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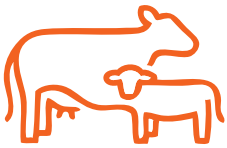
TECHNICAL BULLETIN



INFORCE 3®: Efficacy and Duration of IBR Protection After Intranasal Vaccination of Neonatal Calves

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Summary

- Three research studies[1] investigated the short- and long-term IBR efficacy of intranasal INFORCE 3® (a trivalent modified-live virus vaccine) when administered to young neonatal calves.
  - 120 dairy calves (3 to 8 days old) seronegative for antibody against BoHV-1 (experiments 1 and 2) or seropositive for maternally derived antibody against BoHV-1 (experiment 3), thus allowing evaluation of INFORCE 3 performance in the presence of maternal antibodies.
  - Calves were challenged with virulent BoHV-1 on day 28 or 193 (seronegative calves) or day 105 (seropositive calves) after vaccination.
- Calves challenged 28 days after INFORCE 3 vaccination demonstrated 94.4% less IBR incidence and 97.9% reduction in disease duration vs controls, plus favorable impacts on rectal temperature, nasal shedding of virus, and post-challenge antibody titers.
- Similar results were observed for calves challenged 193 days (over 6 months) after INFORCE 3 vaccination (75.7% less IBR incidence and 63.6% reduction in disease duration vs controls, plus favorable impacts on temperature, nasal shedding, and antibody titers).
- Calves vaccinated with INFORCE 3 in the presence of maternal antibodies and challenged 105 days (15 weeks) later demonstrated shorter disease duration, a trend toward less IBR incidence, less nasal shedding of virus, and higher antibody titers than controls.
- Vaccination of neonatal calves with INFORCE 3 offers an effective strategy for stimulating long-term IBR immunity, even in the presence of maternal antibodies.

Intranasal vaccination of neonatal calves with INFORCE 3® helps provide IBR protection for over 6 months, and can override maternal antibodies to help moderate IBR for at least 15 weeks.

Infectious bovine rhinotracheitis (IBR) is one of several major viral diseases that perpetually threaten cattle productivity, often as a component of viral and bacterial co-infections involved in the bovine respiratory disease complex. IBR is a highly contagious and infectious respiratory disease caused by bovine herpesvirus type 1 (BoHV-1) that can afflict cattle of any age. The viral pathogen can also cause conjunctivitis, abortions, encephalitis, and generalized systemic infections which together can impose substantial economic losses on all segments of the cattle industry.

Vaccination has played an important role in the prevention and control of IBR and has been traditionally recommended as soon as passive immunity in calves has waned (around 4 to 6 months of age).[2] However, the timing of vaccination can be problematic because calves typically receive different levels of maternal antibody from colostrum (differences in quality and amount consumed) and animals may become

Vaccination of young calves can be an important strategy for reducing the window of BoHV-1 vulnerability associated with maternal antibody decay.

susceptible to BoHV-1 prior to complete decay of these maternal antibodies. As the timing of protection loss is not precise, effective vaccination of younger calves can be an important strategy for reducing this window of BoHV-1 vulnerability, helping cattle get off to a healthier, more productive start.

The presence of circulating maternal antibodies in young calves at the time of vaccination can sometimes interfere with the generation of an effective immune response to various bovine viral respiratory disease vaccines.[3,4] However, vaccination of young calves by the intranasal (IN) route may offer the potential to override maternal antibodies that can sometimes interfere with induction of immunity when vaccines are administered via parenteral routes.[5]

Three research studies[1] were conducted to investigate the short- and long-term efficacy of an intranasal IBR vaccine administered to young neonatal calves, and also evaluate efficacy in calves positive for maternal antibodies at the time of vaccination.

INFORCE® 3

INFORCE 3 is a respiratory vaccine that prevents respiratory disease caused by bovine respiratory syncytial virus (BRSV) while also aiding in the prevention of IBR and parainfluenza$_3$ (PI$_3$). Developed specifically for needle-free *intranasal* administration at 1 mL/nostril or 2 mL in a single nostril, INFORCE 3 has shown excellent efficacy in helping prevent respiratory disease and helping reduce viral shedding.[6-9] The modified-live virus vaccine contains proprietary temperature-sensitive IBR and PI$_3$ strains, as well as naturally temperature-sensitive BRSV, so the vaccine strain replicates in the relatively cool nasal passages. INFORCE 3 helps prime the immune system of cattle so a memory response is generated to subsequent vaccinations and disease challenges.

Intranasal vaccination may help override maternal antibodies that can sometimes interfere with parenteral vaccines.

Dairy and beef herds can benefit from this safe, effective, and novel vaccine. INFORCE 3 is licensed for use in all classes of cattle: pregnant heifers and cows, calves 3 days of age or older, at weaning, before commingling, or on arrival.

4-Week Efficacy Study

Experiment Design —

Three challenge studies conducted at a commercial research facility investigated the efficacy of INFORCE 3 against IBR in Holstein calves vaccinated early in life at 3 to 8 days of age.[1] The first study involved 36 neonatal calves (acquired from commercial dairies) that were removed from their dams at birth and *not* fed colostrum. Instead, the calves received oral electrolytes during their first 48 hours of life and then a milk replacer diet until 6 weeks of age (commercial starter available free-choice thereafter). At 3 to 8 days of age (study day 0, Figure 1), calves were randomly assigned to 2 treatment groups and received IN vaccination (2 mL, 1 mL/nostril) with either:

- INFORCE 3, n=18;
- Control vaccine (same as INFORCE 3 but without the BoHV-1 fraction), n=18.

Calves were confirmed seronegative for BoHV-1 and had virus neutralizing (VN) antibody titers <1:2 at vaccination. During this vaccination phase of the study, calves in each treatment group were housed in separate BSL-2 rooms, in individual pens (treatment groups not commingled to avoid

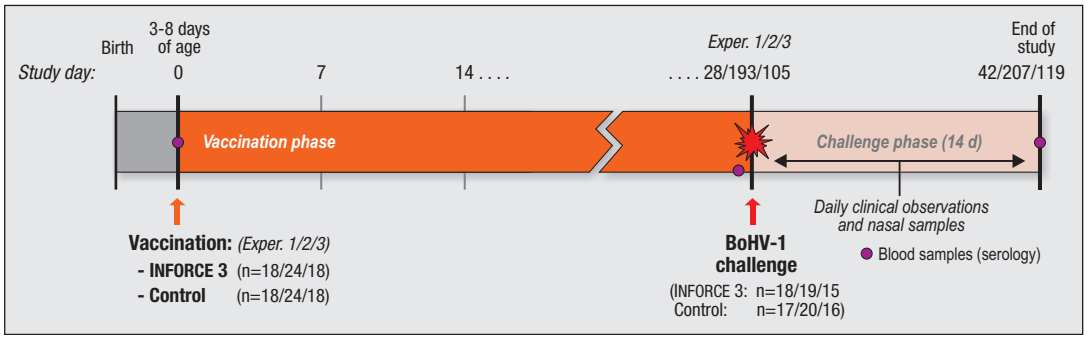

Figure 1 – Summary of design, treatment groups, and timeline for 3 studies involving different BoHV-1 challenge dates (28, 193, or 105 days after vaccination of neonatal calves).

possible viral shedding from vaccinates to control calves).

On day 28, a 14-day challenge phase commenced with IN inoculation of each calf with a 4-mL dose (2 mL/nostril) of virulent BoHV-1 (Cooper strain). The treatment groups were commingled into 2 rooms of a BSL-2 facility at the time of challenge. (The loss of some calves in each study prior to administration of challenge virus was unrelated to vaccination.)

Calves were observed daily and scored for development of acute IBR. Animals were considered to be clinically affected by the virus challenge if they demonstrated depression, increased respiratory effort/sound (dyspnea), and/or mucopurulent nasal discharge at the time of evaluation. Rectal temperatures were also obtained at the time of clinical observation. Nasal secretions were collected prior to challenge and daily after challenge and assessed for virus isolation (shedding of challenge virus). Blood samples were collected from each calf at the time of vaccination, prior to challenge, and on the last day of the challenge phase, with serum analyzed for VN antibodies to BoHV-1.

Collected data were statistically analyzed by appropriate standard methods using each calf as an experimental unit. Least squares (LS) means were calculated for each treatment group, with statistical significance recognized at $P \leq 0.05$. Clinical observations, data collections, and laboratory measurements were conducted by personnel without knowledge of treatment group assignments. The study was conducted in accordance with the Zoetis Institutional Animal Care and Use Committee.

Results —

Health outcomes for the study (Figure 2) showed that a protective effect was conferred by INFORCE 3 vaccination. All 17 control calves (100%) experienced clinical IBR within 14 days of BoHV-1 challenge (clearly demonstrating the virulence of the challenge). In contrast, only 5.6% (1/18) of INFORCE 3 vaccinates experienced clinical IBR. INFORCE 3 vaccination significantly ($P \leq 0.001$) reduced disease incidence by ***94.4%*** compared to controls. The duration of disease was also reduced by INFORCE 3, falling from a mean of 4.8 days for controls to 0.1 for vaccinates (***97.9%*** relative improvement, $P \leq 0.001$). As further evidence of disease moderation, mean rectal temperatures of vaccinates were significantly ($P \leq 0.05$) lower than controls on post-challenge days 3 through 9 (data not shown).

Serology results (Figure 2) confirmed that mean post-challenge VN antibody titers for BoHV-1 were significantly greater ($P \leq 0.05$) at study end for INFORCE 3 vaccinates compared to controls. In addition, vaccinated calves shed significantly less virus in nasal secretions compared to the controls ($P \leq 0.05$) from day 2 through day 13 of the 14-day post-challenge observation period (data not shown).

These outcomes provide clear evidence that early IN vaccination of neonatal calves with INFORCE 3 helped provide a high degree of protective immunity against IBR.

Calves challenged 4 weeks after vaccination with INFORCE 3® showed 94% less IBR incidence and 98% less disease duration than controls, and reduced nasal shedding of virus.

INFORCE 3® helped provide a high degree of protective immunity against IBR in 3 separate challenge studies in young calves.

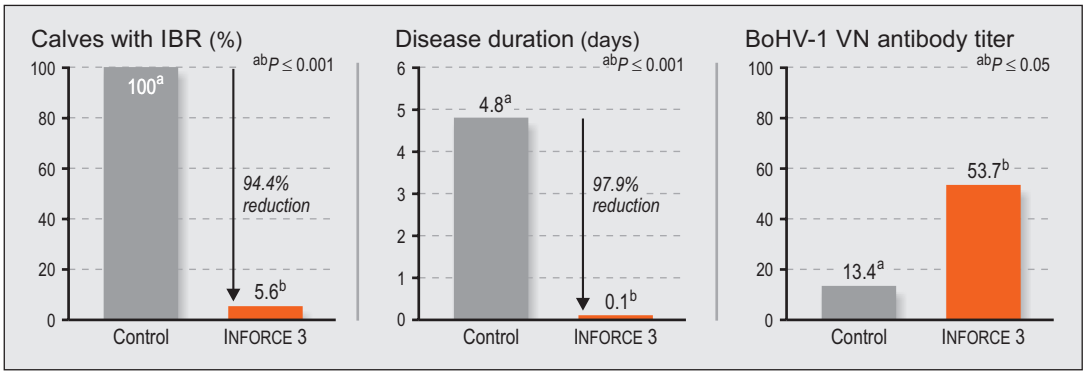

Figure 2 – Four-week efficacy study results; IBR incidence and duration after BoHV-1 challenge 28 days post-vaccination, and geometric LS mean VN serum antibody titers at study end.

In the 6-month DOI study, INFORCE 3® was administered in a single nostril and challenge was 193 days later.

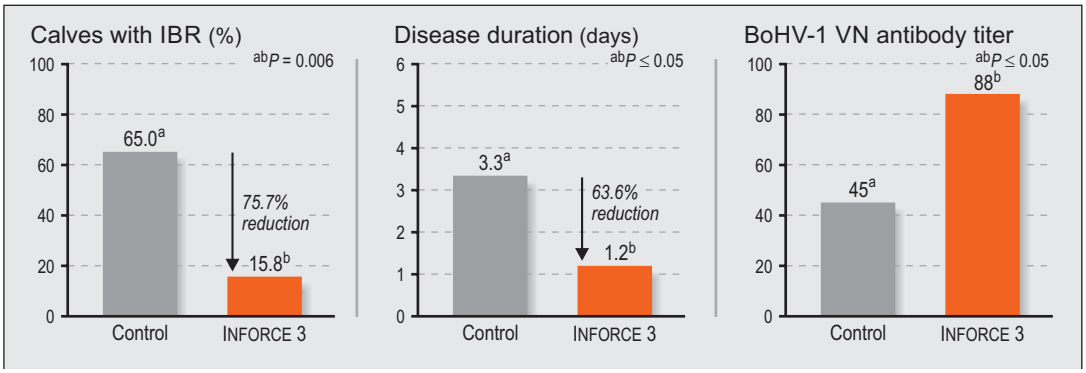

Figure 3 – Six-month DOI study results; IBR incidence and duration after BoHV-1 challenge 193 days post-vaccination, and geometric LS mean VN serum antibody titers at study end.

6-Month Duration of Immunity Study

Experiment Design —

A second challenge study investigated the 6-month duration of immunity (DOI) provided by INFORCE 3.[1] The protocol, timing, location, and conditions for this study were almost identical to the 4-week efficacy study (Figure 1, BoHV-1-negative neonatal calves vaccinated 3 to 8 days of age) but with the following exceptions:

- initially 24 calves/treatment group.
- INFORCE 3 administered in a *single* nostril (2 mL).
- calves housed in outdoor individual calf huts with no nose-to-nose contact (huts grouped by treatment at a separate location on the same farm); calves group-housed by treatment in separate outdoor pens after weaning at 6 to 8 weeks of age.
- virulent BoHV-1 challenge administered *193 days (over 6 months)* after vaccination (controls n=20, vaccinates n=19).

Young calves challenged 6 months after INFORCE 3® vaccination had less IBR incidence, disease duration, and viral nasal shedding than controls. IBR protection persisted for at least 6 months.

Results —

Results summarized in Figure 3 again demonstrated a protective effect provided by INFORCE 3, and that protection extended to ***at least 6 months*** after early neonatal vaccination. Mean IBR incidence was 65.0% (13/20) in the control group compared to 15.8% (3/19) for INFORCE 3 vaccinates. Thus, INFORCE 3 significantly (P = 0.006) reduced disease incidence by ***75.7%*** compared to controls. The duration of disease (Figure 3) was again reduced by INFORCE 3 (mean 3.3 days for controls vs 1.2 days for vaccinates, ***63.6%*** relative improvement, $P \leq 0.05$), and mean rectal temperatures of vaccinates were again significantly ($P \leq 0.05$) lower than controls on post-challenge days 4 through 10 (data not shown).

Mean post-challenge VN antibody titers to BoHV-1 were significantly greater ($P \leq 0.05$) at study end for INFORCE 3 vaccinates compared to controls (Figure 3), indicative of an anamnestic antibody response specific for BoHV-1. Similar to the first study, vaccinated calves shed significantly less virus in nasal secretions compared to the controls ($P \leq 0.05$) on day 3 and days 6 through 11 post-challenge (data not shown).

Results of this long-term DOI study confirmed that early IN vaccination with INFORCE 3, even when dosed in neonatal calves, provided a high degree of protective immunity against IBR that persisted for at least *6 months* after vaccination.

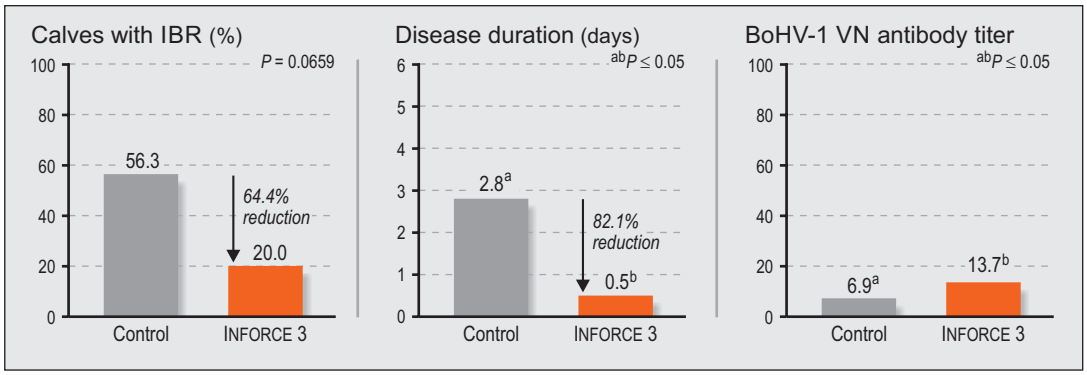

Figure 4 – Maternal antibody non-interference results; IBR incidence and duration after BoHV-1 challenge 105 days post-vaccination, and geometric LS mean VN serum antibody titers at study end.

INFORCE 3® was administered in a single nostril of neonatal calves fed colostrum and positive for BoHV-1 maternal antibodies at the time of vaccination.

Maternal Antibody Non-Interference Study

Experiment Design —

A third challenge study further investigated the IBR efficacy provided by INFORCE 3, this time involving colostrum-fed calves positive for BoHV-1 antibodies when vaccinated.[1] Again, the protocol, timing, location, and conditions for this study were almost identical to the initial 4-week study (Figure 1) but with the following exceptions:

- within 6 hours of birth, calves were fed reconstituted spray-dried pooled commercial colostrum replacer containing antibodies specific to BoHV-1 and other common respiratory pathogens (100 g of IgG), thus ensuring development of a consistent level of serum antibodies before vaccination.
- INFORCE 3 administered in a *single* nostril (2 mL).
- virulent BoHV-1 challenge administered *105 days (15 weeks)* after vaccination (serum VN antibodies at background levels, titers of ≤1:4); (controls n=16, vaccinates n=15).

Results —

Study results (Figure 4) again revealed an IBR-protective effect conferred by single-nostril vaccination with INFORCE 3, even in the face of maternal antibodies at the time of vaccination. Mean IBR incidence of 20.0% (3/15) for INFORCE 3 vaccinates represented a numerical reduction of ***64.4%*** compared to the 56.3% (9/16) rate observed in the control group ($P = 0.0659$). The duration of disease severity was significantly reduced ($P \leq 0.05$) by INFORCE 3, from a mean 2.8 days for controls to 0.5 days for vaccinates (***82.1%*** relative reduction). Mean rectal temperatures of both groups were variable over the course of the post-challenge observation period (higher in vaccinates on days 2 and 11; higher in controls on days 4-6; data not shown).

Serology data (Figure 4) confirmed that post-challenge BoHV-1 VN antibody titers were significantly greater ($P \leq 0.05$) at study end for INFORCE 3 vaccinates compared to controls (though overall titers were generally lower than those observed in the other studies). INFORCE 3 vaccinates demonstrated an anamnestic response with greater increase in anti-BoHV-1 antibodies compared to the controls. Vaccinated calves also shed significantly less virus in nasal secretions compared to the controls ($P \leq 0.05$) from day 5 through day 14 of the post-challenge period (data not shown).

Study outcomes support the use of INFORCE 3 for early IN vaccination of neonatal calves fed colostrum. Even in the presence of maternal antibodies, single-nostril vaccination with INFORCE 3 helped generate a protective IBR immune response that extended for at least *15 weeks* after vaccination.

Even in the presence of maternal antibodies, INFORCE 3® induced a protective IBR immune response that extended for at least 15 weeks after vaccination.

Conclusions

Use of intranasal INFORCE 3® in neonatal calves offers a valuable and effective strategy for early life, long-term induction of IBR immunity.

IBR vaccination of neonatal calves can help provide immunological protection against a window of disease vulnerability caused by maternal antibody decay, thus helping cattle get off to a healthy and more productive start. Results of these 3 studies confirmed that IN vaccination of young calves (3-8 days of age, seronegative or seropositive for BoHV-1) with INFORCE 3 induced clinically relevant protective immunity against BoHV-1 respiratory challenge. Reductions in IBR incidence and severity were demonstrated in BoHV-1 seronegative vaccinates as early as 1 month and as late as 6 months following a single dose of INFORCE 3. In addition, IN vaccination with INFORCE 3 was able to override the presence of maternal antibodies in colostrum-fed calves and provided a disease-sparing effect that extended at least 15 weeks.

Use of intranasal INFORCE 3 in neonatal calves offers a valuable and effective strategy for early life, long-term induction of IBR immunity, even in the presence of maternal antibodies.

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