Comparison of *Salmonella* Dublin SRP vaccination programs on the development of immunity in calves

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Introduction

Salmonella Dublin is a common cause of calf illness and is endemic in many regions of the U.S. dairy industry. A 2016 report from the National Veterinary Services Lab found *S.* Dublin was the most commonly isolated *Salmonella* serotype obtained from ill cattle in the U.S. This serotype is also considered to be host-adapted in bovine and therefore carrier animals that appear normal can be shedders and a source for maintaining the infection within a herd. As such, finding mechanisms to protect naïve animals from clinical disease can reduce losses in dairy herds. The objective of this work was to describe the immune response stimulated by experimental *Salmonella* Dublin SRP vaccines.

Materials and methods

Prior to initiation of the study, protocols were approved by Iowa State University's Institutional Animal Care and Use Committee (protocol number 22-157). A total of 78 calves from a commercial dairy farm were randomly enrolled into 1 of 3 treatment groups: 1) placebo (saline only; n = 24; Gr1); 2) *S.* Dublin SRP in adjuvant A (n = 26; Gr2); or 3) *S.* Dublin SRP in adjuvant B (n = 28; Gr3).

Calves were enrolled weekly over the course of 8 weeks if they had a serum protein value > 5.4 mg/dL and were healthy at first vaccination. At an average of 1 week (4-10 days of age) and 4 weeks of age, calves from Gr2 and Gr3 were vaccinated with 1 mL subQ, while Gr1 received 1 mL of saline. Prior to each vaccination, at 4 and 8 days after the second vaccination, and at 90-120 days of age, serum was collected for antibody (Ab) titer analysis. Additionally, at 4 and 8 days after the second vaccination, peripheral blood monocytes (PBMC) were collected in Cell Preparation Tubes (CPT Tubes, BD Biosciences) for cell-mediated immunity analysis.

At each timepoint, bovine Interferon-gamma (IFN-γ) ELISPOTs (Mabtech) were performed to quantify the number of SRP-responsive cells. In parallel cultures, PBMC were also stimulated with SRP antigens and concentrations of IL-17 and IFN-γ were measured by ELISA. ELISA and ELISPOT data were log transformed and analyzed by ANOVA using commercially available software.

Four times throughout the study and then 1 month after the study was completed, bulk tank milk from the source herd was tested via a *Salmonella* ELISA (PrioCHECK) to assure *S.* Dublin negative status. Additionally, all calves were checked for ELISA status on blood collected between 90-120 days of age. All health events for trial calves were captured on all calves through 90 days of age by farm staff.

Results

Initial analysis using Tukey's post-hoc on log-transformed values indicated that results obtained for Gr2 and Gr3 did not differ, and both were significantly different from Gr1. Therefore, Gr2 and Gr3 were combined as vaccinates. At 4 and 8 days after the second dose of vaccine, vaccinates had significantly higher numbers of IFN-γ producing cells and concentrations of IFN-γ and IL-17 ($P < 0.01$) than controls. Prior to each vaccination and at 4 days post-second vaccination, Ab titer levels were not significantly different between groups, but at 8 days post-second vaccination and 90-120 days of age, Ab titer levels remained significantly higher in vaccinates ($P < 0.01$). Data regarding calf health is currently being analyzed. All bulk tank and serum *Salmonella* ELISA tests were negative.

Significance

Vaccination with experimental *Salmonella* Dublin SRP vaccines (unlicensed) stimulates both cellular and humoral immunity in young calves when vaccinated at 1 and 4 weeks of age. This data demonstrates that immune responses expected, and needed to protect against bacterial invaders, developed in a short period following vaccination. This data will help veterinarians develop vaccine protocols against *S.* Dublin. Further research is needed to determine if the level of protection developed following vaccination will be protective against *S.* Dublin challenge.

