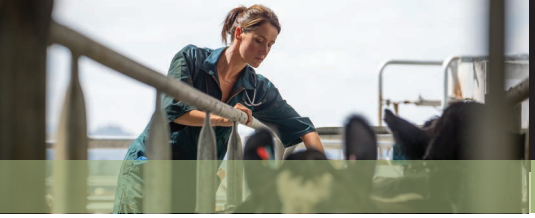

EQUIVALENT ANTIBODY CONCENTRATIONS FOLLOWING IM OR SC VACCINATION WITH BIOBOS RCC

Background

BioBos RCC was registered in New Zealand in 2024 as a single-dose vaccine, designed to induce specific colostral antibodies against Bovine Rotavirus (BRV), Bovine Coronavirus (BCV) and *E. coli* F5 (K99) when administered to pregnant cattle. Originally registered for intramuscular injection in the anterior neck, this method may not always be practical or safe for cattle handlers in NZ farming systems.

Study objective

To compare BRV, BCV and *E. coli* serum antibody concentrations and injection site tolerance, in previously unvaccinated heifers, in response to subcutaneous (SC) administration of BioBos RCC in the ischiorectal fossa (IRF) or anterior neck, with intramuscular (IM) administration in the anterior neck.

Study design

The study population was a mob of 9 to 10 month-old, Friesian/ Jersey cross, non-pregnant heifers, that had no prior vaccination with BRV, BCV or *E. coli* antigens, on a commercial dairy farm in Waikato, New Zealand (NZ).

Animals in the study sample of 36 heifers, were in normal health and body condition based on visual inspection, and had no abnormalities at the possible injection sites. These selected animals were randomly allocated to one of three treatment groups (n = 12/group). The animals were grazed as a single mob and managed as per standard farming practice for the duration of the study.

Animals in groups 1 (IM) and 2 (SCn) were injected in the anterior neck by IM and SC injection, respectively, whereas animals in group 3 (SCi) were injected subcutaneously in the IRF as indicated in Figure 1. Each 2mL vaccine dose was drawn from the same vial using a new syringe and needle per animal. The injection site was neither clipped or cleaned. The Study Director (SD, a veterinarian) used 18G X 1” needles for IM injections and 18G X 3/8” needles for SC injections.

Group	Group code	Location	Administration
1	IM	Anterior neck	Intramuscular
2	SCn	Anterior neck	Subcutaneous
3	SCi	Ischiorectal fossa	Subcutaneous

Table 1: Treatment groups

The SD observed each animal, and palpated its injection site six times (Days 2, 7, 14, 21, 28, and 35) post-vaccination. The concentration of serum antibodies specific to the three vaccine antigens was tested by ELISAs using blood samples collected pre-treatment and 21, 28, and 35 days after vaccination. The ELISAs for both BRV and BCV used whole viral particles as antigen and a direct competitive platform. The *E. coli.* ELISA used F5 fimbria as antigen and an indirect non-competitive platform. Hence the results were reported as % inhibition and % positivity, respectively.

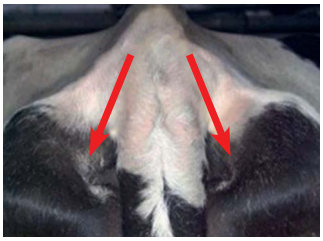

Figure 1: Location of the ischiorectal fossa injection site

Note, based on the calculated sample of 10/ group, and as all animals had completed the animal phase, the blood samples of two animals per group were randomly selected for removal from the study.

The primary question whether injection into the alternative SC sites was at least as good at inducing antibodies as IM injection was examined under a non-inferiority framework using a non-inferiority margin of -30%. This margin was chosen based on the expected variability (~ 2 × SD), and biologically, on this difference being 1.5 times the cut-points used to interpret the ELISAs.

Longitudinal analysis of covariance models that included the fixed effects of group and pre-treatment % inhibition/positivity, with animal as a random effect, were built for each vaccine antigen. Also, as a simple check, the maximum post-vaccination % inhibition/positivity values for each antigen were modelled using OLS regression with pre-vaccination % inhibition/positivity as a covariate.

The study was conducted with AgResearch animal ethics approval (No. 2394) and according to the ACVM Research Standard.

Results

i. Safety All animals remained visibly healthy. No abnormalities were detected at the IM or SCn injection sites. At the SCi injection site, very slight palpable swelling (diffuse, soft and oedematous) was detected in 3 of the 12 animals on Day 2. At the next inspection on Day 7, the swelling had resolved and no further anomalies were observed for the duration of the study. Contingency table analysis concluded, that there was no evidence of a relationship between injection site and the minor swelling observed (p=0.092).

ii. Antibody Pre-vaccination sampling indicated few animals had prior natural exposure to BCV or *E. coli*, however the BRV % inhibition of 12/30 animals was >40%, suggesting recent exposure. Although the animals were run together, 5 of these 12 animals were in the IM group, of which 3 had high values.

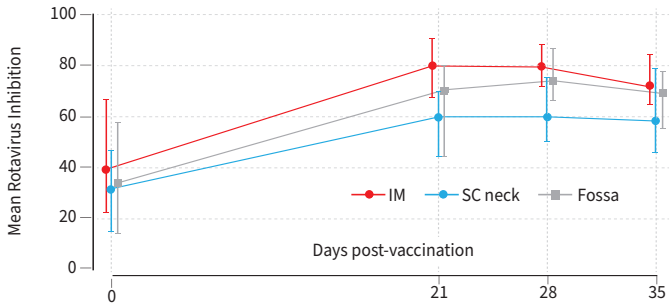

Figure 2a: Mean BRV % inhibition and inter-quarter range by treatment group prior to vaccination (0) and 21, 28 and 35 days after vaccination

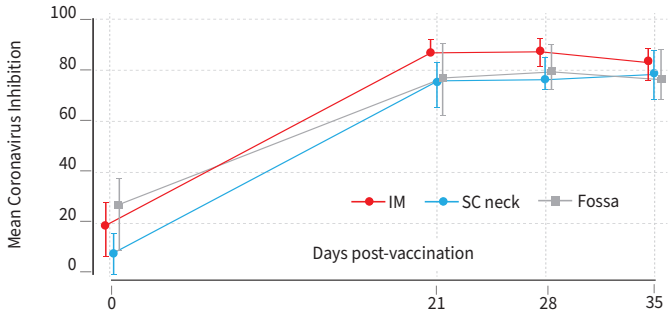

Figure 2b: Mean BCV % inhibition and inter-quarter range by treatment group prior to vaccination (0) and 21, 28 and 35 days after vaccination

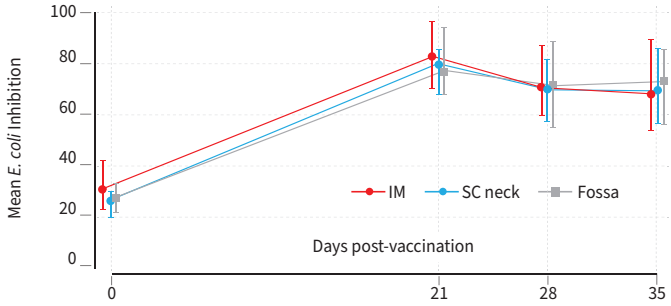

Figure 2c: Mean E. coli positivity % and inter-quarter range by treatment group prior to vaccination (0) and 21, 28 and 35 days after vaccination

	Group	Mean	95% CI
BRV	IM neck	76.0	66.7 to 85.4
	SC neck	59.7	50.4 to 69.0
	SC IRF	71.1	61.8 to 80.4
BCV	IM neck	85.5	78.3 to 92.7
	SC neck	78.6	71.0 to 86.3
	SC IRF	75.7	68.1 to 83.2
E. coli	IM neck	72.2	63.8 to 80.5
	SC neck	74.0	65.7 to 82.3
	SC IRF	74.0	65.8 to 82.3

Table 2: Average post-vaccination % inhibition/positivity, adjusted for day and pre-vaccination antibodies, and associated 95% CI.

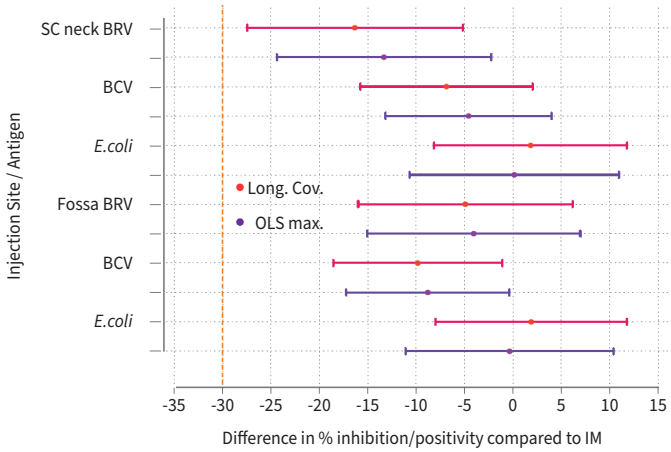

Figure 3: Mean and 90% CI intervals for the differences in mean % inhibition between subcutaneous injection in either the anterior neck (SC neck) or ischiorectal fossa (Fossa) and intramuscular injection sites, adjusted for pre-vaccination % inhibition and day.

Vaccination increased antibody concentrations in all animals against *E. coli* and BCV, and 29/30 against BRV. Simple mean and inter-quartile range percent inhibition/positivity for each antigen, by time point, are shown in Figures 2a, 2b, 2c.

The post-vaccination mean % inhibition/positivity, adjusted for day and pre-vaccination antibodies, with their associated 95% confidence intervals are shown in Table 2. As the model assumptions were met, confidence in the robustness of the calculated confidence intervals is high.

The lower-bound of the confidence limits from the longitudinal covariance and regression models (the red and purple lines, respectively, in Figure 3), illustrate non-inferiority was achieved for all antigens.

Discussion

Injection site lesion rates were very mild and short-lived in the IRF group. Resolution of the diffuse swelling by day 7 confirmed that there are no animal safety or food quality issues likely to arise following hygienic vaccination of animals in the proposed subcutaneous sites.

Despite between-group differences in pre-vaccination mean antibody concentrations (due to natural exposure), the post-vaccination antibody concentration values were similar across the three injection site groups for all three vaccine antigens, hence demonstrating non-inferiority, between subcutaneous and intramuscular injection sites.

Conclusion

This study showed that BioBos RCC is safe and effective when administered subcutaneously in either the anterior neck or the ischiorectal fossa, and supports vaccination by these routes. Subcutaneous administration of BioBos RCC in the ischiorectal fossa provides a practical injection site for safe handling of dairy cows by veterinary and dairy farm staff in New Zealand.

TBV 17 - 03.25