**Project Title:** Investigation into the Efficacy of *bdellovibrio bacteriovorus* as a Novel Pre-Harvest Intervention to Control *Escherichia coli* O157:H7 and *Salmonella spp*. in Cattle Using an *in vitro* Model

**Principle Investigator(s):** Jennifer A. Page, Brian Lubbers, Joshua Mahe, Linda Ritsch and Sara E. Gragg

**Institution(s):** Kansas State University

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## Background

*Salmonella* and *Escherichia coli* O157:H7 are just 2 of the many pathogens responsible for several foodborne illness outbreaks each year. Cattle commonly harbor these pathogens in their gastrointestinal tracts; thus, these pathogens are capable of contaminating beef products.

Interventions administered to cattle prior to slaughter are aimed at reducing or eliminating pathogen populations within the animal, thereby reducing the opportunity for our beef supply to be contaminated. *Bdellovibrio bacteriovorus* is a gram-negative microorganism that preys upon other gram-negative microorganisms, including *Salmonella* and *E. coli* O157:H7. Fortunately, *B. bacteriovorus* is also harmless to human and animal tissues. Given the previously published effectiveness of *B. bacteriovorus* to reduce gram-negative populations, the potential of this microorganism to reduce foodborne pathogen populations warrants investigation.

The objective of this study was to mimic the digestive system of a cow to investigate the effectiveness of *Bdellovibrio bacteriovorus* to control *E. coli* O157:H7 and *Salmonella* populations cattle feces and rumen fluid.

## Methodology

*Bdellovibrio bacteriovorus* was grown per ATCC guidelines to create a stock culture that was stored in the -80°C freezer. Fecal and Rumen fluid samples were collected from dairy heifers at the Kansas State University dairy unit. The fecal and rumen fluid samples were inoculated with antimicrobial resistant or susceptible strains of *E. coli* O157:H7 and *Salmonella*. All experimental samples were mixed with 10 ml of *B. bacteriovorus* to create a final target concentration of 1.0x104 CFU/ml. At 0-, 24-, 48- and 72-hours following inoculation, the samples were analyzed to determine the surviving populations of antimicrobial susceptible and resistant *E. coli* O157:H7 and *Salmonella* in cattle rumen fluid and feces.

## Findings

*Bdellovibrio bacteriovorus* was not considered to be effective at reducing antimicrobial resistant or susceptible *E. coli* O157:H7 populations in cattle rumen fluid or feces. However, as graphs C and D illustrate below, *B. bacteriovorus* was effective at reducing *Salmonella* populations in cattle feces in comparison to the control samples. Other studies have shown *B. bacteriovorus* to be less effective against *E. coli* O157:H7, as this pathogen produces a compound called indole, which interferes with the ability of *B. bacteriovorus* to prey upon *E. coli* O157:H7.



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## Implications

Reductions in *Salmonella* populations indicate that *B. bacteriovorus* may have the potential to inhibit *Salmonella* survival and growth in cattle. Although these findings are preliminary, our research does suggest promise for *B. bacteriovorus* as a novel intervention. Future studies are necessary to determine the survivability and effectiveness of *B. bacteriovorus* throughout the entire digestive system of a cow. Eventually, studies will be required to determine efficacy within a live animal. Using this and future studies as the foundation, the long- term goal is to develop a novel intervention that will reduce *Salmonella* populations within the cow and, thus, lead to fewer Salmonellosis outbreaks.

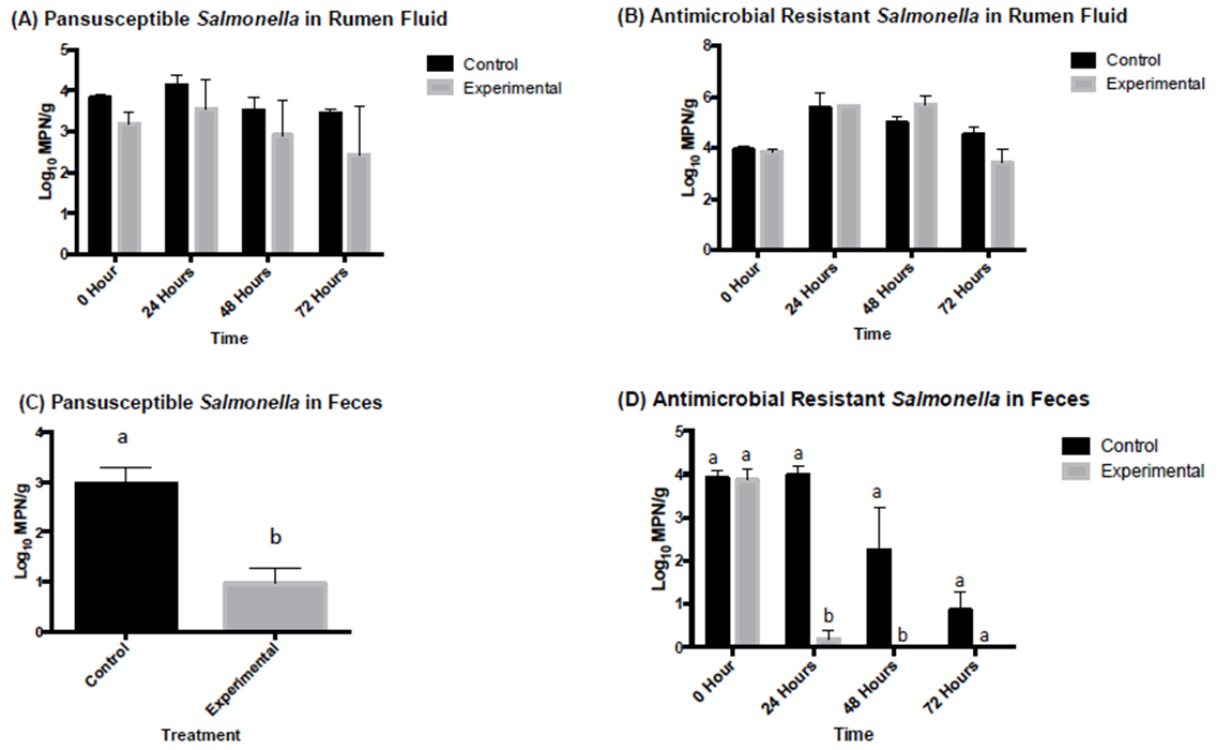


Figure 1. (A) Pansusceptible *Salmonella* in Rumen fluid, (B) Antimicrobial resistant *Salmonella* in rumen fluid, (C) Pansusceptible *Salmonella* in feces, and (D) Antimicrobial resistant *Salmonella* in feces.

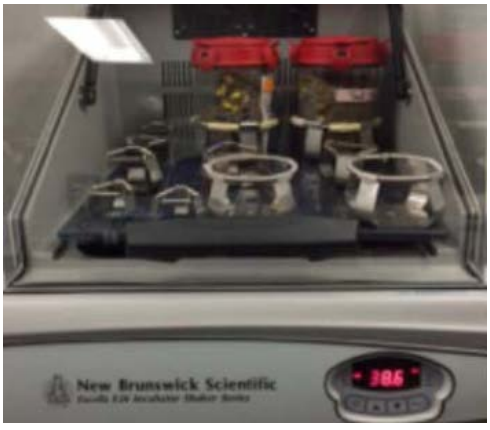


Figure 2. Simulation of the cow digestive system with an anaerobic atmosphere, typical cow body temperature and slight shaking to mimic peristaltic motion.



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Figure 3. A typical laboratory setup to analyze feces and rumen fluid for *Salmonella*.

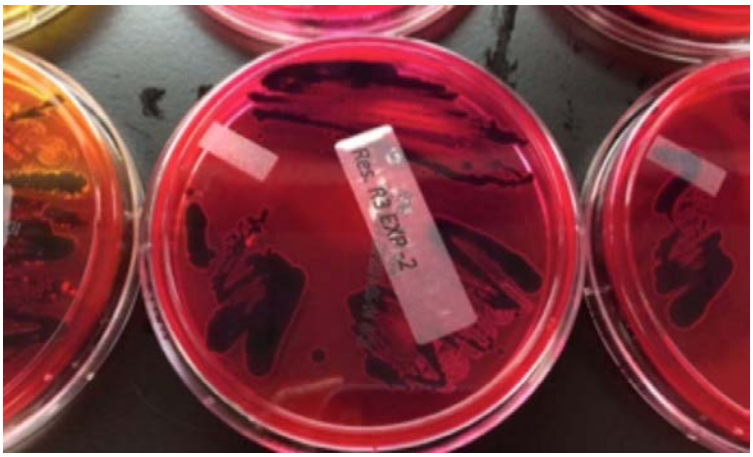

Figure 4. *Salmonella* growth on petri dishes.



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