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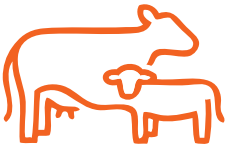
TECHNICAL BULLETIN



Benefits of vaccinating peri-parturient dairy cows with INFORCE 3® and improved health outcomes that indicate intranasal vaccination circumvents the immunosuppressive effects of gestation and calving

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SUMMARY

- Peri-parturient dairy cows generally experience a profound suppression of the systemic immune system as a result of the hormonal and metabolic stress of gestation, calving, and lactation.
- Peri-parturient immune suppression in dairy cows is associated with increased susceptibility to infectious diseases and an impaired ability to respond to parenteral vaccination.
- Prior studies have shown that intranasal (IN) vaccination with INFORCE 3® stimulates antigen-specific and non-specific secretory immune responses in respiratory tissue of peri-parturient dairy cows.
- In this study, peri-parturient cows vaccinated with INFORCE 3® had improved health outcomes.
- Cows were randomly assigned to one of three vaccinated groups or a control group; vaccinated cows received either a single dose of INFORCE 3® 18-24 days pre-calving (group PC), a single dose on the day of calving (DC), or two doses given pre-calving and the day of calving (PC/DC). Outcomes were compared for the vaccinated and nonvaccinated control groups.
- Compared to control cows, vaccinated cows had a lower incidence of pneumonia approaching statistical significance (P =0.06).
- The cumulative impact of the lower rates of adverse health events in vaccinated cows resulted in a significantly lower rate (P=0.028) of culling and mortality compared to control cows.
- Although the differences in results between the three vaccinated groups were not significant, the study generally supported INFORCE 3® vaccination on the day of calving (DC) vs. pre-calving (PC), and vaccination with two doses (PC and DC) instead of a single dose.
- The large study population (> 1,100 cows in each treatment group) and the controlled experimental design gave statistical power to the study results.
- A herd health strategy that includes IN vaccination with INFORCE 3® of adult dairy cows shortly before or on the day of calving, either with one or two doses, can help offset the risk of adverse health events during the peri-parturient period.

INFORCE 3® (Zoetis), an intranasal (IN) vaccine for prevention of respiratory disease caused by infectious bovine rhinotracheitis (IBR) virus, parainfluenza type-3 (PI_3) virus, and bovine respiratory syncytial virus (BRSV), met the challenge-of-immunity thresholds for these three commonplace infectious diseases of cattle that continue to affect the beef and dairy industries.[1-5]

The ability of modified live-virus IN bovine respiratory vaccines to stimulate local expression of interferon and specific anti-viral nasal IgA is well established.[6-12] For example, investigators have demonstrated high levels of interferon (IFN) in nasal secretions, consistent seroconversion, and protection against virulent challenge following vaccination of calves with a live, temperature sensitive strain of IBR virus, a precursor to the strain used in INFORCE 3®.[10]

More recently, Cortese et al measured significant increases in nasal IFN-gamma, total IFN, and nasal IBR virus IgA levels in dairy cows (N = 32) given peri-parturient vaccination with INFORCE 3®.[12] This outcome was noteworthy, not only for the consistency of the immune response in a sizeable test population but also because IN vaccination helped circumvent the systemic immune suppression that occurs as a result of peripartum stress associated with gestation, calving and lactation.[13-16] Unlike previous studies in seronegative calves,[1-5] the study in peri-parturient cows demonstrated that a post-vaccination secretory immune response occurred in adult dairy cattle exposed to immunosuppressive hormonal and metabolic changes.

The study described in this report further establishes the value of INFORCE 3® by measuring the impact of vaccination on several herd health parameters in adult dairy cows. The incidence were measured in cows following peri-parturient vaccination with either one or two doses of INFORCE 3®. The study was conducted in a large, well managed dairy with a relatively low potential for adventitious disease exposure or other confounding factors to occur.

Study Design

Test animals

All cows enrolled in the study were pregnant Holsteins. Cows with acute or chronic health conditions were excluded from the study, as were any cows not calving within 30 days of vaccination with INFORCE 3®. All cows were multi-parous (parity ≥2). In addition to INFORCE 3®, cows were vaccinated in accordance with the dairy's standard protocol. This included a multi-valent parenteral vaccine against viral respiratory-reproductive pathogens, a five-way leptospirosis bacterin, a multi-valent clostridial vaccine, a viral-bacterin combination calf scours vaccine, and an *E. coli* bacterin-toxoid for mastitis control.

Test site and facilities

The study was conducted on a large, pasture-based organic dairy located in Colorado. Treatment groups were separated to avoid nose-to-nose contact and sharing of feed and water. Cows were maintained in separate pens according to treatment classification. A minimum of 25 feet of free air space between treatment pens was maintained for 2 weeks following vaccination to avoid horizontal transfer of the live-virus vaccine agents between test groups.

Treatment groups and study parameters

INFORCE 3® was administered with a provided cannula as a 2 cc IN dose according to label directions. Vaccination was conducted either by or under the supervision of the attending veterinarian. Cows were randomly assigned to one of four treatment groups **(Figure 1)**. Group T1 consisted of unvaccinated controls (C); group T2, cows vaccinated with a single dose 18-24 days pre-calving (PC); group T3, cows vaccinated with a single-dose on the day of calving (DC); and group T4, cows vaccinated with two doses, 18-24 days pre-calving and on the day

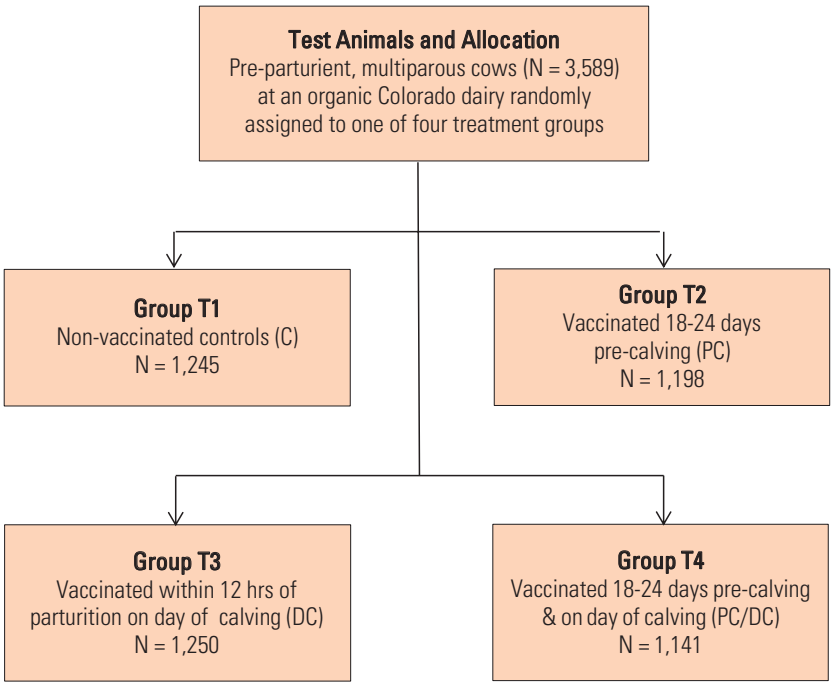

Figure 1 – The experimental design is shown for cows in the INFORCE 3® peri-parturition study. Pregnant adult cows (parity ≥ 2) were blocked by parity and calving date and randomly assigned within the block to one of the four treatment groups shown. Groups T2 and T3 were vaccinated with a single dose, group T4 with two doses.

of calving (PC/DC). Cows given a DC dose were vaccinated within 12 hours of calving.

Several post-calving parameters were measured for each treatment group. The incidence of cow removal due to mortality and culling and the incidence of pneumonia were determined for the first 60 DIM and for the full lactation period. Individual cows may have experienced recurring cases of mastitis or uterine infections, all of which were included for purposes of statistical analysis.

Treatment groups and study parameters

The study utilized a randomized complete block experimental design. Animals were individually identified and blocked by parity and calving date and then assigned to one of the four treatment groups within a block. The primary variables for the study were disease morbidities during the lactation period, production responses, culling and death losses.

Results

Cow removal

The rate of cow removal for the four treatment groups is shown in **Figure 2**. During the first 60 DIM, there was little variation in the cow removal rate among the four treatment groups. Among the three vaccinated groups (PC, DC, and PC/DC), the overall cow-removal rate (culls and mortalities) during the first 60 DIM varied by <1.0%, and the combined cow-removal rate for the three vaccinated groups differed by <0.5% from the rate for the control group (4.23% vs. 4.72%, *P*=0.49).

When the entire lactation period is considered, the overall rate of cow removals due to mortality and culling was significantly lower in PC/DC cows given two doses of INFORCE 3® vs. the control group, 19.4% vs. 22.1% (*P*=0.028), a 13.9% relative increase in the removal rate for control cows.

Among the three vaccinated groups, the cow-removal rate for the full lactation period varied by <0.35% (19.24% to 19.58%), a non-significant difference. However, compared to the control group, the overall cow-removal rate during the full lactation period increasingly declined for cows given a single dose pre-calving (PC), a single dose on the day of calving (DC), and two doses at PC/DC.

Pneumonia

The incidence of pneumonia was lower in each of the vaccinated groups compared to controls, both for the first 60 DIM and for the full lactation period (**Figure 3**). The differences in incidence rates were not significant for the first 60 DIM. When the combined incidence rate for all INFORCE 3® vaccinated groups (5.71/100 cows) was compared to control group (7.56/100 cows) for the full lactation period, the difference approached statistical significance (*P*=0.06). When expressed as the relative difference in pneumonia incidence rates for the full lactation period, the control group had a 24.5% higher rate of pneumonia compared to the combined vaccinated groups.

Discussion

Peri-parturient cows vaccinated with INFORCE 3® had improved health outcomes for several commonplace disease conditions that adversely affect dairy operations. Vaccinate cows had a reduction in the incidence of pneumonia approaching significance. The cumulative impact of these favorable health outcomes in vaccinated cows was a significant lower rate of culling and mortality compared to the overall

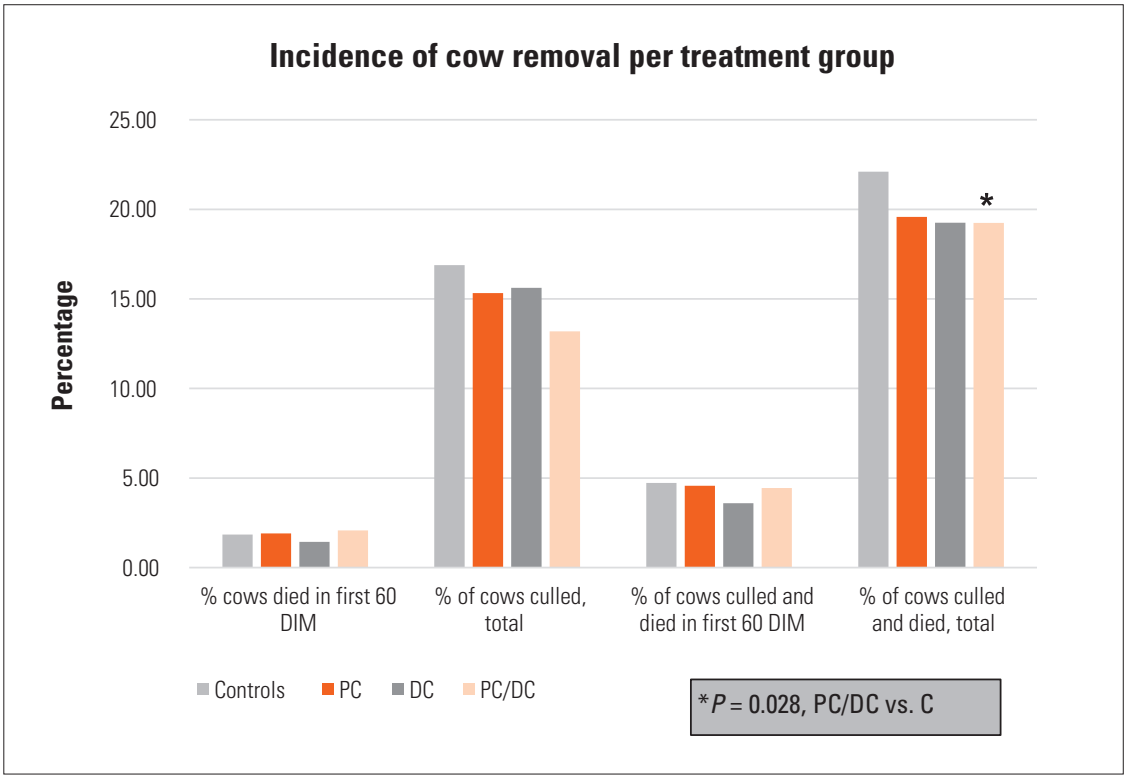

Figure 2 – The chart shows the cow-removal percentage for the four test groups. Cows given two doses of INFORCE 3® (group PC/DC) had the lowest percentage of removals when the number of culls and mortalities were combined for the full lactation period. The overall cow-removal rate for the PC/DC group was significantly lower for the full-lactation period vs. controls (19.40% vs. 22.10%), a significant difference, *P* = 0.028.

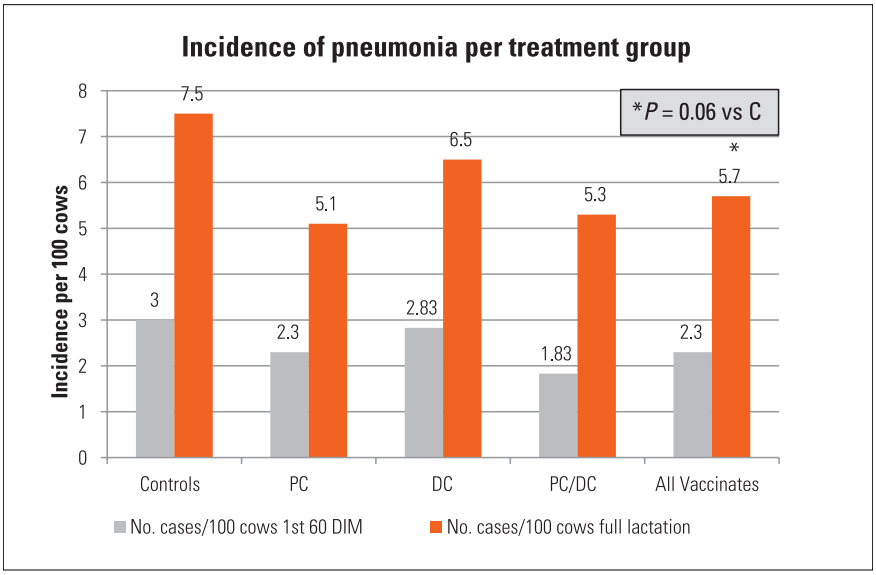

Figure 3 – The chart shows the incidence of pneumonia per 100 cows in the four treatment groups during the first 60 days in milk (DIM) and through the entire lactation period. Compared to controls, the incidence of pneumonia trended lower for each of the INFORCE 3® vaccination groups, including cows given a single dose pre-calving (PC), a single dose on the day of calving (DC), or two doses PC and DC, both for the 60-day and full lactation periods. When the incidence per 100 cows for all vaccinated groups was combined (5.71/100 cows), the rate approached significance (*P*=0.06) when compared to the rate for controls (7.56/100 cows) for the full lactation period.

cow-removal rate in the control group (**Figure 2**). Mortality is an objective measure of health status. Although the difference was not statistically significant, cows in the DC vaccinated group had a lower death loss vs. the control group, both during the first 60 DIM and for the full lactation period.

The relatively low rate of pneumonia in vaccinated cows was noteworthy because the herd had been previously vaccinated for bovine respiratory disease pathogens, and the adult cows had a strong likelihood of previous natural exposure and seropositive immune status. Despite the probability of some degree of pre-existing systemic immunity, cows vaccinated with INFORCE 3® had improved protection against pneumonia, the probable result of a mucosal immune response following IN vaccination. In this respect, INFORCE 3® functions as a vaccine and an immune modulator, boosting the local mucosal immune response even in peri-parturient cows with impaired systemic immune function and in cows with residual serologic immunity. The results of the study described here are a clinical validation of prior research demonstrating that INFORCE 3® elicits specific and non-specific secretory immune response in the nasal mucosa of peri-parturient cows.[12]

Although the differences in results among the three vaccinated groups were not significant, the study generally supported INFORCE 3® vaccination on the day of calving (DC) vs. 18-24 days pre-calving (PC), and vaccination with two doses (PC and DC) instead of a single dose. For example, the overall rate of cow removal was lower in DC vs. PC cows, and lowest in the PC/DC group.

A recent immunologic study offers an intriguing explanation for how a mucosal immune response at the respiratory point of entry can stimulate protection at other mucosal sites such as the GI and reproductive tracts.[17] The investigators found that pulmonary immunization in lab animals produced

increased expression of mucosal homing receptors and durable cytotoxic T-cell expression both in the lungs and reproductive mucosa. Thus, a mucosal immune response at one site resulted in cross-presentation of vaccine antigens at other mucosal sites, producing in a robust, local protective immune response in multiple secretory surfaces. This apparent "crosstalk" between mucosal compartments was enabled by persistence of antigen in draining lymph nodes and cross-presentation at other mucosal sites.

These findings suggest that "aerosol or intranasal delivery might be capable of protecting against not only airway or systemic infections but also gastrointestinal or reproductive tract pathogens." While this is possible following local presentation of antigen, very few antigen presenting cells in lymph nodes acquire immunizing antigen after parenteral vaccination. In other words, mucosal cross-protection is possible following local immunization but not systemic immunization.

The degree of immune suppression and its consequences in dairy cows during the peri-parturient period is often profound. Investigators have found that dairy cows during parturition may exhibit a 3 to 4-fold increase in plasma levels of the stress hormone cortisol, and hypocalcemic cows at calving may experience a 5 to 7-fold increase in plasma cortisol.[18] This is accompanied by severe depression of lymphocyte proliferation and cytotoxic activity and reduced phagocytosis by neutrophils, the principal cellular component of the innate immune response to mastitis.[19] The peri-parturient stress response can increase adrenalin production, which results in loss of uterine tonality and impaired ability to expel fetal membranes and the placenta. This increases the risk of retained placenta, which then becomes a culture medium for bacterial infections, occurring in >10% of cows in high-producing herds.[18] Most peri-parturient dairy cows experience reduced feed intake, negative energy balance, insulin resistance, hypocalcemia, and overall reduction in immune function as well as a high likelihood of bacterial contamination of the udder and uterus.[18] The combination of these factors places adult dairy cows at risk of various acute and chronic diseases, suboptimal production, and reduced longevity, ultimately leading to removal of under-performing cows from the herd.

There has been limited research on the nature of the mucosal respiratory immune response in at-risk adult cattle during the peri-paturient period. However, recent data has demonstrated that IN vaccination with INFORCE 3® stimulates innate and antigen-specific local mucosal immunity in adult dairy cows.[12] This mode of action helps circumvent the effects of systemic immune suppression resulting from the stresses of gestation and parturition. A herd health strategy that includes IN vaccination with INFORCE 3® of adult dairy cows shortly before or on the day of calving, either with one or two doses, can help offset the risk infectious respiratory disease.

Acknowledgment

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