TECHNICAL BULLETIN

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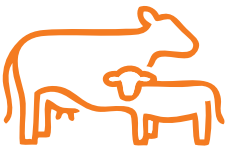

## INFORCE™ 3:
## Safety demonstrated in three separate studies.

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**SUMMARY**

- USDA field safety study summary:[1]
  - INFORCE™ 3 was administered intranasally to 0- to 8-day-old calves, weaned and high-stressed calves, and pregnant cows from all three trimesters.
  - A total of 1,873 animals in three geographic locations and in several different categories (e.g., pregnant) were enrolled in the study, of which 1,253 were vaccinates and 620 were nonvaccinated controls.
  - No local or systemic post-vaccination (within four hours of vaccination) adverse events were reported in any animal in the study.
  - There were no adverse events directly related to INFORCE 3 vaccination under field conditions.
- Intense safety in young calves:[2]
  - INFORCE 3 and a commercial 5-way intranasal vaccine were both administered intranasally using a 10X concentrated dose to 3- to 10-day old colostrum-deprived calves. In addition, a booster dose of a commercial vaccine was administered parenterally to all animals 14 days post-intranasal dose.
  - INFORCE 3 was safe and did not induce any clinical disease, increased rectal temperature, viremia or leukopenia.
- High-risk stocker cattle study:[3]
  - INFORCE 3 and a commercial 5-way intranasal vaccine were both administered intranasally to high-risk stocker calves on arrival to the feedlot. In addition, a booster dose of a commercial 5-way vaccine was administered parenterally to all calves 14 days post-intranasal dose.
  - The safety and demonstrated efficacy of INFORCE 3 has earned the trust of veterinarians and cattle producers and made it one of the leading vaccines in the United States.

INFORCE 3 aids in the prevention of respiratory disease caused by IBR and PI3 and prevents BRSV respiratory disease. Extensive safety studies were conducted with INFORCE 3, demonstrating safety in all ages and classes of animals, including newborn calves and high-stress stockers.[2]

*USDA requirements for field safety studies (FSS) are conducted in accordance with Veterinary Services Memorandum 800.204. FSS are evaluated under typical field husbandry conditions. The objective of such trials is to detect adverse events of unexpected type or frequency that might indicate the need for further investigation. More than one serial (batch or lot) of product must be tested and the product must represent the product that will be produced once it's licensed. Studies need to be conducted in multiple geographic regions; typically, three distinct regions of the United States (U.S.) are required with a minimum of 200 animals per region (600 in toto). The product must be tested under various conditions of husbandry and all types of animals (age, breed, sex, pregnancy and/or lactation status, and any other distinguishing features) that are to be included in label recommendations should be included.*

## Overview of the INFORCE 3 USDA field safety study

The rigor and commitment to ensure the safety of INFORCE 3 went beyond the USDA regulations. In addition, during the development of INFORCE™ 3 it became apparent that a competitor had to issue a temporary stop sale and distribution of its 5-way intranasal vaccine only six months post-launch to address a safety issue. It was required by the USDA to add the following warning statement to its label: "Do NOT vaccinate high-stress stocker cattle or high-risk cattle on arrival."

When designing the USDA required field safety study (FSS), Zoetis decided to evaluate more than double the number of required animals (1,253 in toto) and ensured all ages and classes of animals were tested, including newborn calves and high-stress stockers. INFORCE™ 3 also was tested for safety in all stages of pregnancy (200 per trimester). The extent of the number and type of animals included in the FSS was strongly influenced by customer experiences with a recently approved intranasal vaccine.

**Table 1 – USDA field safety study — animals enrolled by category**

| Category | Location* | Placebo Controls | INFORCE 3 PLS 1 | INFORCE 3 PLS 2 | Total Number | Sex |
|---|---|---|---|---|---|---|
| Calves (0- to 8-day-old) | CA | 54 | 53 | 53 | 160 | ♂ |
| | IN | 54 | 53 | 53 | 160 | ♂ |
| Calves (2 to 3 months) | CA | 26 | 27 | 27 | 80 | ♀ |
| | IN | 26 | 27 | 27 | 80 | 68♂ 12♀ |
| Calves (6 to 12 months) | CA | 26 | 27 | 27 | 80 | ♀ |
| | IN | 26 | 27 | 27 | 80 | ♀ |
| High-stress stockers | MO | 100 | 100 | 100 | 300 | ♀ |
| Pregnant animals | CA | 151 | 134 | 150 | 435 | ♀ |
| | IN | 101 | 121 | 106 | 328 | |
| | MO | 56 | 57 | 57 | 170 | |
| Total for all groups | - | 620 | 626 | 627 | 1873 | - |

*CA = California, IN = Indiana, MO = Missouri
PLS = Prelicense Serial

**Table 2 – USDA field safety study — pregnant animals enrolled by trimester**

| Treatment Group | Location* | Trimester 1 | Trimester 2 | Trimester 3 | Total |
|---|---|---|---|---|---|
| Placebo Control | CA | 55 | 27 | 69 | 151 |
| INFORCE 3: PLS 1 | CA | 60 | 22 | 52 | 134 |
| INFORCE 3: PLS 2 | CA | 61 | 24 | 65 | 150 |
| Placebo Control | IN | 47 | 39 | 15 | 101 |
| INFORCE 3: PLS 1 | IN | 44 | 50 | 27 | 121 |
| INFORCE 3: PLS 2 | IN | 42 | 45 | 19 | 106 |
| Placebo Control | MO | 2 | 36 | 18 | 56 |
| INFORCE 3: PLS 1 | MO | 0 | 32 | 25 | 57 |
| INFORCE 3: PLS 2 | MO | 1 | 37 | 19 | 57 |
| Total for all groups | - | 312 | 312 | 309 | 933 |
| Total Vaccinates/Trimester | - | 208 | 210 | 207 | 625 |

*CA = California, IN = Indiana, MO = Missouri
PLS = Prelicense Serial

**Table 3 – USDA field safety study design**

| Treatment | Vaccine | N | Approximate Study Days | | |
|---|---|---|---|---|---|
| | | | Vaccination | Dose | General Health Observation |
| T01 | None or licensed parenteral vaccine | 620 | Day 0* | 2 mL | Day 0 through Day 28/29 |
| T02 | INFORCE 3: PLS 1 | 626 | | | |
| T03 | INFORCE 3: PLS 2 | 627 | | | |

*Day 0 was staggered depending upon age group availability
PLS = Prelicense Serial

## STUDY DESIGN

### *Animal Categories*

A total of 1,873 animals (1,485 females and 388 males) were enrolled in the study and included 1,402 Holsteins, one Holstein-cross and 470 beef breed cattle. Tables 1 and 2 summarize the animals enrolled by study location, animal category and the respective pregnancy trimester.

A nonvaccinated control group was included for each animal category to provide an estimate of the current disease rate at each site. These rates supplemented the historic disease rates that the investigators observed at each site.

### *Test vaccines*

Prelicense serials (PLS) are a requirement of the USDA and can be evaluated for safety under field conditions. INFORCE™ 3 PLS 1 and 2 were produced under routine manufacturing conditions and met all the requirements necessary for commercial released serials.

### *Vaccination*

All vaccinated animals given either INFORCE 3 PLS 1 or 2 were vaccinated once intranasally with 2 mL using a cannula attached to a syringe. With the exception of the stocker calves in Missouri, which were vaccinated with 5 mL of a commercial vaccine, the placebo control animals received no other vaccine (Table 3).

### *Post-vaccination observations*

Within four hours post-vaccination, all animals were observed by the investigator and any untoward local or systemic reactions were noted. In addition, for 28 days post-vaccination, all animals were observed daily for any clinical disease or adverse events, which were recorded and summarized by site, animal category, diagnosis and whether related to INFORCE 3. Any dead animals were necropsied and a likely cause of death was determined. Pregnant animals were monitored for abortion or parturition. Calves that were born during the study were observed for the duration of the study.

## Results — USDA field safety study

Within four hours after vaccination, no local or systemic reactions were observed in any animal. During the 28-day observation period, 101 of 620 controls (16.3%), 118 of 626 of the INFORCE 3 PLS 1 vaccinates (18.8%) and 105 of 627 INFORCE 3 PLS 2 vaccinates (16.7%) had an adverse event; however, none of these adverse events were determined to be related to INFORCE 3 by the investigators.

## Discussion — USDA field safety study

The rate of adverse events observed were consistent with expectations and were not directly related to administration of INFORCE 3. Adverse events that were unrelated to administration of INFORCE 3 were observed at all three sites and bore a

unique association with the type of animal, site management protocols and the season when the study was conducted. The overall frequency of these adverse events was distributed evenly in the placebo controls and vaccinates.

Adverse events included gastroenteritis, otitis media, bacterial conjunctivitis and *Mannheimia haemolytica* pleuropneumonia. Tissues from animals that died in all calf categories were negative for IBR, PI3 and BRS viruses, and the investigators determined that the cause of these adverse events were not related to the test product.

## Overview of additional safety studies with INFORCE™ 3

### *Intense safety study in young calves*

As previously discussed, during the development of INFORCE™ 3, it became apparent that a competitor had to issue a temporary stop sale and distribution of its 5-way intranasal vaccine only six months post-launch to address a safety issue. It was required by the USDA to add the following warning statement to its label: "Do NOT vaccinate high-stress stocker cattle or high-risk cattle on arrival."

Zoetis' commitment to test and ensure the safety of INFORCE 3 prior to commercializing the product was demonstrated in two additional independent clinically controlled safety studies. The first of which is the Safety in Young Calves and is described below (Table 4).

### *Animals*

Healthy colostrum-deprived Holstein calves were selected for this study. The calves were 3 to 10 days old at the time of vaccination and were sero-negative (serum neutralizing [SN] antibody titer of ≤ 1:4 for IBR, PI3 and BVDV Types 1 and 2, and ≤ 1:8 for BRSV). The calves also were not persistently infected (PI) with bovine viral diarrhea virus.

## STUDY DESIGN

### *Vaccine/Vaccination*

The sentinel calves did not receive any vaccine. The INFORCE 3 and commercial 5-way vaccinated calves received vaccines that were reconstituted at the time of vaccination (Day 0) with liquid diluent to contain a 10X concentrated dose. On Study Day 14, all calves except sentinels received a 5-way MLV vaccine at the recommended dose by the subcutaneous route.

## Pertinent variables measured

### *White blood cell counts*

EDTA blood was collected for determination of white blood cell (WBC) levels before and after IN vaccination to determine if either vaccine induced leukopenia. Leukopenia was defined as a post-vaccination drop of WBC counts of ≥ 40% of the baseline level. The baseline count was determined by averaging the individual counts for each calf on the three days prior to vaccination.

**Table 4 – Study design**

| Treatment | Number of Animals on Day 0 | Formulation | Intranasal Vaccination (Day 0) | Parenteral Vaccination (SC) (Day 14) |
|---|---|---|---|---|
| INFORCE 3 | 10 | 10X concentration | 2 mL | 5-Way MLV vaccine |
| Sentinels | 3 | - | - | - |
| Commercial 5-way Intranasal | 9 | 10X concentration | 2 mL | 5-Way MLV vaccine |
| Sentinels | 3 | - | - | - |

### *Rectal temperatures and clinical observations*

Rectal temperatures were collected from Days -2 through 14 and clinical observations were recorded from Days -2 through 25. Fever was defined as any rectal temperature ≥ 103.3°F following vaccination. An animal was considered to have clinical disease if it exhibited symptoms, including abnormal nasal discharge, abnormal respiration and/or lethargy.

### *Necropsy*

On Days 7, 14 and 18, one calf from each group was euthanized and necropsied for determination of gross pathology and histopathology. At the end of the study, all remaining calves were euthanized and necropsy findings recorded. Immunohistochemistry (IHC) was conducted on lung tissues for detection of IBR or BVD antigen.

### *Masking*

All clinical observations and laboratory testing were conducted and recorded without the knowledge of treatment group assignment.

## RESULTS

Mild to moderate clinical disease as defined above was observed in five out of nine (55.6%) and fever in four out of nine (44.4%) commercial vaccine IN vaccinated calves while only one calf vaccinated with INFORCE 3 was clinically ill and no fever was recorded for calves vaccinated with INFORCE 3 (Table 5). Neither INFORCE 3 nor the commercial vaccine vaccinated calves developed leukopenia following IN vaccination. Gross pathology and histopathology results of all calves were either normal or mild, and not specific for any particular etiology. Tissues from only one calf were positive for BVDV antigen, a calf in the INFORCE 3 group that was necropsied at the end of the study. No tissues collected during the study were positive for IBR antigen.

**Table 5 – Summary of leukopenia, clinical disease and fever**

| Treatment | N | Leukopenia (40% drop) | Mild to Moderate Clinical Disease | Moderate Fever (≥ 103.3°F) |
|---|---|---|---|---|
| INFORCE 3 | 10 | 0 | 1 | 0 |
| Commercial 5-way Intranasal | 9 | 0 | 5 | 4 |

## DISCUSSION

In this intense safety study in young calves, it appears that INFORCE 3 is safe. INFORCE 3 did not induce any clinical disease, increase rectal temperature or cause leukopenia. In contrast, the commercial 5-way intranasal vaccine induced clinical disease and fever.

## Overview of additional safety studies with INFORCE™ 3

The third safety study conducted was designed to validate the safety of INFORCE 3 in high-risk cattle. The study was conducted according to a typical feedlot entry vaccination regimen as described below:

**Table 6 – Study design**

| IN Vaccine on Arrival (Day 0) | Number of Animals | SQ Vaccine (Day 14) |
|---|---|---|
| None | 20 | Commercial 5-way MLV vaccine |
| INFORCE™ 3 | 20 | Commercial 5-way MLV vaccine |
| Commercial 5-way Intranasal | 20 | Commercial 5-way MLV vaccine |
| Sentinels | 4 | None |

### *Animals*

Healthy cross-bred beef heifers at 4 to 5 months of age and weighing approximately 400 pounds were obtained from multiple sources for this trial.

At arrival, animals were allotted to treatment group and pen and received doramectin, ceftiofur crystalline free acid and ULTRABAC® 8 per label directions. An ear notch sample also was collected to test for BVDV persistent infection (PI). Multiple animals of the same treatment were housed in the same pen, with three pens for each treatment (seven, seven and six animals per pen). The sentinel animals were housed in the pens with the animals that received no IN vaccine on arrival.

## STUDY DESIGN

### *Vaccine/Vaccination*

The sentinel calves did not receive any vaccine (Table 6). The calves vaccinated with INFORCE™ 3 and the commercial vaccine received vaccines containing commercial release levels of all fractions. On Study Day 14, all calves except sentinels received a subcutaneous dose of the 5-way commercial MLV vaccine.

## Pertinent variables measured

### *Clinical observations*

All animals were observed once daily for clinical signs of respiratory disease and overall health, and given a clinical score related to severity of disease according to the following criteria:

No = Normal animal, no clinical signs.

1 = Nonspecific clinical signs. Clinical signs as a whole are not specific for acute viral infections by IBR, BRSV or BVDV. Clinical signs may include nasal discharge, abnormal respiration and mild lethargy.

2 = Acute clinical disease. Clinical signs as a whole are moderate in degree and specific for acute infection. Clinical signs may include nasal discharge, abnormal respiration, lethargy, gauntness, ocular discharge, hypersalivation, diarrhea, dehydration, lameness and/or reluctance to move.

3 = Severe clinical disease. Clinical signs as a whole are severe in degree and specific for acute IBR, BRSV or BVD infection. Clinical signs may include nasal discharge, abnormal respiration, lethargy, gauntness, ocular discharge, hypersalivation, diarrhea, excessive bruising, dehydration, recumbency, lameness and/or reluctance to move. Animals that are moribund or unable to reach food or water were euthanized.

### *Rectal temperatures*

Rectal temperatures were only collected and recorded if an animal qualified to be pulled from the pen in order to check if it qualified for treatment.

### *Concurrent treatments and samples*

Animals were not pulled (examined) for the first five days following the arrival dose of ceftiofur crystalline free acid. After the first five days, animals were pulled and received treatment if they met the following qualifications:

1. Animals that had a clinical score of 2 or greater

2. Pulled animals had rectal temperatures taken and those with temperatures ≥104.0°F qualified for treatment

Animals that qualified for treatment received tulathromycin and were not pulled for 10 days. Those animals that re-qualified for treatment following tulathromycin were given danofloxacin mesylate and were not pulled for 48 hours. Animals could have received one additional dose of danofloxacin mesylate if they re-qualify for treatment following the first 48-hour period. If an animal re-

qualified for treatment following 48 hours after the second dose of danofloxacin mesylate, the animal was humanely euthanized. If animals had < 104.0°F on examination, they were returned to the pen without treatment. All animals were returned to their pen of origin following examination and/or treatment unless they qualified for euthanasia.

### *Samples collected*

As an animal qualified and received treatment for the first time, blood and nasal secretions were collected from the animal (first time only).

### *Necropsy*

All animals that were found dead or euthanized were necropsied. On Day 28, animals that were considered to have respiratory disease or in poor health by the person scoring the animals were necropsied and assessed for gross lesions. Samples were collected from specific sites for histopathology, PCR (IBR, BVDV, PI3 and BRSV) and immunohistochemistry (IHC).

## RESULTS

Forty-five percent (9 of 20) of the animals that received the commercial 5-way intranasal vaccine had a clinical disease score of ≥ 2 at some point during the study (Table 7). From those nine animals, five qualified for at least one extra treatment during the study and three animals were euthanized due to respiratory distress or poor health. With INFORCE 3, 15% (3 of 20) of animals had a clinical score of ≥ 2, two required treatment and one animal was found dead the day after the study was over.

## DISCUSSION

The objective of this study was to determine if INFORCE 3 was safe for use in high-stress stocker cattle. The study was conducted in advance of the USDA field safety study and following the voluntary change to the product usage caution statement of a relatively new 5-way intranasal MLV vaccine. On arrival, vaccination with INFORCE 3 resulted in fewer pulls and treated animals compared with the group receiving the commercial 5-way intranasal vaccine. The necropsies performed on the four animals that were euthanized/died showed chronic respiratory disease. The one animal vaccinated with INFORCE 3 that died the day following the end of the study was included in the report in an attempt to obtain as much information as possible about vaccine safety. Tissue IHC was positive for IBR in three of four animals; however, no attempt was made to determine if the IBR was a wildtype or vaccine strain. A fresh sample of lung collected at necropsy was PCR negative for all five respiratory viruses. Due to the chronicity of disease in these animals, the exact cause of respiratory disease could not be determined.

Following this study, the safety of INFORCE 3 in high-stress stocker cattle was further demonstrated in the USDA field safety study. INFORCE 3 does not have any product use caution statement

**Table 7 – Summary of animals being pulled (examined) and treated for clinical disease and total number of dead animals by treatment group**

| Initial Treatment | N | Pulled | Treated | Dead |
|---|---|---|---|---|
| None | 20 | 6 | 3 | 0 |
| INFORCE 3 | 20 | 3 | 2 | 1* |
| Commercial Vaccine | 20 | 9 | 5 | 3 |
| Sentinels | 4 | 2 | 2 | 0 |

*Found dead after study was over

relative to use in high-stress stocker cattle or high-risk cattle.

## Overall Conclusions

The outstanding data generated from these studies demonstrated the safety of INFORCE 3 in very young high-stress calves, weaned and high-stress stocker calves, and pregnant heifers and cows under field conditions.

In addition, INFORCE 3 was found to be safe in both colostrum-deprived calves given a 10X concentrated dose and in high-risk stocker cattle vaccinated according to a typical feedlot entry vaccination regimen.

## REFERENCES


1. Data on file, Study Report No. 3437R-60-09-716, Zoetis, Inc.
2. Data on file, Study Report No. 3131R-60-09-671, Zoetis, Inc.
3. Data on file, Study Report No. 3131W-60-09-672, Zoetis, Inc.