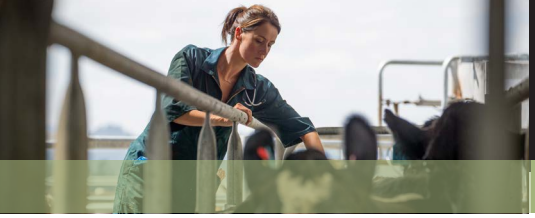

EFFICACY OF BIOBOS IBR MARKER VACCINE

Background

Biobos IBR Marker Vaccine is a vaccine for cattle containing inactivated bovine herpesvirus type 1 (BoHV-1). BoHV-1 is the infectious agent of bovine rhinotracheitis (IBR). Clinical symptoms of BoHV-1 infection are highly variable in clinical manifestation and severity; symptoms of the disease can range from very moderate and localized, up to generalised infection leading in certain cases to death of the affected animals. Sometimes the infection can run sub-clinically and spread amongst the herd unnoticed.

Clinical manifestations of the disease include:

- respiratory syndrome
- infectious pustular vulvovaginitis
- infectious pustular balanoposthitis
- pharyngitis
- enteritis
- conjunctivitis

BoHV-1 can have a negative impact on cattle health and breeding:

- reduced milk production
- low in-calf %, especially early pregnancy rates
- lower weight gain
- infection persists for life in infected cows (as latent infection which can be reactivated)

Marker vaccines utilise the DIVA (Differentiating Infected from Vaccinated Animals) principle. In combination with diagnostic ELISA testing, cattle vaccinated with a marker vaccine (glycoprotein E negative (gE-)) are differentiated from cattle that have been naturally infected (glycoprotein E positive (gE+)).

In New Zealand, BoHV-1 and IBR are widespread within the dairy and beef cattle populations. A positive serological test for BoHV-1 infection generally excludes live cattle and gametes from export to China and other markets. Registration of a marker vaccine where the DIVA principle enables substantial reduction of transmission of virus within and between herds, allows reduction of prevalence of BoHV-1 infection to improve farm productivity, and increases the number of IBR sero-negative cattle available for export.

Vaccination reduces clincal symptoms of infection with BoHV-1 and reduces excretion of the field virus. Studies have shown that vaccination of latently infected animals reduces excretion of virus by up to 10,000 times, compared to unvaccinated animals.

Study Objective

To determine the onset of immunity and efficacy of Biobos IBR Marker Vaccine in a challenge study.

Study Design

Seven Holstein calves aged 3 months of age were enrolled in the study. 5 calves were vaccinated, and 2 left as unvaccinated controls. The calves were healthy, without antibodies against IBR.

Calves were vaccinated with a 2mL dose of Biobos IBR Marker Vaccine with a second dose administered 21 days after the first.

Vaccinated and control calves were all challenged with IBR virus via intranasal administration into both nostrils (2mL into each nostril) 21 days following booster vaccination.

Local (injection site) reactions were evaluated on the days calves were vaccinated, and then daily for 14 days. Clinical observations of animals post vaccination were made daily. Clinical observations after challenge with IBR virus were undertaken from the day of challenge and for the following three weeks. Clinical observations of fever, apathy, nasal discharge, eye discharge and dyspnoea were all scored on a 0 to 3 scale.

Blood samples were collected from animals to determine levels of antibodies against IBR virus by serum neutralisation test. Blood samples were taken before administration of the vaccine and on days 21, 42 and 63. Nasal swabs were collected daily to determine the virus titre on Days 42 to 63 of the study.

Results

There were no local or systemic reactions, or clinical observations in any of the calves after vaccination or after revaccination. There were no significant changes in rectal temperature in any of the calves after vaccination or revaccination.

Daily and overall clinical scores after challenge were much lower in the vaccinated calves compared to the unvaccinated controls. Clinical scores peaked in the unvaccinated control calves 6 – 7 days after challenge. There was a significant difference in the mean total clinical scores between the vaccinated animals (score 3.2) and the unvaccinated animals (score 42).

Virus excretion after the challenge was monitored by isolation of IBR virus from nasal swabs. Virus titres in nasal swabs were lower and persisted for shorter duration in the vaccinated calves, compared to the unvaccinated controls, refer Figure 1. In the vaccinated calves virus was detected from day 1 to day 6 following challenge. The maximum titre in all vaccinated calves ranged from $10^{4.3}$ $TCID_{50}$ to $10^{4.8}$ $TCID_{50}$ from Day 2 to Day 4 after challenge.

VIRAL TITRE FROM NASAL SWABS FOLLOWING CHALLENGE OF TRIAL CALVES

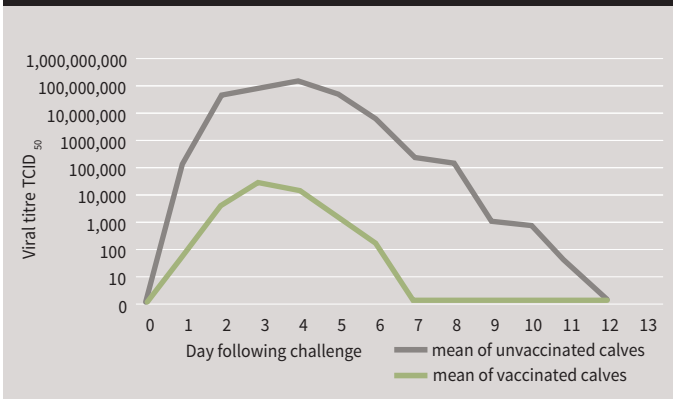

Figure 1. Viral titre $TCID_{50}$ from nasal swabs (log scale)

DETERMINATION OF ANTIBODIES AGAINST IBR BY SERUM NEUTRALISATION TEST (LOG_2 SNT IBR)

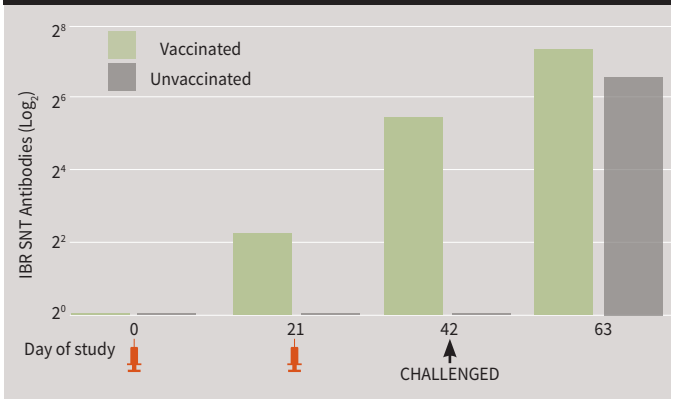

Figure 2. Determination of antibodies against IBR by serum neutralisation test

The unvaccinated control animals showed viraemia from Day 1 to Day 11 after challenge with the maximum average titre $10^{8.1}$ $TCID_{50}$ on Day 4 after challenge (with total virus detected in nasal secretions 10,000 times higher in the unvaccinated animals).

Rectal temperatures after challenge were higher, and persisted high for longer in the unvaccinated controls, compared to the vaccinated calves. Rectal temperatures of vaccinated calves were slightly elevated from Day 5 to 8 after challenge, with maximum recorded temperature 39.8°C on Day 7 after challenge. Rectal temperatures in the unvaccinated control animals increased significantly between Day 5 and Day 11 after challenge, with maximum temperature 41.2°C on Day 7.

After the administration of one dose of vaccine, animals seroconverted with titres 2-3 (log_2) on Day 21 of the study and with titres 5-6 after revaccination (Day 42 of the study). The serological profile of the unvaccinated control animals did not change during the monitored period before challenge.

On Day 42 the vaccinated and control animals were challenged and three weeks later antibodies to IBR were examined in the vaccinated and control animals. All the animals had high levels of specific neutralisation antibodies to IBR with titres 7-8 (log_2) after challenge, refer Figure 2.

Discussion

IBR virus detected in nasal swabs following challenge was significantly lower in vaccinated animals compared with unvaccinated calves, and viral shedding was also of shorter duration in the vaccinated calves. The overall level of IBR virus detected in nasal secretions was greatly reduced in vaccinated calves compared with the control animals, by almost 10,000 times. In a field situation, reducing the quantity of virus in the environment can play an important role in reducing spread of infection within groups of animals, and to neighbouring herds.

In this study, the vaccine meets the European Pharmacopoeial test for efficacy of IBR vaccines, as

- vaccinated calves showed only mild symptoms of IBR and unvaccinated controls showed typical symptoms of the disease
- the average number of days of virus excretion in vaccinated calves was at least three days shorter than in the control group
- in at least 80% vaccinated calves the maximum virus titre found in nasal swabs was at least 100 times lower than the average maximum titre found in control calves

Conclusion

Biobos IBR Marker Vaccine was shown to be efficacious in this challenge efficacy test performed according to the European Pharmacopoeial monograph for IBR vaccines, following administration of 2mL intramuscular doses given at 21 day intervals to calves aged 3 months of age.

Vaccination with Biobos IBR Marker Vaccine reduces IBR virus in nasal secretions by almost 10,000 times, which impacts on viral secretion and disease spread.

Biobos IBR Marker Vaccine is a Restricted Veterinary Medicine registered pursuant ACVM ACT 1997, No. A11239