

EFFICACY OF KOLIBIN NEO UNDER CHALLENGE MODEL CONDITIONS

Introduction

Challenge study models are the 'gold-standard' tests for efficacy of a vaccine. A challenge study to prove vaccine efficacy involves vaccination of naive animals within the target age bracket, followed by an induced artificial exposure to the target antigen by administering an infective dose of the virulent antigen(s) via the natural route for infection.

In this case the challenge studies involved oral gavage with sufficient pathogen to subsequently cause disease (scours) in calves born to unvaccinated dams. Kolibin Neo is administered to the pregnant dam with vaccinal protection via passive transfer of immunity during colostrum ingestion and absorption of antibodies in the newborn calf. Consequently, the vaccination program was administered to pregnant dams and the challenge studies were then undertaken in their neonatal calves.

The following are three challenge studies performed to prove the efficacy of Kolibin Neo to each of the antigens in the vaccine. In each study, fifteen pregnant dairy cows, aged 2 to 5 years, with serology demonstrating a lack of antibodies against bovine rotavirus, bovine coronavirus and *E. coli* were enrolled in the Study.

Ten cows were administered two doses of 2mL Kolibin Neo by intramuscular injection, three weeks apart, concluding 2 – 4 weeks prior to calving. The remaining five cows were assigned to the untreated control group. Blood samples for antibody measurement were collected from each of the cows prior to sensitiser vaccination and one week following booster vaccination, as well as on the day of calving.

Calves from all cows were fed colostrum from their dam on their day of birth, and were thereafter maintained in a separate vaccinated calf group being fed colostrum and milk from only vaccinated dams, and a control calf group being fed only colostrum and milk from unvaccinated (control) dams.

Study 1: Bovine Rotavirus Potency Test by Challenge

Blood samples were collected for antibody evaluation from the dams prior to sensitiser vaccination, one week after booster vaccination, and on the day of calving. After calving the calves were maintained in an environment where they were not exposed to infection by bovine rotavirus. At 5 - 7 days after birth, each calf was orally challenged with $10^{6.0}$ TCID₅₀ of a virulent strain of bovine rotavirus.

Challenged calves were observed for 7 days after challenge. The incidence, severity and duration of diarrhoea and the duration and quantity of virus excretion was evaluated. A comparison of both groups (vaccinated (V) and control (C)) was undertaken and statistical evaluation was performed.

Results

The cows responded to vaccination and had high titre levels on the day of calving, as shown in Figure 1.

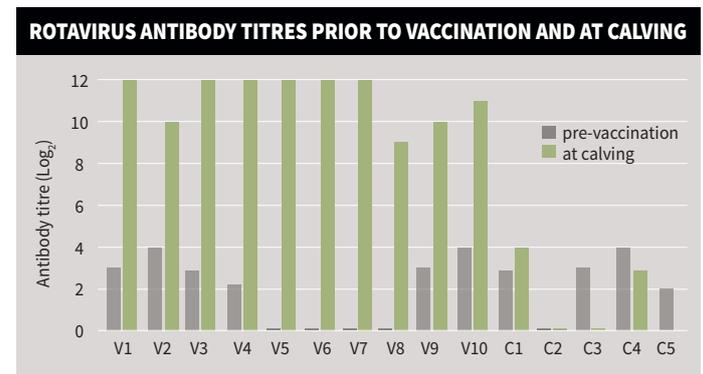


Figure 1: Rotavirus antibody titres in dams

One of ten calves fed colostrum and milk from vaccinated dams had slight diarrhoea on the second day after challenge, and bovine rotavirus appeared in the faeces of this calf for 24 hours. The other calves in the vaccinated group appeared normal for all days after the challenge, with no abnormal clinical signs or diarrhoea.

All five calves from the control group fed colostrum and milk from unvaccinated dams had slight diarrhoea for four to six days, and one calf had watery diarrhoea. All five control calves shed rotavirus in their faeces from the second to sixth day after challenge.

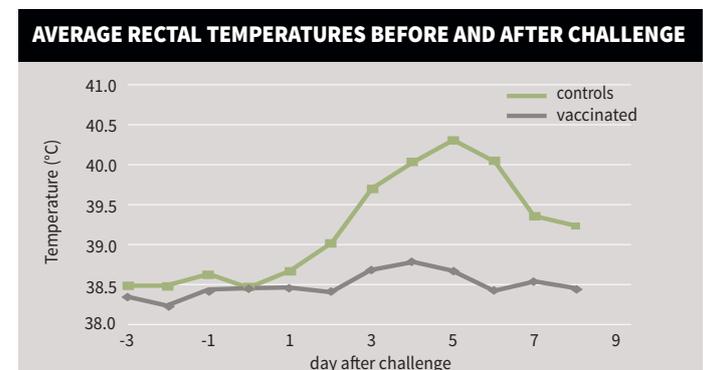


Figure 2: Average temperatures of calves after rotavirus challenge

The rectal temperature of calves from vaccinated dams did not exceed normal at any stage after the challenge. Rectal temperatures were significantly higher in challenged calves from unvaccinated dams than calves from vaccinated dams (Figure 2).

Study 2: *E. coli* Potency Test by Challenge

Blood samples were collected for antibody titre evaluation from the dams prior to sensitiser vaccination, one week after booster vaccination, and on the day of calving. After feeding colostrum from their dam, the calves were orally challenged with 50×10^8 CFU of K32 : K99 strain of enteropathogenic *E. coli* within 12 hours of birth. Calves were observed for the following 10 days.

Clinical signs of *E. coli* infection were evaluated each day for each calf according to the following scale

- 0 no signs
- 1 slight diarrhoea
- 2 marked diarrhoea (watery faeces)
- 3 dead

The total post-challenge clinical score for each calf over 10 days was calculated.

A comparison of both groups (vaccinated and control) was performed and statistical evaluation was performed.

Results

Antibody titres in vaccinated cows showed a significant increase and remained high at calving (Figure 3).

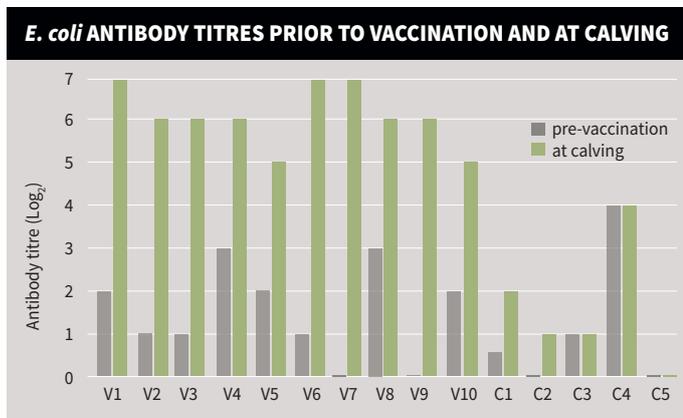


Figure 3: *E. coli* antibody titres in dams

Two of the ten calves fed colostrum and milk from their vaccinated dams had slight diarrhoea on the second and third day after challenge, but were otherwise healthy, as were the other eight calves in the group. All five calves from the control group fed colostrum and milk from unvaccinated dams had marked watery diarrhoea on days one to three after challenge. Three calves from the control group subsequently died within 72 hours of the *E. coli* challenge. The remaining two calves were apathetic and had marked or slight diarrhoea from days one to six after challenge.

The challenge test showed a significant decrease in total *E. coli* clinical score for the calves fed colostrum and milk from vaccinated dams, compared to the control calves (Figure 4).

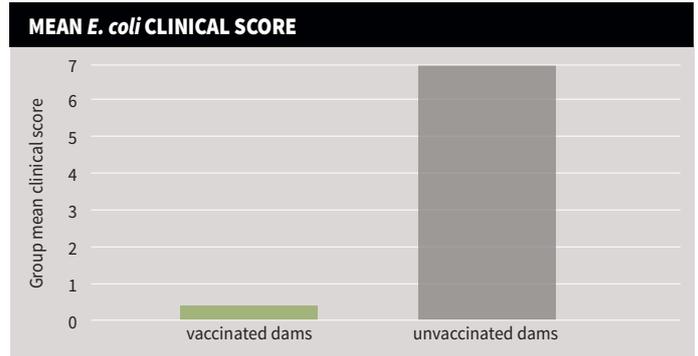


Figure 4: Mean clinical score in calves challenged with enteropathogenic *E. coli*

Scores in the calves from vaccinated cows were evaluated for the full 10 days (range 0 - 2) with nil mortality. Scores in the negative control group ranged from 5 - 10 with only two calves scored for the entire 10 days, due to mortality suffered by remainder of the group. NB: once a calf dies it ceases to contribute to group scores.

Rectal temperatures in challenged calves from unvaccinated dams were significantly higher than those from vaccinated dams from one day to eight days after challenge (Figure 5).

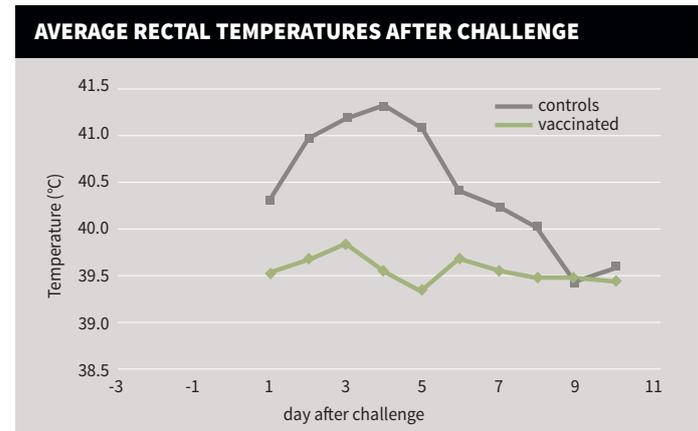
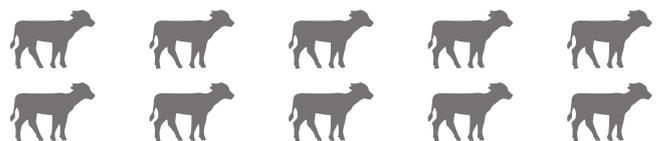


Figure 5: Average temperatures of calves after *E. coli* challenge

Calves from vaccinated dams (treatment)

Calves from vaccinated dams, fed colostrum from their dam then challenged with enteropathogenic *E. coli* and observed for 10 days remained healthy. There was nil mortality in this group.



Calves from unvaccinated dams (control)

Three of the five calves from unvaccinated dams, fed colostrum from their dam then challenged with enteropathogenic *E. coli* developed diarrhoea and died within 4 days of the challenge.



Study 3: Bovine Coronavirus Potency Test by Challenge

Blood samples were collected for antibody evaluation from the dams prior to sensitiser vaccination, one week after booster vaccination and on the day of calving. At 5 - 7 days after birth, each calf was orally challenged with $10^{6.5}$ TCID₅₀ of a virulent strain of bovine coronavirus. Challenged calves were observed for 8 days after challenge. The incidence, severity and duration of diarrhoea along with the duration and quantity of virus excretion was evaluated.

A comparison of both groups (vaccinated and control) was performed and statistical evaluation was performed.

Results

Antibody titres following vaccination can be seen in Figure 6. The cows responded to vaccination and their titre remained high on the day of calving.

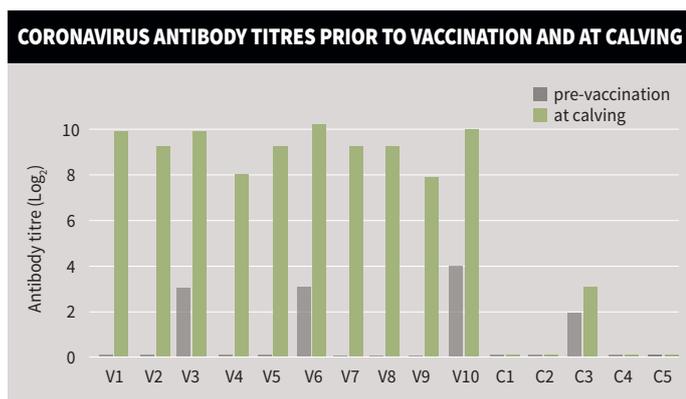


Figure 6: Coronavirus antibody titres in dams

In the challenged calves, no abnormal rectal temperature values were recorded for calves born to vaccinated dams after administration of the coronavirus challenge dose.

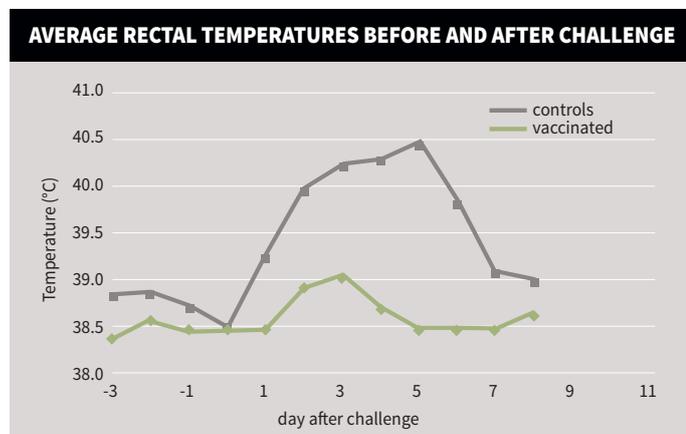


Figure 7: Average temperature of calves after bovine coronavirus challenge

Rectal temperatures were significantly higher in challenged calves from unvaccinated dams than calves from vaccinated dams (Figure 7). Five out of ten calves fed colostrum and milk from vaccinated dams had slight diarrhoea on the second and third day after challenge. All five calves from the control group fed colostrum and milk from unvaccinated dams had marked watery diarrhoea for 2 - 3 days. All calves from the negative control group either died within 96 hours of the challenge, or were euthanased, as they failed to respond to treatment.

Challenged calves from unvaccinated dams shed bovine coronavirus (BCV) in their faeces for five to six days, with titres in faeces as high as 2048. In contrast, challenged calves from vaccinated dams shed BCV only at a very low level (max titre 32) for a shorter time (maximum of 4 days).

Calves from vaccinated dams (treatment)

Calves from vaccinated dams, fed colostrum from their dam then challenged with bovine coronavirus and observed for 8 days remained healthy. There was nil mortality in this group.



Calves from unvaccinated dams (control)

Calves from unvaccinated dams, fed colostrum from their dam then challenged with bovine coronavirus developed marked watery diarrhoea. Three calves died within 4 days of the challenge, with the other two euthanased as they failed to respond to treatment.



Overall Conclusions

This series of challenge model efficacy tests showed that Kolibin Neo is highly effective at minimising the impact of disease from *E. coli*, bovine coronavirus and bovine rotavirus, even under highly virulent challenge conditions. Feeding of colostrum and milk from vaccinated dams is extremely important to obtain these results.

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