The Impact of Vaccination Against *Lawsonia intracellularis* on Shedding of *Salmonella enterica* serovar Typhimurium and the Gut Microbiome

Fernando L. L. Leite[1], Randall S. Singer[1], Tonya Ward[2], Connie J. Gebhart[1], Richard E. Isaacson[1]

1: Department of Veterinary and Biomedical Sciences; 2: Biotechnology Institute, University of Minnesota, St. Paul, MN, USA

INTRODUCTION

- *Salmonella enterica* serovar Typhimurium and *Lawsonia intracellularis* are two of the most prevalent intestinal pathogens of swine. *L. intracellularis* causes proliferative enteropathy, a disease which leads to decreased weight gain, diarrhea and production loss. *Salmonella* Typhimurium causes diarrhea but also results in subclinical persistent colonization of pigs and can lead to food borne illnesses. *Salmonella enterica* is a leading cause of foodborne illness worldwide and is also a leading cause of death due to food borne illnesses[1,2]. It has been estimated that the economic losses due to salmonellosis in the USA exceeds $3 billion per year[3]. Strategies aimed at reducing the burden of *Salmonella enterica* in all meats are crucial, including pork. *L. intracellularis* infection has been found as a risk factor for increased *S.* Typhimurium shedding in swine[4].
- The objective of this study was to investigate if oral live vaccination against *L. intracellularis* could lead to decreased *S.* Typhimurium shedding and if vaccine induced changes were related to changes in the gut microbiome.

MATERIALS AND METHODS

Animals

Three replicates of 3 animals were housed in separate rooms for a total of 9 animals in each of 5 treatments

Treatments

1. **Non-infected control (n=6) (Control)**
2. **S. Typhimurium infection (Sal)**
3. **S. Typhimurium, *L. intracellularis* co-infection (Sal Law)**
4. **S. Typhimurium infection and vaccination with Enterisol® Ileitis* (Sal Vac)**
5. **S. Typhimurium, *L. intracellularis* co-infection and vaccination with Enterisol® Ileitis*(Sal Law Vac)**

*** Boehringer Ingelheim Vetmedica**

Timeline of Treatments

Days post Salmonella Infection (dpi) and Treatment

- 28 dpi | **Enterisol® Ileitis vaccination**
-7 dpi | ***L. Intracellularis* challenge with 2 x 10^9 cfu**
0 dpi | ***S.* Typhimurium challenge with 1 x 10^8 cfu + Sample collection**
2 dpi | **Sample collection**
7 dpi | **Sample collection**
14 dpi | **Sample collection**
21 dpi | **Sample collection**
28 dpi | **Sample collection**
49 dpi | **Last collection time point and euthanasia of animals**

***Salmonella* quantification**

Feces collected from rectum → Serial dilution and incubation in tetrathionate for 48hrs → Incubation in Rappaport-Vassiliadis for 24 hrs → Plating and quantification in XLT4 Plates → Positive colonies confirmed by *invA* gene PCR

Most Probable Number (MPN) enrichment quantification method

Microbiome Analysis

Feces collected from rectum → Extract DNA, and amplify the V1-V3 region of 16S rRNA gene → Sequencing with MiSeq 300bp paired end reads → Microbiome data analysis

RESULTS

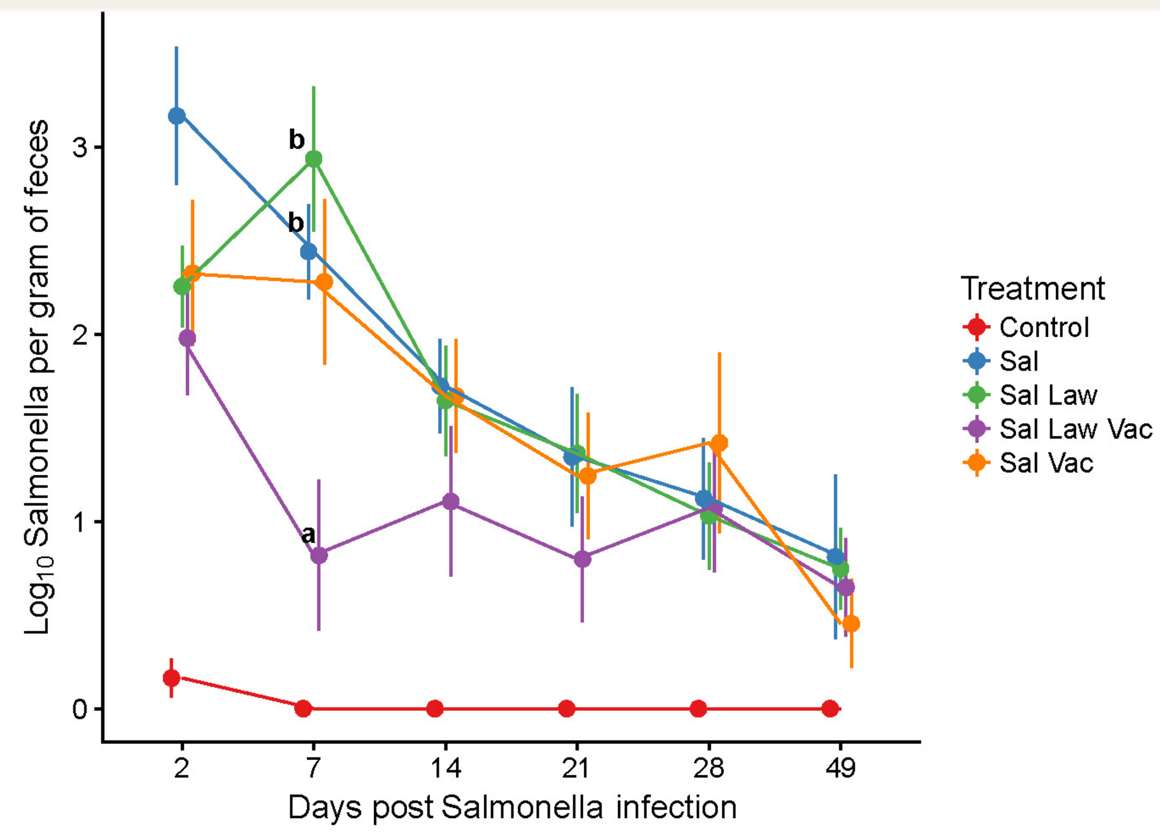

Figure 1. Fecal shedding of *Salmonella enterica* serovar Typhimurium over time. Significant differences between treatment groups are designated by different letters ($p < 0.05$).

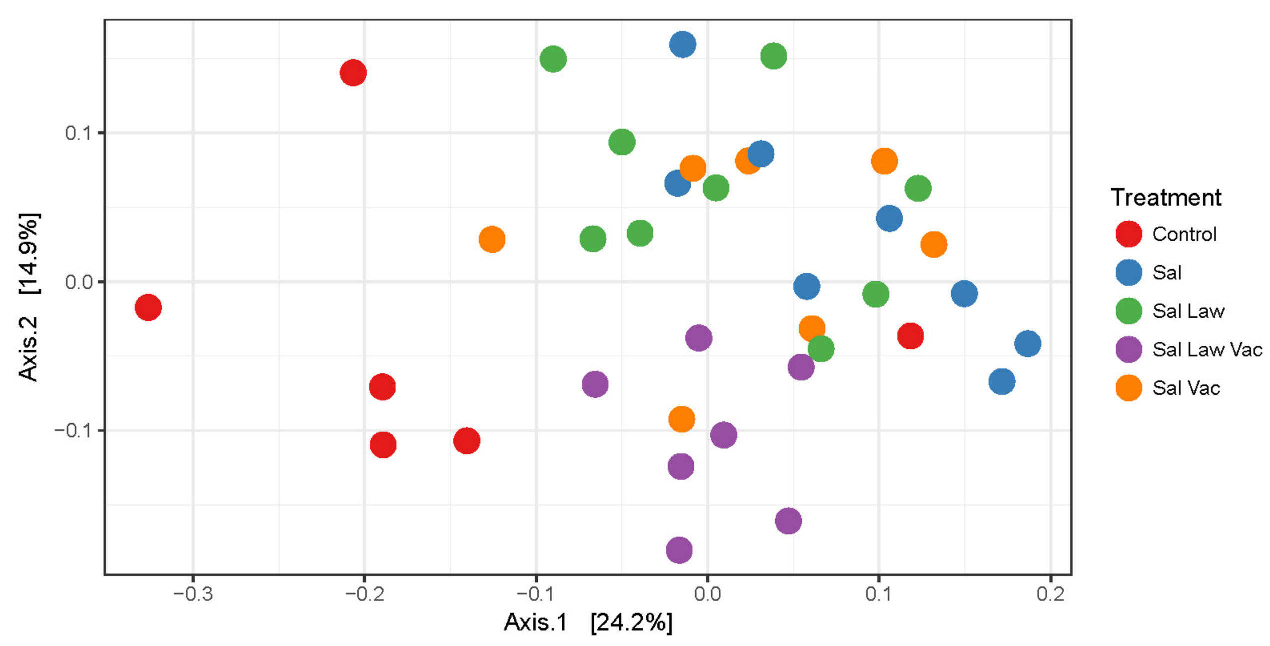

Figure 2. Principal coordinate analysis of microbiome composition among different treatment groups at 7 das post *Salmonella* infection (ANOSIM $p < 0.05$). Note the distinct clustering of the Sal Law Vac group.

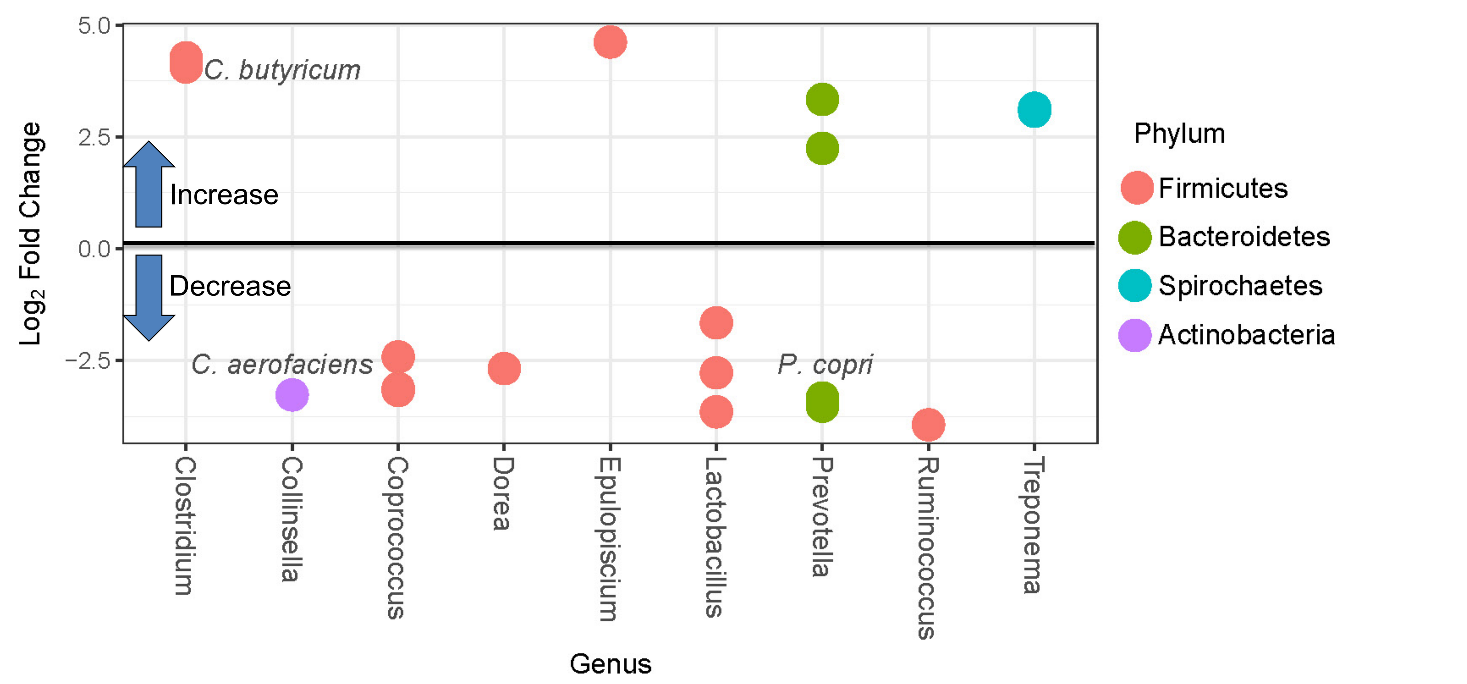

Figure 3. Differentially abundant bacteria identified comparing Sal Law to Sal Law Vac treatment at 7 days post *Salmonella* infection. Vaccine increased abundance of *C. butyricum* and decreased abundance of *C. aerofaciens* and *P. copri.*

DISCUSSION AND CONCLUSIONS

- Vaccination against *L. intracellularis* significantly reduced *Salmonella* shedding ($p<0.05$, Figure 1) in co-infected animals.
- Significant differences in beta diversity were found (ANOSIM $p<0.05$, Figure 2). The Sal Law Vac group had a distinct microbiome community structure from other groups demonstrating that vaccination led to a different gut microbiome response to *Salmonella* infection.
- Vaccination increased *Clostridium* and *C. butyricum* which produce butyrate, a short chain fatty acid that can down regulate *Salmonella* invasion genes[5]. *Prevotella corpri* and *Collinsella aerofaciens* were decreased, these are pathobionts that can induce high inflammatory responses that could favor *Salmonella* infection[6,7].
- These results indicate that vaccination against *L. intracellularis* in co-infected herds may provide a new tool to increase food safety and animal health by decreasing *Salmonella* shedding and transmission without the need for antibiotics.

References:
1. Kirk, PLoS Med. 12, 1–21 (2015).
2. Scallan, Emerg. Infect. Dis. 17, 7–15 (2011).
3. Hoffmann, Food Prot. 75, 1292–1302 (2012).
4. Beloeil, Prev. Vet. Med. 63, 103–120 (2004).
5. Gantois, Gene Expression. 72, 946–949 (2006).
6. Zhang, Cell Walls. 69, 7277–7284 (2001).
7. Dillon, Mucosal Immunol. 9, 24–37 (2016).

USDA NIFA
United States Department of Agriculture
National Institute of Food and Agriculture

UNIVERSITY OF MINNESOTA