



PROFESSIONAL SERVICES

Boehringer Ingelheim Vetmedica, Inc.

TECHNICAL BULLETIN

An Immunogenicity Study To Evaluate The Efficacy Of A *Salmonella dublin* Aromatic Dependent (*aroA*-) Modified-Live Vaccine For Protection Against Disease Associated With *Salmonella dublin* Infection In Cattle

INTRODUCTION

Salmonellosis is a problem of increasing magnitude. This is especially true for *Salmonella dublin*, which is host adapted for cattle and can develop readily into the carrier state in cattle. One way cattle producers and dairies have tried to prevent or control salmonellosis is through vaccination. However, vaccination with commercially available killed *S. dublin* bacterins have had variable success rates in protecting calves against infection.

ABSTRACT

In this study, we evaluated the safety and efficacy of the avirulent live *Salmonella dublin* strain as a vaccine to protect calves against salmonellosis caused by *S. dublin*. Thirty-four (34) 2-week-old calves were used for this study. Each of these calves were culture negative for all salmonella and came from dairies that didn't use salmonella bacterins or vaccines. Calves were randomly assigned to two test groups consisting of 22 vaccinates and 12 controls. Vaccinations were given subcutaneously at 2 and 4 weeks of age. Fecal cultures were taken on a weekly basis after vaccination to determine if the vaccine was being shed or calves were exposed to virulent salmonella prior to challenge. All calves were challenged orally with a virulent strain of *S. dublin* 16 days after the second vaccination. Clinical signs, salmonella shedding and blood culture were monitored daily for 16 days post challenge. At 16 days post challenge, all surviving calves were necropsied and various organs were cultured for salmonella. All calves that died prior to day 16 were also cultured for salmonella.

The vaccinated calves had significantly fewer clinical signs less ($p < 0.05$) mortality, recovery of *S. dublin* from the blood, fecal shedding of *S. dublin* and recovery of *S. dublin* at necropsy from certain internal organs. The Enterovene D vaccine strain was never recovered from the calves' fecal material or blood samples after inoculation or in the organs at necropsy. This study indicates this vaccine is a safe and effective aid in the prevention of salmonellosis caused by *S. dublin* in calves.

RESULTS

The most clinically significant finding in this study was the reduction in mortality of the vaccinates when compared to the controls ($p = 0.0007$). Only 10% (2/20) of the vaccinates died after challenge while 73% (8/11) of the controls did not survive *S. dublin* infection.

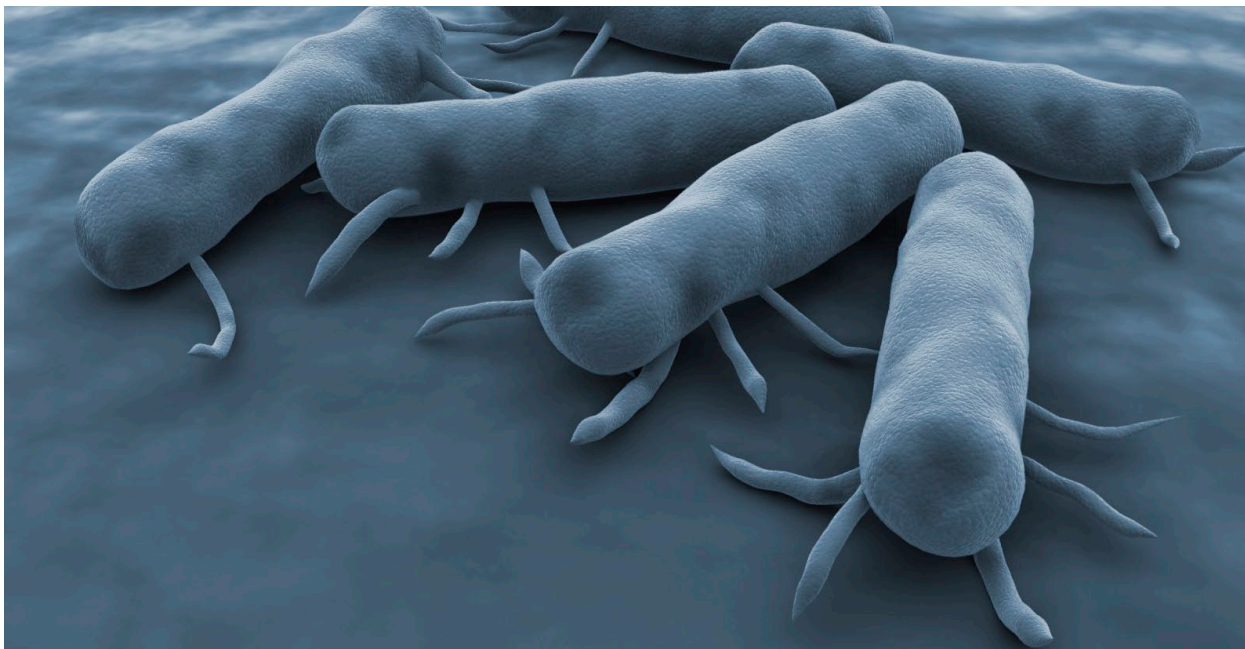
The remaining clinical signs, other than death, that were recorded were fever, inappetance, depression and diarrhea. The vaccinated calves displayed some of these clinical signs. However, the severity in the vaccinated calves, as indicated by the cumulative clinical score, was far less than the control calves ($p < 0.0001$).

Scores for each clinical sign were low due to the early onset of death in the controls. However, even though the controls died early and therefore, were observed for fewer days than the vaccinates, there was a significant reduction in all of the clinical signs observed. This included temperature ($p = 0.0244$), appetite ($p = 0.0195$), attitude ($p = 0.0025$) and fecal consistency ($p = 0.0219$).

Shedding of the virulent *S. dublin* strain was also examined after challenge. One gram of fecal samples were cultured daily from the 20 vaccinates for a total of 289 cultures following challenge. Similarly, 110 samples were taken from the 11 control calves. *Salmonella dublin* was recovered from 96% (106/110) of the cultures taken from the control calves. In contrast, 80% (231/289) of the cultures from the vaccinates after challenge were positive for *S. dublin* isolation. The difference is significant ($p < 0.0001$).

In addition to determining the shedding of *S. dublin* in the fecal material, the incidence of bacteremia was also investigated. Bacteremia was significantly reduced in the vaccinates ($p = 0.0006$). While 91% (10/11) of the control calves were bacteremic and blood culture positive for *S. dublin* during the study, only 25% (5/20) of the vaccinates had bacteremia as evidenced by positive blood culture. In addition to the 24 hours post challenge that was required by the submitted protocol, blood cultures were continued for the entire observation period. *Salmonella dublin* was isolated from 54% (6/11) of the blood cultures from the controls and none (0/20) of the vaccinates at 24 hours post challenge ($p = 0.0006$). Of the 289 blood cultures taken from the vaccinates, only 3% (9/289) were positive for *S. dublin* isolation. This is in sharp contrast to the 48% (53/110) of the control cultures that were culture positive for *Salmonella dublin*. Again, these results are significant ($p < 0.0001$). Of the nine positive cultures obtained from the 289 samples taken from the vaccinated group, four of them are from one of the two vaccinates that died after challenge.

Calves that died as a result of challenge were necropsied at the time of death. All others were posted at 16 days after challenge. *Salmonella dublin* was isolated from 85% (56/66) of the samples taken from the non-vaccinated controls at necropsy. In comparison, 17% (21/120) of the samples taken from the vaccinates were positive for *S. dublin* isolation at necropsy ($p < 0.0001$). In addition, more than half 12/21 of these positive isolations came from the two vaccinates that died. No other vaccinated calf had more than two organs culture positive at 50% (10/20) of the calves had no positive cultures taken from any organs at necropsy. In contrast, 100% (11/11) of the control calves had positive cultures at necropsy and 73% (8/11) of these calves had all (6/6) organs culture positive. The culture of each organ was examined for significant differences between controls and vaccinates. This included duodenal contents ($p = 0.0001$), ileal contents ($p = 0.0068$), spleen culture ($p < 0.0001$), liver culture ($p < 0.0001$) and bile culture ($p = 0.0001$). The culture of the colon contents was the only sample where the difference between controls and vaccinates was not significant ($p = 0.0570$).



CONCLUSION

In this study, we demonstrated EnterVene d Salmonella dublin modified-live vaccine is extremely effective at reducing mortality and clinical signs caused by challenge with a virulent wild-type field isolate of Salmonella dublin.

In addition, use of this vaccine reduces:

- Shedding of *S. dublin*
- The recovery of *S. dublin* from the blood, and
- The number of tissues/organs infected with *S. dublin*.

When used as described in this study, the Salmonella dublin Vaccine Live Culture is an effective aid in the prevention of salmonellosis caused by *S. dublin*.



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