

## EFFECTS OF SRP VACCINE IN REDUCING *E. COLI* O157:H7 IN CATTLE

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### Introduction

Cattle are the main reservoir of *Escherichia coli* O157:H7, which is a food-borne pathogen that causes bloody diarrhea in adults and kidney damage in children. *E. coli* O157 is shed in the feces of cattle, which can be a contamination source of water, ground beef, fresh vegetables, and unpasteurized milk and fruit juices. In 2003, shiga-toxin producing *E. coli* O157:H7 caused 73,000 illnesses, which resulted in over 2,000 hospitalizations and 60 deaths in the United States. The estimated annual cost of this illness was \$405 million, which included \$370 million for premature deaths, \$30 million for medical care, and \$5 million for lost productivity. Strategies to reduce this food borne illness must be further investigated.

A new vaccine technology targeting *E. coli* O157:H7 in hopes of reducing the colonization of this pathogen in beef cattle has been developed (Epitopix, LLC, Wilmar, MN). This vaccine was designed to block the transport of iron into the bacterial cell, which is an essential nutrient needed for the survival of this microorganism. Previous trials showed that this vaccine elicited an immune response and reduced fecal shedding of the pathogen in

five-month old Holstein steers. The purpose of this experiment was to further evaluate the efficacy of this new siderophore receptor/porin protein (SRP) technology by analyzing fecal shedding and immune responses of mixed-breed calves orally inoculated with *E. coli* O157:H7.

### Experimental Procedures

Thirty beef calves (3 to 4 months old) that were pre-tested and shown to be free of *E. coli* O157:H7, were processed and allowed to acclimatize as a herd at a local Manhattan, Kansas farm. Calves were treated with One Shot Ultra 7, Bovi-Shield Gold 5, Micotil 300, and Dectomax. Calves were fed a starter ration at approximately 7 lbs per head per day and had *ad libitum* access to water and brome grass hay.

Approximately one month after arrival, calves were placed into one of two treatment groups and administered either the SRP vaccine or the placebo on day 0 of the trial. Twenty-one days after the first injection was administered, the calves were revaccinated and transported to a BL-2 facility where they were confined to individual pens and allowed to acclimatize for one

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week. On day 36, calves from both treatment groups were orally inoculated with a mixture of five strains of *E. coli* O157:H7, which were made resistant to nalidixic acid.

Cattle fecal samples and rectoanal mucosal swab (RAMS) samples were collected once a day for five consecutive days after oral challenge and then sampled three times a week for the following five weeks. Blood samples were collected on day 1, just prior to vaccination, and at weekly intervals to monitor antibody response to SRP vaccination in calves. At the end of the study, calves were euthanized and samples were taken from the rumen, omasum, abomasum, cecum, colon, and rectum, along with tissue samples from the gall bladder mucosa and the rectoanal mucosa, to determine the presence and concentration of *E. coli* O157:H7. Detection of this microorganism was performed by transferring each sample to a selective enrichment broth, by making serial 10-fold dilutions to allow for quantification of the microorganism, and by transferring each dilution to a selective agar containing nalidixic acid to simplify detection of the challenge organism. Further antigenic testing was performed to confirm proper identification of the microorganism, and concentration of the bacteria from each sample was calculated.

### **Results and Discussion**

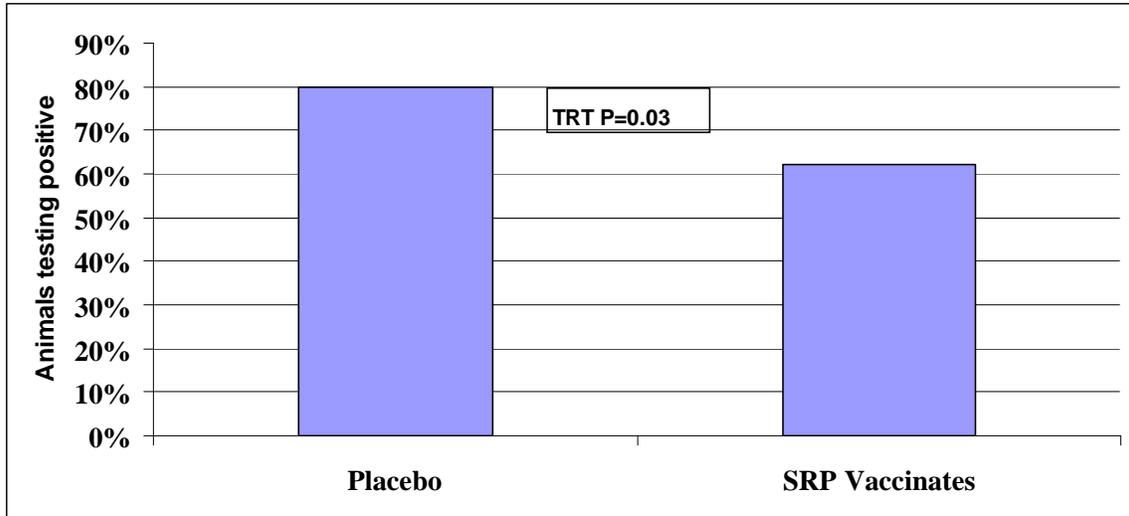
The presence and concentration of *E. coli* O157:H7 in the two treatment groups

are illustrated in Figures 1-5. From day 11 to day 35 post challenge, there was a decrease ( $P<0.04$ ) in fecal shedding of *E. coli* O157:H7 over time when the “SRP” treatment group was compared to the control group. There was no significant difference between the two treatment groups when evaluating the concentration of the bacteria using the RAMS (rectoanal mucosal swab) sampling technique. There was a difference ( $P<0.05$ ), however, when the prevalence in both the fecal and RAMS samples were combined (Figure 3), showing that there were twice as many positive samples in the control group when compared to the “SRP” treatment group by day 32 post challenge.

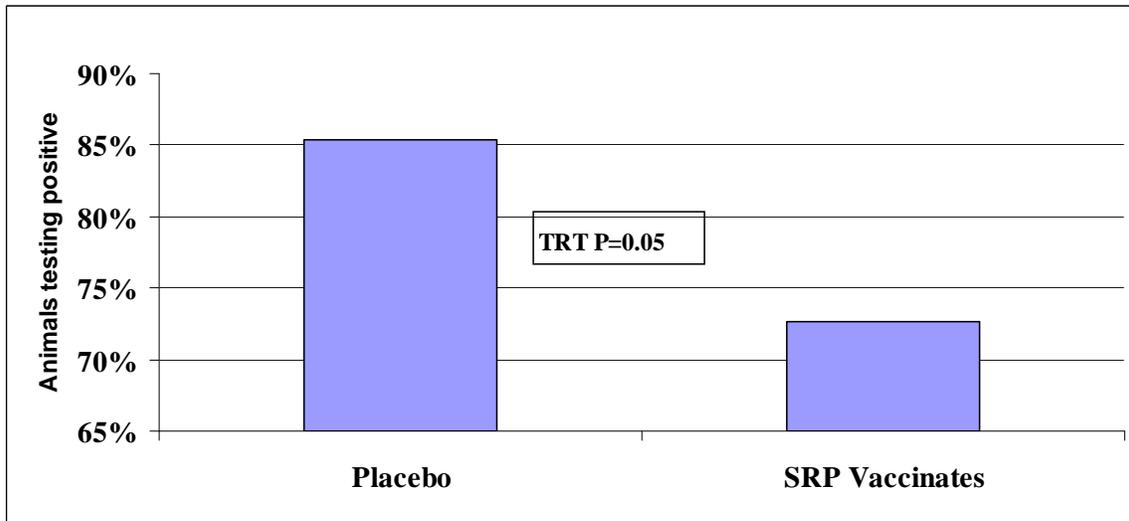
The necropsy data also illustrated the efficacy of this vaccine showing the mean proportion of samples testing positive in the SRP treatment group were less ( $P<0.01$ ) than those found positive in the non-vaccinate control group. The SRP vaccinate group had higher ( $P<0.01$ ) concentrations of anti SRP antibodies compared to the control group.

### **Implications**

Overall, vaccination of calves with the SRP vaccine reduced the prevalence of *E. coli* O157:H7 in cattle. This not only defines a new potential strategy to reducing food borne illness, but also increases the consumer’s confidence when purchasing quality meat.



**Figure 1 Average Percent of Animals Positive with Nal<sup>R</sup> *E. coli* O157:H7 from Day 11 Post Challenge to the Day of Necropsy.**



**Figure 2 Nal<sup>R</sup> *E. coli* O157:H7 Positive Cattle in Either the Fecal or Rectoanal Mucosal Swabs Samples from Day 11 Post Challenge to the Day of Necropsy**

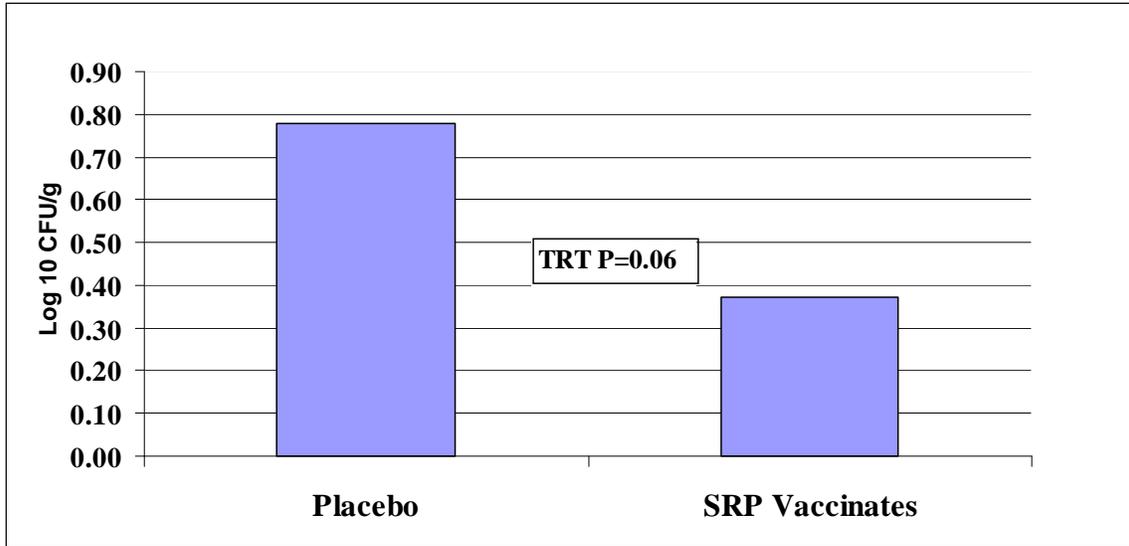


Figure 3 Average Fecal Concentration of NaI<sup>R</sup> *E. coli* O157:H7 in Cattle from the Day of Challenge to the Day of Necropsy

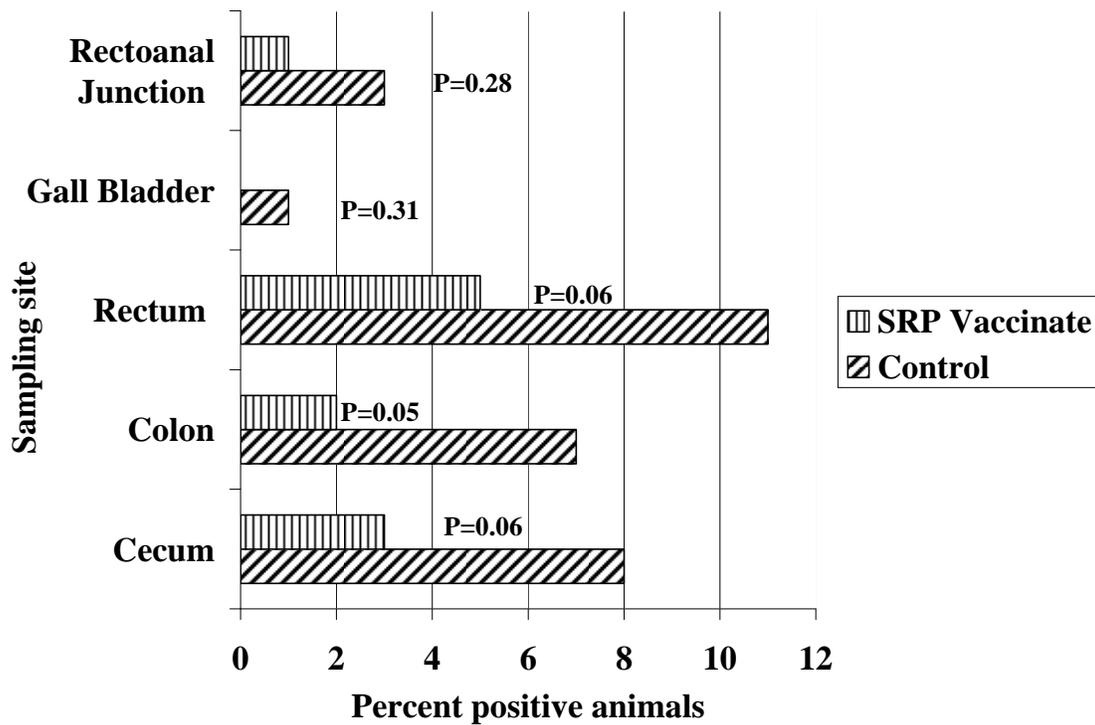
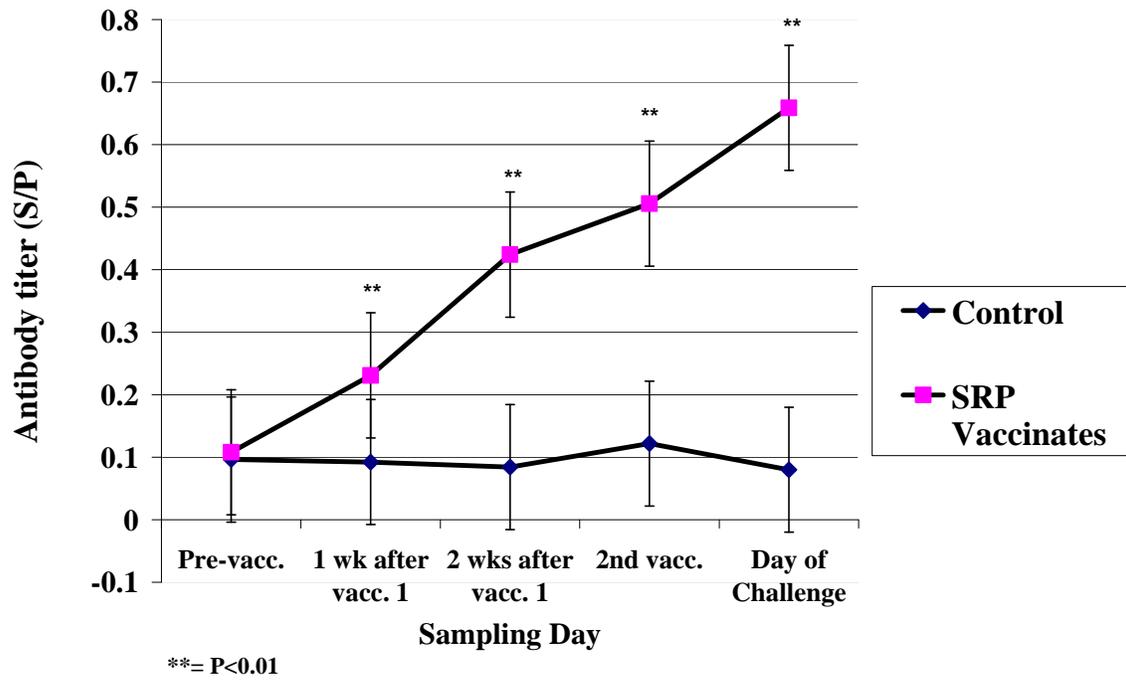


Figure 4 The Number of Animals from Each Treatment Group that had NaI<sup>R</sup> *E. coli* O157:H7 Present in the Selected Sampling Site



**Figure 5 Calves' Immunological Responses from the Day the First Vaccination was Administered to the Day of Challenge.**