DISSERTATION

A COMPREHENSIVE STUDY OF *SALMONELLA* INFECTIONS AND MICROBIAL ANALYSIS OF PROBIOTICS ON BEEF CATTLE

Submitted by

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ABSTRACT

A COMPREHENSIVE STUDY OF *SALMONELLA* INFECTIONS AND MICROBIAL ANALYSIS OF PROBIOTICS ON BEEF CATTLE

Non-typhoidal *Salmonella* remains a significant concern for food safety in the United States, causing millions of infections, hospitalizations, and deaths yearly. The Healthy People 2030 initiative set forth by the U.S. Department of Health and Human Services aims to address this issue by establishing goals and objectives for national health promotion and disease prevention, including two objectives focused on *Salmonella* control in the food supply. The recent declaration of *Salmonella* as an adulterant in certain poultry products by the U.S. Department of Agriculture further highlights the urgency of this issue. To align with the Healthy People 2030 goals and achieve a 25% reduction in salmonellosis, the U.S. Department of Agriculture's Food Safety and Inspection Service (FSIS) implemented new performance standards for beef products. However, such policies must be supported by quantitative microbial risk assessments (QMRA) to determine their impact on *Salmonella* infections. Therefore, these analyses would benefit from a systematic review examining existing literature on *Salmonella*, considering factors such as illness rates, exposure, and bacterial loads. This review included 42 articles that provided data necessary for fitting a dose-response model to empirical data that describes how dose, virulence group, and food vector affect illness (attack) rates. Results from the mixed-effects logistic regression model showed significant impacts of log dose consumed, virulence group, and food vector on illness rates. Notably, *Salmonella* serogroups of “Higher” virulence were found to be associated with greater odds of illness than “Lower” virulence strains. The study highlights the need for improved data

reporting and standardized outbreak investigations to enhance the fitting of models to outbreak data. By considering factors like serovar group and food vector in the modeling process, regulators can demonstrate what influences attack rate to frame more effective food safety policies. In conclusion, this systematic review provides valuable insights into *Salmonella* infection risk from food sources and emphasizes the importance of evidence-based policies to reduce the burden of *Salmonella*-related illnesses and improve food safety in the United States.

Liver abscesses in beef cattle are a common problem associated with highly-fermentable carbohydrate diets during finishing, leading to decreased production efficiency and aggregate carcass value. Dietary antimicrobial supplementation, such as tylosin, helps to control liver abscesses but raises concerns about selection for antimicrobial resistance. This study examined the impact of a probiotic mixture of propionic and lactic acid bacteria on microbial communities and antimicrobial resistance genes (ARGs) in fecal and liver abscess samples from beef cattle alongside *Salmonella* populations of mesenteric lymphatic tissues.

Treatment diets fed in this study included a probiotic mixture alone (DFM), inclusion of Tylosin (TYL), a combination of including both (DFM+TYL), and a control group diet that did not include any supplements (CON). Fecal samples were collected at the time that feeding started, and then 28 d before arriving at the abattoir, where liver abscesses and mesenteric lymph nodes were sampled. Fecal and liver abscess samples were subjected to 16S rRNA and targeted enriched shotgun metagenomics to evaluate microbial communities and resistance genes of bacteria present. A portion of the liver abscess and mesenteric lymph nodes were tested for presence of *Salmonella* using PCR with further analysis of enumeration and serotype classification for mesenteric lymph nodes. Results showed no differences ($P > 0.05$) between the fecal microbiomes of the different treatment groups, and the addition of tylosin or probiotic mixture did not impact the fecal

resistome. Similarly, no differences ($P > 0.05$) were observed between the liver abscess microbiomes of the different ($P > 0.05$) treatment groups, with Fusobacteria and Bacteroidetes being the dominant phyla in liver abscesses. Results indicated that incorporating DFMs did not affect *Salmonella* prevalence in the cattle's mesenteric lymph nodes or liver abscesses. Presence of *Salmonella* was found at low levels in only 22% of samples (91 positive out of 503 samples), just below 1 log CFU/g, and was predominantly represented by the C1 serogroup in mesenteric lymph nodes. These findings suggest that while diet interventions may not have a substantial impact, *Salmonella* can colonize mesenteric lymphatic tissues in cattle at low frequencies and concentrations. Treatment groups tested had no impact ($P > 0.05$) on fecal and liver abscesses microbiomes and resistance gene presence, along with no impact on *Salmonella* prevalence in liver abscesses or mesenteric lymphatic tissues.

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CHAPTER 1

REVIEW OF LITERATURE

Due to the nature of this dissertation, the literature review will be split into two parts, with the first focusing on *Salmonella* and the second focusing on liver abscesses in cattle.

PART I

Foodborne Illness in the United States

In the United States, the Centers for Disease Control and Prevention (CDC) estimates that 48 million people get sick, 128,000 are hospitalized, and 3,000 die from foodborne diseases yearly (CDC, 2022). While public health is a major concern, foodborne illnesses also have dire economic impacts. The Economic Research Service reported that, in 2018, a total of $17.6 billion was lost due to foodborne infections (USDA ERS, 2021). While many pathogens play a role in food-borne illnesses, *Salmonella* (non-typhoidal) is reported to be the second highest in illnesses and the most common bacterium associated with hospitalizations and deaths in the United States (CDC, 2022). It is apparent that *Salmonella* plays a critical role in public health.

Salmonella in Food Systems

Salmonella is a facultative, anaerobic, rod-shaped, and predominantly motile gram-negative bacterium and a member of the *Enterobacteriaceae* family. *Salmonella* can be classified into two major groups as typhoidal or non-typhoidal, with most foodborne infections arising from non-typhoidal *Salmonella*. In the U.S. alone, and estimated 1.35 million infections, 26,500

hospitalizations, and 420 deaths occur per year occur due to *Salmonella* spp. (CDC, 2023), making it a bacterium of concern, especially in food safety.

Infection with *Salmonella* starts as cells enter the digestive tract and penetrate the epithelial cells lining the intestinal wall. This process consists of *Salmonella* pathogenicity islands that encode for type III secretion systems and multi-channel proteins that allow *Salmonella* to inject its effectors across the epithelial cell membrane into the cytoplasm (Eng et al., 2015). *Salmonella* then becomes engulfed by the cell, which is believed to be vital for pathogenesis (Bakowski et al., 2008). *Salmonella* is unique because the host cell would typically elicit an immune response, and lysosomes would begin to degrade the intracellular bacteria. However, with the pathogenicity islands, the type III secretion genes fill the vacuole with other effector proteins, inhibiting lysosomes from degrading the vacuole (Eng et al., 2015).

Salmonellosis can vary in onset, but typically those in the at-risk demographic consisting of individuals aged under 5 or over 65 years of age, people that are immuno-compromised (either by illness or medical treatment), or pregnant women are more susceptible to infection (ASPA, 2019). Symptoms of salmonellosis typically occur within 6 – 12 hours and are usually self-limiting, consisting of diarrhea, fever, and stomach cramps (CDC, 2023; Eng et al., 2015). While this infection can vary in severity and symptoms, a typical infection lasts 4 – 7 days (CDC, 2023).

Not All Salmonella are Equal

Salmonella enterica serovars have been found to differ in virulence. Fenske et al. (2023) identified a virulence group of *Salmonella* that had higher rates of infections leading to hospitalization. This group consisted of *S. enterica* ser. Litchfield, *S. enterica* ser. Typhimurium, *S. enterica* ser. Enteritidis, *S. enterica* ser. Agona, *S. enterica* ser. Dublin, *S. enterica* ser. Newport,

S. enterica ser. Muenchen, *S. enterica* ser. Reading, and *S. enterica* ser. Saintpaul (Fenske et al., 2023). These authors concluded that by having this information, foodborne surveillance could best target these serovars as they have the greatest potential to impact human health (Fenske et al., 2023).

USDA Regulations Regarding Salmonella

On August 1, 2022, the U.S. Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) declared *Salmonella* to be regarded as an adulterant in breaded and stuffed raw chicken products (USDA, 2022). While the USDA has had performance standards such as FSIS directive 10250.2 that allow for 5 of 51 broiler carcasses to test positive for *Salmonella*, no strict requirements were previously in place for further processed poultry (FSIS, 2021).

This declaration generated some concern with beef processors as there have been several large outbreaks of *Salmonella* in ground beef. While *Escherichia coli* O157:H7 and Shiga toxin-producing *E. coli* (STEC) have been a concern since the Jack in the Box outbreak in 1993, *Salmonella* is generally not associated with ground beef. However, in CDC reports from 1973 to 2011, Laufer et al. (2015) found that 4.8% of *Salmonella* outbreaks in the U.S. were associated with beef products. In addition, these authors identified a change in the food vector with outbreaks shifting from whole muscle cuts (such as roast) to ground beef. To further support this point, it was reported that over the 2002-2011 timespan, ground beef was identified as the food vector for 45% of all *Salmonella* beef infections, with 3 of those large outbreaks resulting in the same number of infections from the years prior (CDC, 2023; Laufer et al., 2015). Therefore, this has generated

some concern among beef processors that *Salmonella* could be labeled as an adulterant in ground beef.

While historically, *Salmonella* was not typically associated with beef, *Salmonella* is commonly identified in cattle and the environments they are raised (Webb et al., 2017; Dodd et al., 2011). While ground beef is a common vector identified for *Salmonella*, *Salmonella* also has been identified at 27.5% in liver abscesses of fed cattle and 16.5% in market cows and bulls (Herrick et al., 2022). While liver abscesses cause condemnation of the product as edible tissue, it still holds importance in animal health. Therefore, it is apparent that a collection of articles regarding *Salmonella* outbreaks is necessary to accurately model *Salmonella* outbreaks and factors that can contribute to them.

PART II

Liver abscesses are commonly identified in feedlot cattle. The disease typically starts with ruminal acidosis, then results in rumenitis, eventually leading to a liver abscess. The disease matters to the industry because liver abscesses account for $60 million a year of economic loss (Jaborek, 2023; Herrick et al., 2022).

Ruminal Acidosis

The rumen is a complex site of anaerobic fermentation of feedstuffs into fatty acids that the animal can utilize. Assuming this constant rate of breakdown of feed by the bacteria and the rate of absorption by the animal are equal, the pH ranges from 5.8 to 6.6.8 in grain-adapted cattle (Nagaraja and Titgemeyer, 2007). With pH ranging daily from 5.6 to 6.8 in the rumen, factors such as intake of food, the buffering capacity of the animal (through saliva), and absorption of the fatty acids generated play significant roles in ruminal pH (Gonzáles et al., 2012; Nagaraja and Titgemeyer, 2007). While many factors can result in liver abscess occurrence, feeding a high-

energy, readily fermentable diet low in roughage is often a major component of liver abscess occurrence (Nagaraja and Chengappa, 1998). When evaluating cattle fed a high energy, highly fermentable diet (such as in a feedlot), rations typically contain less digestible fiber and more highly fermentable products such as grain to help animals rapidly gain weight. However, less saliva is generated by decreasing rumination, and the saliva's ability to buffer ruminal fluid is limited (Gonzáles et al., 2012). While this is just a small part of a very complex environment, saliva plays a major role in buffering the rumen due to its high levels of bicarbonate and phosphate (Bailey and Balch, 1961).

As the reduction in buffering ability begins, rumen pH declines. Whenever pH falls below 5.6, it is considered a benchmark for ruminal acidosis, with pH 5.6-5.0 considered subacute, and anything below pH 5.0 regarded as acute acidosis. While both sub and acute acidosis are similar in increased lactic acid-producing bacteria, a full list of the differences identified by Nagaraja and Titgemeyer (2007) can be found in Table 1. In summary, as the pH of the rumen drops, there is an increase of lactic acid-producing bacteria, but bacteria with the ability to buffer and utilize that lactic acid are killed off, resulting in a higher concentration of lactic acid, shifting the whole rumen microbiome to favor bacteria that can withstand low pH levels. With lower pH and no mucosa to protect epithelial cells, the ruminal wall becomes damaged, generating a lesion (Abdela, 2016; Nagaraja and Chengappa, 1998). This condition is often referred to as rumenitis.

Bacteria Involved in Liver Abscess Formation

While many bacteria are associated with liver abscess formation, the most identified one is *Fusobacterium necrophorum* (Reinhardt and Hubbert 2015; Nagaraja and Chengappa, 1998). *Fusobacterium necrophorum* is an obligately anaerobic, non-spore-forming, non-motile, gram-

negative bacterium that is endemic to the rumen. While typically occurring at low levels, switching cattle from roughage to a grain-based diet has been shown to increase *F. necrophorum* levels by 10-fold (Tan et al., 1994). This likely occurs due to *F. necrophorum* being able to utilize lactate for fermentation. As the rumen drops in pH, more lactate is produced by bacteria, which the rumen cannot buffer, resulting in more readily available lactate for *F. necrophorum* to utilize (Nagaraja and Chengappa, 1998). Furthermore, with a decrease in pH and the occurrence of rumenitis, *F. necrophorum* has an opportunity to colonize lesions and gain access to the bloodstream. It is hypothesized that after *F. necrophorum* gains access to the bloodstream, it enters the portal vein, which is filtered by the liver (Nagaraja and Chengappa, 1998).

F. necrophorum and the Liver

With *F. necrophorum* being an obligate anaerobe, the highly oxygen-rich liver is not ideal for colonizing (Reinhardt and Hubbert, 2015). Therefore, it is suggested that other bacteria, such as *Trueperella pyogenese* are present and produce needed substrates, such as lactic acid for *F. necrophorum* to assist with the colonization of the liver (Reinhardt and Hubbert, 2015). As *F. necrophorum* can replicate in the liver, the leukotoxin, and endotoxic lipopolysaccharide may protect it from phagocytosis (Nagaraja and Chengappa, 1998). While defending itself, *F. necrophorum* begins killing phagocytes by releasing cytolytic products such as lysosomal enzymes and oxygen metabolites (Nagaraja and Chengappa, 1998). To react to this and contain the infection, the body develops a fibrin-encapsulated abscess (Forrester et al., 1985).

However, recent research has identified that the hindgut may play a significant role in liver abscess formation (Pinnell et al., 2022a). Pinnell et al. (2022a) found that some microbial

communities in abscesses mirrored those in the hindgut more so than those of the rumen, suggesting that bacteria migrated from the hindgut rather than the rumen.

Microbiome of Liver Abscesses

While *F. necrophorum* is the primary organism associated with liver abscesses, there is an established microbiome that sets up following the infection. While the order of abundance may change, in general, the abundance typically consists of Fusobacteria, Bacteroidetes, Proteobacteria, Firmicutes, and Actinobacteria (Pinnell and Morley, 2022ab; Weinroth et al., 2017; Amachawadi et al., 2016). However, Pinnell et al. (2022a) found two distinctive community makeups of liver abscesses, with communities either dominated by Fusobacteria or Bacteroidetes. Those communities dominated by Fusobacteria commonly included the *Acinetobacter*, *Lactobacillus*, *Pseudomonas,* and *Psychrobacter* genera, while Bacteroidetes-dominated abscesses contained *Atopobium*, *Campylobacter*, *Filifactor*, *Helcococcus*, *Parvimonas*, and *Trueperella* genera (Pinnell et al., 2022a). Upon comparing these bacterial communities to similar communities through the digestive tract, it was hypothesized that those liver abscesses consisting of Bacteroidetes might originate from the hindgut rather than the rumen (Pinnell et al., 2022a). Further, those abscesses consisting of Fusobacteria most likely originate from the rumen as those additional microbes identified are found in low pH rumen environments (Pinnell et al., 2022a; Nagaraja and Titgemeyer, 2007).

Additional Factors Contributing to Liver Abscess Formation

As mentioned previously, a high grain, low forage diet that drops ruminal pH is often implicated in liver abscess formation; however, growth; other factors, such as cattle breed and

management practices can also contribute to the occurrence of liver abscesses. While liver abscesses are common in beef-breed steers in the feedlot, the prevalence among dairy types (such as Holstein) is far more rampant (Herrick et al., 2022). In fact, , it has been reported that prevalence of liver abcesses in dairy cattle increased from 12% in 2003-2004 to up to 55% in 2015 (Reinhardt and Hubbert, 2015). In addition, Holsteins are fed a high-grain diet for a much longer duration than beef steers. Due to this, many speculate that the greater amount of time on feed plays a major part in the increased prevalence of liver abscesses often found in Holstein cattle (Herrick et al., 2022; Nagaraja and Chengappa, 1998).

Furthermore, management practices such as feeding frequency have been found to impact the prevalence of liver abscesses. As feeding behaviors are dependent on feeding management, intensive management should be utilized as disruptions in feeding behaviors can negatively impact ruminal pH (Gonzáles et al., 2012). Research into producing the acidosis condition in cattle was successful when feed was withheld for 24 h and then followed by overfeeding an all-grain diet (Nagaraja and Titgemeyer, 2007). Additional research has confirmed these claims and includes ration control (large variations of forage in the diet) to be another potential control point in regulating ruminal pH (Soto-Navarro et al., 2000; Grant and Albright, 1995). In fact, Gonzáles et al. (2012) reported that frequent feeding throughout the day caused greater saliva production and passage of feed stuffs, which resulted in a more stable ruminal pH.

Production Impacts

Liver abscesses have been identified as the largest contributor (67%) to liver condemnations (Eastwood et al., 2017; Brown and Lawrence, 2010). The National Beef Quality Audit reported that liver abscess occurrence had increased greatly since 1991 and about 30% of

cattle exhibited liver abscesses in 2016 (Eastwood et al., 2017). Just based on liver condemnations alone, completely disregarding impacts from reduced carcass weights, a total of $60 million a year has been hypothesized for total visceral losses (Jaborek, 2023; Harrick et al., 2022). Additionally, adhesion of severe liver abscess to the carcass can directly impact dressing percentage, with a trim loss of 0.43% to 0.26% being suggested (Brown and Lawrence, 2010). While these percentages are low, liver abscesses can occur anywhere from 0-100% of cattle slaughtered, resulting in major economic losses (Reinhardt and Hubbert 2015). While the loss of the liver is a huge loss in profits, the losses associated with decreased daily gain and animal performance should be considered to understand the economic impacts of liver abscesses fully.

As the rumen pH decreases, the animal's feed intake is slowed, resulting in the slower passage of feed which negatively affects average daily gain (Gonzáles et al., 2012). While the cause of this reduction is unclear, three theories are outlined in the literature. The first theory outlined by Gonzáles et al. (2012) is that due to the high accumulation of organic acids in the rumen, epithelial cells send a feedback signal to the brain to reduce intake. Due to this, a decrease in rumen motility is observed that slows the passage of feed, resulting in decreased food intake. The second is about the accumulation of organic acids and glucose, resulting in increased osmolality of the ruminal fluid and blood. While this hypothesis has been tested in vivo, experiments have trouble reproducing the findings in vitro due to the animal's self-limiting food intake to avoid a further pH drop in the rumen (Gonzáles et al., 2012). The final theory is similar to the first theory in that a high accumulation of organic acids occurs, but Allen et al. (2005) suggest that propionate is the organic acid having the greatest impact. Due to an accumulation of propionate in the blood and then in the liver, the liver sends feedback signals to the nervous system

resulting in the animal stopping eating even within the course of a meal (Allen et al., 2005). Gonzáles et al. (2012) suggested that the third theory is the most plausible theory.

Economic Impacts

Due to decreased intake, liver abscesses impact average daily gain (ADG), with increasing severity of liver abscesses resulting in a greater depression of average daily gain (Rezac et al., 2014; Brown and Lawrence, 2010). While Brown and Lawrence (2010) found that mild and moderate liver abscess occurrence did not impact ADG, the severe occurrence of liver abscess can reduce ADG by 0.17kg/d (Rezac et al., 2014). Furthermore, these economic losses are estimated to range from $20-$80 per head (Brown and Lawrence 2010). In addition to the economic issues, welfare of these animals is also something to consider. An additional point of focus with liver abscesses is that cattle typically show no symptoms, with the condition only identifiable during the slaughter process (Gonzáles et al., 2012; Nagaraja and Chengappa, 1998). While ultrasonography has been tested, application in a feedlot setting is unrealistic as the whole liver cannot be easily visualized due to other organs getting in the way (Nagaraja and Chengappa, 1998).

Tylosin for Prevention of Liver Abscesses

Tylosin is a bacteriostatic macrolide antibiotic and feed additive used commonly in veterinary medicine. Tylosin works against gram-positive organisms in the rumen with limited impacts on gram-negative organisms. Tylosin is believed to impact liver abscesses through its inhibitory effects on *F. necrophorum* (Nagaraja and Chengappa, 1998). While this is unexpected as *F. necrophorum* is a gram-negative organism, literature commonly finds Tylosin lowering liver abscess occurrence in grain-fed cattle (Nagaraja and Chengappa, 1998). Additionally, Tylosin

inhibition of *F. necrophorum* is slightly disputed as it is unclear whether its greatest impacts occur in the blood, liver, or rumen (Reinhardt and Hubbert, 2015; Nagaraja and Chengappa, 1998). However, most hypothesize that the main effects of limiting *F. necrophorum* occur in the rumen (Nagaraja and Chengappa, 1998). Furthermore, although Tylosin has been shown to reduce liver abscess prevalence in feedlot cattle, liver abscesses are still found in 15% of feedlot cattle (Davedow et al., 2020; Reinhardt and Hubbert, 2015; Nagaraja and Chengappa, 1998). While a complete removal of abscesses in feedlot cattle is uncommon, Nagaraja and Chengappa (1998) have hypothesized that due to low concentrations of *F. necrophorum*, other bacteria are able to cause liver abscesses. Additionally, increased antimicrobial resistance or delivery of Tylosin in the feed may not provide the concentrations necessary to remove all *F. necrophorum* populations (Reinhardt and Hubbert, 2015; Nagaraja and Chengappa, 1998).

Regulations Regarding the Use of Tylosin

Tylosin is a macrolide antibiotic in the same class of antimicrobials important for human health in combating foodborne infections such as *Campylobacter jejuni* and *Salmonella* (Cazer et al., 2020; Roberts, 2008). Hence, recent restrictions have been implemented requiring a veterinary prescription throughout the United States and Canada for using antibiotics such as Tylosin (Davedow, 2020; U.S. Food and Drug Administration, 2019). These restrictions were put into place as of January 1, 2017, together with removal of the growth promotants claim from the label alongside more intensive input from the veterinarian prescribing the antibiotic (Dewell et al., 2022). However, these restrictions only apply to antibiotics deemed medically important (such as Tylosin) and not to ionophores commonly used in beef production systems (Dewell et al., 2022). With the growth promotant claim removed and more intensive oversight being required for

feeding, sales of tetracycline-class antimicrobials dropped from 5.8 to 3.5 million kg (Dewell et al., 2022).

While current regulations were established based on antimicrobial resistance threats, literature on the topic reflects an unclear reality to Tylosin's impact on resistance gene presence in bacteria (Cazer et al., 2020; Weinroth et al., 2019). A meta-analysis conducted by Cazer et al. (2020) found there is potential for increased resistance to macrolides. However, more studies with thorough reporting are required to support their findings.

Factors Affecting Resistance Gene Prevalence

One of the factors reported to be responsible for bacterial antimicrobial resistance development in cattle is the duration of feeding Tylosin (Czaer et al., 2020; Müller et al., 2018; Beukers et al., 2015). Reducing the duration of feeding Tylosin has been found to help decrease pressures selecting for antimicrobial resistance (Beukers et al., 2015). However, the impacts on liver abscess prevalence results are mixed. Upon comparing control cattle (cattle that did not receive Tylosin) to those that received Tylosin in the first 78% of duration in the feedlot, the severity of liver abscesses was greater, while those that received Tylosin in the later 75% of feedlot duration had no differences in severity (Davedow et al., 2020). In contrast, Müller et al. (2018) reported that intermittent feeding of Tylosin during the finishing stage of production decreased the total percentage of liver abscess occurrence. This conflicts with the results reported by Davedow et al. (2020). When evaluating resistance gene presence, both studies sampled fecal bacteria and found that feeding at the beginning or end of the time in the feedlot had no difference in the prevalence of antimicrobial-resistance genes. However, another study found conflicting results such that, upon feeding Tylosin, fecal enterobacteria showed higher prevalence of copper and

macrolide resistance genes (Amachawadi et al., 2015). While the copper was supplementarily added to the rations, unlike the other studies, it suggests that adding antimicrobial agents to a diet might encourage resistance genes to be more prevalent in bacteria.

Examinations of the Resistance Gene Community

When evaluating soil and fecal resistomes of cattle exposed to Tylosin, no differences for both sample types were identified by Weinroth et al. (2019). While this approach is different from traditional independent culture studies, with the implementation of metagenomics, a "snapshot" of resistance genes of all bacteria present in the samples is evaluated compared to a single organism. Using this approach, Weinroth et al. (2019) found that the location of cattle production had a greater impact on the resistance genes present than administration of Tylosin in a feedlot. This suggests that even bacteria sourced from animals that did not have Tylosin administered to them, a naturally occurring resistance may be present. When examining antimicrobial resistance genes of wild Elk, Bison, and feedlot cattle, tetracycline resistance genes were dominant across all species examined suggesting that tetracycline resistance is endemic among the animal and may not be impacted by antibiotic use (Pinnell et al., 2023). However, cattle tended to have lower microbial diversity and higher resistance gene diversity alongside greater resistance gene presence to macrolides, lincosamides, and streptogramins compared to wild elk and bison populations (Pinnell et al., 2023). While these finding contradict those of Czaer et al. (2021), Müller et al. (2018), and Beukers et al. (2015) this might originate due to the focus on *Enterococcus* spp., and as a result, the resistance genes of all bacteria present were not captured.

Alternatives for Tylosin in the Feedlot

Regardless of contrary findings of Tylosin use and antimicrobial resistance in the literature, regulations could limit its use. As discussed, Tylosin can greatly reduce the prevalence of liver abscesses, making production more economically sound. Therefore, a replacement to help reduce liver abscess incidence that does not contribute to antimicrobial resistance is needed (Rezac et al., 2014; Brown and Lawrence 2010). The most actively researched preventatives focus on vaccine and probiotic use.

Vaccines

When evaluating efficacy of vaccination against liver abscesses in the feedlot, results are mixed (Reinhardt and Hubbert, 2015; Checkley et al., 2005). Checkley et al. (2005) were only able to decrease severity of liver abscesses with vaccination with no impacts on liver abscess occurrence. This suggest that the vaccine could only decrease severity and not control for the prevalence of liver abscesses. Saginala et al. (1997) reported that liver abscesses still occurred regardless of vaccine usage but found that cattle without abscesses possessed higher levels of antileukotoxin antibodies, suggesting that antileukotoxin antibodies might have a protective effect against the condition. Later published research supported the finding that antileukotoxin expressed a protective effect, but when applied in a vaccine, no differences could be identified (Amachawadi and Nagaraja, 2016). A review by Reinhardt and Hubbert (2015) suggested that methods that worked in a controlled setting were often found ineffective upon application in the feedlot environment.

Probiotics

Probiotics can modulate the digestive tract microbiota and benefit the host animal (Uyeno et al., 2015). With cattle, it has been established that the bacterial and fungal communities of the rumen can be modulated and shifted with probiotics (Mansilla et al., 2022). With this modulation, feed efficiency, the prevalence of pathogenic bacteria, and ruminal pH can be controlled without contributing to antimicrobial resistance (Mackeyet al., 2023; Mansilla et al., 2022; Arowolo and He, 2018; Uyeno et al., 2015). However, efficacy of probiotics in beef cattle is highly debated, while changes have been identified, practical application is unclear.

Typically, the main types of probiotics used in cattle consist of yeast cultures (typically *Saccharomyces cerevisiae*) or *Lactobacillus* strains of bacteria (Uyeno et al., 2015). Literature suggests that the use of probiotic yeast often results in improved fiber digestion and improving fungi populations in the rumen (Arowolo and He, 2018). At the same time, use of *Lactobacillus* strains results in higher amounts of volatile fatty acids and greater protein synthesis (Arowolo and He, 2018). Furthermore, yeast has been investigated for its probiotic capabilities. However, most probiotics in beef cattle focus on bacteria (Getabalew et al., 2020).

When looking at controlling acute acidosis in the rumen, Goto et al. (2016) identified that 20 to 50 g of a multi-strain probiotic for 7 days might improve low pH and high lactic acid levels in the rumen. Mixed probiotics utilized consisted of *Lactobacillus plantarum* strain 220 (9×10^6 colony-forming units (CFU)/g), *Enterococcus faecium* strain 26 (9×10^5 CFU/g), and *Clostridium butyricum* strain MiyariJ (9×10^4 CFU/g; Goto et al., 2016). Additionally, when supplemented with lactobacilli probiotics, Mansilla et al. (2022) found overall increased cattle performance with higher weights and suggested that the probiotics reduced shedding of *E. coli* O157:H7. Furthermore, another suggested approach is to combine antibiotics with probiotics to

limit the total use of the antibiotic. Feed efficiency was found to decrease when looking at a symbiotic approach (one combining pro- and pre-biotics) to replace antibiotic use (Mackey et al., 2023). While probiotics can improve cattle performance, little work has been published to suggest they can control liver abscess prevalence in feedlots. Therefore, additional research is required to evaluate the impact of probiotics on the prevention of liver abscesses in cattle.

CHAPTER 2

REVIEW OF PUBLISHED *SALMONELLA* FOODBORNE INFECTIONS

Introduction

In the U.S., *Salmonella* is estimated to be responsible for 1.35 million infections, 26,500 hospitalizations, and 420 deaths every year, making it a bacterium of concern, especially regarding food safety (CDC, 2023). In 2020, the U.S. Department of Health and Human Services released the Healthy People 2030 (HP2030), an initiative started in 1980 that sets goals and objectives for national health promotion and disease prevention. Healthy People 2030 has nearly 360 objectives, with two regarding *Salmonella* controls in food supply systems. The first objective is to reduce the occurrence of *Salmonella* as the causative organism in foodborne outbreaks. Currently, there are an estimated 13.3 diagnosed infections per 100,000 people, with the goal by 2030 to get to a rate of 11.5 per 100,000 people. The second objective focuses on the same pathogenic bacteria but highlights the presence of *Salmonella* in beef production and the need to reduce its prevalence.

As of August 1, 2022, the U.S. Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) declared *Salmonella* to be regarded as an adulterant in breaded and stuffed raw chicken products (USDA, 2022). While *Salmonella* is typically associated with poultry production, the literature suggests Salmonella outbreaks from beef products are becoming more common (CDC, 2023; Laufer et al., 2015). In an evaluation of CDC reports from 1973 to 2011, Laufer et al. (2015) found that 4.8% of *Salmonella* outbreaks in the US were associated with beef products. In addition, Laufer et al. (2015) identified a change in the food vector with outbreaks shifting from whole muscle cuts (such as roasts) to ground beef. To further support this point, it

was reported that over the 2002 to 2011 timespan, ground beef was identified as the food vector for 45% of all beef-related *Salmonella* infections, with three large outbreaks resulting in the same number of infections from all the years prior (CDC 2023; Laufer et al., 2015).

With FSIS aligning alongside the HP2030 goals, a 25% reduction of salmonellosis is targeted for the upcoming years. To help achieve this, FSIS has established new performance standards in beef at 2 positive samples out of 48 for facilities generating > 50,000 lbs of ground beef or beef trimmings for grind. With these standards in place, the FSIS projects a total of 8,900 illnesses avoided each year (FSIS, 2021). However, to generate these policies, quantitative microbial risk assessment (QMRA) must explore the impacts of different microbiological criteria. For this modeling to occur, a dose-response model must be generated first. These models work by taking information from published investigations of foodborne outbreaks identified by the researcher. However, paper selection for model generation is up to the researcher, possibly resulting in biased results due to the lack of inclusion of information. Therefore, the primary objective is to review and summarize the literature on *Salmonella* with factors such as those ill, exposed, and bacterial loads consumed and then to determine factors such as strain virulence, food matrix, and dose to see how they impact the occurrence of illness.

Methodologies

This systematic review followed the PRISMA (Preferred Reporting for Items for Systematic Reviews and Meta-analyses) protocol. The review team comprised four people screening titles and two reviewing articles to ensure parameters were met. Three databases were searched on March 3, 2023, excluding all articles before 1930. Databases utilized included Pub Med, Web of Science, and CAB Direct (search string can be found in Appendix A). The search

string consisted of ("Salmonella") AND ("outbreak" OR "foodborne illness") AND ("exposure" OR "infected" OR "dose" OR "ill") NOT ("pet" OR "zoonoses" OR "resistance").

Screening Process

The screening process is outlined in Figure 1, with a more detailed explanation below. A total of 1932 studies were imported into Zotero (Corporation for Digital Scholarship; Fairfax, Virginia) and duplicates (505) were removed. Following deduplication, four reviewers (TT, SG, CP, JD) independently screened titles and abstracts. The following five parameters were used to determine if the records would be further evaluated:

1. Does the bacterial infection originate from a food source of consumption of the bacteria?
2. Is there a food vector identified?
3. Was *Salmonella* confirmed in the food or those infected?
4. Was *Salmonella* identified at the genus level?
5. Is the paper in English?

Following the prior parameters, a total of 974 articles were removed from the Zotero file. The paper was admitted to the full-text stage if the prior questions could not be answered from a title or abstract. The remaining 453 articles were reviewed at the full-text level by two reviewers (TT, SG) and were included if they had at least two or more of the following parameters:

1. Is the infectious dose consumed or identified in the food product stated?
2. How many people were exposed to the infected food product?
3. How many people consumed the infected food product?

From this final step, a total of 427 papers were removed, leaving 42 papers meeting at least two of the required parameters.

Data Extraction and Risk of Bias Assessment

All reviewers discussed the data extraction process and procedures in Zotero to clarify any areas of confusion. Articles were assigned to two teams of reviewers (TT, SG and CP, JD) for title screening. After the screening, the articles were combined into the same Zotero file, and duplicates were removed. Two reviewers (TT and SG) independently screened the remaining 427 papers and combined the results. They removed duplicates to generate a master list with all appropriate information (author, year, country, food matrix, dose, exposed, and ill). Any disagreements along both processes were resolved by consensus. Of the 42 papers identified, a total of 98 data points were generated. Bias assessment consisted of meeting with both screening teams to ensure documents matched from both teams. If a match did not occur, all reviewers would review the document and either omitted or accepted the document through a unanimous decision. Additionally, as foodborne outbreaks can occur and not be reported, this review is only able to observe published outbreaks.

Statistical Analysis

Utilizing R (v4.1.0), the "glmer" function from the lme4 package was used to generate a mixed-effects logistic regression model (Bates et al., 2015). The model generated had log dose, virulence group, and food animal or other as variables with the article the data was collected from as a random effect [glmer(cbind(Ill, non_ill) ~ log_dose + severity_group + food_animal_other+ (1|Paper), family = binomial("logit"))]. Using article as a random effect, the study could control for variation in how food products were sampled and enumerated to generate the needed information. Log dose was converted into log CFU/g or /mL as needed per study to ensure units were the same and could be compared. The serovar virulence group was assigned to "Higher" or "Lower" virulence groups of serovars identified by Fenske et al. (2023). In summary, the "Higher"

virulence group (by serovar) consisted of *Salmonella* Litchfield, *Salmonella* Typhimurium, *Salmonella* Enteritidis, *Salmonella* Agona, *Salmonella* Dublin, *Salmonella* Newport, *Salmonella* Muenchen, *Salmonella* Reading, and *Salmonella* Saintpaul, with all other *Salmonella* serovars being grouped into the "Lower" virulence classification (Fenske et al., 2023). Food animal or other consisted of food vectors that either originated from animals (such as eggs and dairy products) or from their tissues (meat and/or organs), while "other" consisted of anything that did not meet the food animal classification (i.e., vegetables, soups, grains, etc.). A relation of those ill and non-ill upon exposure that resulted in an attack rate was used as the response variable. Upon model generation, the logit function was used in R (v4.1.0) to generate odds ratios to determine the impacts of variables. Furthermore, visualizations were generated in R (v4.1.0) with the ggplot2 package (Wickham, 2016).

Results

General Summary of Salmonella Identified

A summary of the 42 papers utilized in this study is provided in Tables 2 and 3. From the final 42 papers selected for the study, the serotype and country in which the outbreak was identified arein Figure 2A. As for the country of origin, most outbreaks identified in the current search occurred in the United States or Japan. While the majority of European countries identified in the study averaged around 1.5 studies.

The most common food vector identified in the review was eggs, and desserts (e.g., cakes and ice cream) were the most common foods for outbreaks (Figure 2B). A total of 62 observations came from animals, and 36 were from non-animal originating foods. When focusing on the serotypes identified, *Salmonella* Enteritidis (69 observations) and *Salmonella* Typhimurium (23 observations) were reported in the greatest numbers (Figure 2C). Upon grouping the *Salmonella*

based on virulence levels defined by Fenske et al. (2023), a total of 72 serovars were identified as "Higher" virulence, while 24 were identified as "Lower" virulence. Evaluating the dose needed to cause an infection, the average $\log_{10}$ dose CFU/gram was 4.29 ± 2.733 (mean ± sd), with doses ranging from -0.87 to 11 $\log_{10}$ dose CFU/gram of *Salmonella*.

Mixed-Effects Logistic Regression Model Results

From the model generated, there were differences ($P < 0.05$) associated with each independent variable evaluated, suggesting that all the variables (virulence group, log dose, and food vector type) had an impact on illness (Table 4). Assuming all other variables held constant odds of being ill increased by approximately 50.54% for a one-unit increase in log dose (95% CI 50.24, 50.85). This suggested that initial dose of bacteria in a food product can play a major role in illness. There was a difference in odds ratios ($P < 0.05$) identified between the "Higher" and "Lower" virulence groups. Individuals exposed to the "Lower" virulence group had 36.2% less odds of becoming ill when compared to individuals exposed to the high virulence group (95% CI 32.4, 40.3). With this knowledge concerning microbial controls in food production, data suggested that regulators should consider controlling highly virulent strains of *Salmonella* as not all *Salmonella* are similar in virulence. Furthermore, when evaluating the food vector origin (Food Animal or Other) when the product was classified as other, we found that the odds of becoming ill were 45.07% lower than a food animal product (95% CI 43.77, 46.38).

Discussion

While most of the sampled studies reported outbreaks, two inoculation challenge studies were included. These studies might introduce bias as the participants were most likely a very homogenous mixture of healthy 20–30-year-old males, which does not properly represent the entire population. Additionally, the dose variable is constant in challenge studies whereas in

outbreak studies, the dose would likely vary as those exposed might intake different amounts of the infected product. Furthermore, many studies captured in this review were outbreaks at home within a family. Due to this occurrence, attack rates generated may be inflated as the entire family gets sick, making the attack rate 100%, which is not typical. These small outbreaks of 1 or 2 people might skew results to attack rates that do not represent those for *Salmonella*. With only 42 studies being evaluated, many published reports do not report all the criteria we were searching for (dose, ill, exposed). This might be because of differences in how countries require reporting of outbreaks and the types of information they collect. Many of the studies reporting all the variables often originated from Japan. A key component of this may be due to the regulatory requirements in Japan, with the Food Sanitation Act requiring the sampling of products through the Food Recall and Reporting section. While a few studies from the USA could determine the concentration of Salmonella (CFU/g) in the infected products, food vectors could not be collected most of the time, resulting in metrics such as CFU/g not being reported. These outbreaks reported in the U.S. typically focused on genetically identifying the outbreak strain rather than the infectious dose. Implementing a system like Japan's in the USA might theoretically allow more data points to be captured, resulting in more accurate modeling for food safety regulators to base policy.

Log dose of *Salmonella* consumed, virulence group, and food matrix have a major impact on *Salmonella* infection from food products. While log dose consumed is to be expected as the more bacterial cells consumed, the more likely an illness is to occur. The virulence group findings are interesting in that they further support the results from Fenske et al. (2023) in that the "Higher" virulence group was found to cause illness at a greater rate compared to the "Lower" virulence groups. Regarding public policy, this suggests that when modeling food safety regulations, serotypes must be accounted for and framing regulations to target those *Salmonella* of greatest

concern regarding public health. Food matrix found that those of animal origin are at a higher risk for *Salmonella* infection than non-animal food vectors. These results are unsurprising as *Salmonella* are endemic to most animal microbiomes. It can still occur in non-animal food vectors; however, the dose consumers ingest is often lower.

When generating dose-response models, authors typically rely on data generated in 1951 consisting of feeding *Salmonella* from spray-dried whole eggs to human volunteers (Teunis et al., 2010; Oscar, 2004; Latimer et al., 2001). With these approaches, bias can be generated as the subjects were homogenous in makeup, consisting of males in good health (McCullough and Eisele, 1951a,b). As foodborne outbreaks affect a larger population demographic, alongside the studies utilizing stereotypes that may not commonly occur in foodborne outbreaks, the need for summarization of outbreak data is reinforced (Teunis et al., 2010). When outbreak data are utilized in modeling, search protocols or how papers are obtained are often based on convenience, and no formal review is conducted to generate the data (Teunis et al., 2010).

Conclusion

In conclusion, a total of 42 articles were identified that provided data necessary to fit a model describing the associations between dose, virulence, food vector, and attack rate that have the potential to generate data useful for regulation implementation. Additionally, virulence group, log dose consumed, and food vector were found to impact illness rates of *Salmonella* and should be considered when generating regulations.

CHAPTER 3

EXAMINATION AND CLASSIFICATION OF *SALMONELLA* IDENTIFIED FROM MESENTERIC LYMPHATIC TISSUES OF BEEF CATTLE FED A PROBIOTIC MIXTURE OF PROPIONIC AND LACTIC ACID BACTERIA

Introduction

The *Escherichia coli* outbreak in 1993 associated with Jack in the Box significantly impacted food safety regulations in the United States. While that outbreak reframed the food safety system, it also drew attention to other pathogens, such as *Salmonella*, which can contaminate ground beef. Historically (1970-2000), *Salmonella* infections originating from beef products were associated with whole muscle cuts (Laufer et al. 2015). However, in recent years (2002-2011), ground beef has made up 45% of outbreaks associated with beef (Laufer et al., 2015). While cross-contamination may play a role in these infections, beef lymphatic tissues have been identified as potential pathways for this pathogen to make it into ground beef (Arthur et al., 2008; Webb et al., 2017). As the bacteria are held in lymphatic tissues, pathogen interventions such as carcass sprays have little to no effect on reducing lymphatic tissue bacterial loads (Edrington et al., 2020). Upon grinding, these lymphatic tissues in beef trim burst, contaminating the freshly ground product.

While postmortem intervention steps may have little to no effect, antemortem interventions such as direct-fed microbials and vaccines have been identified as potential interventions (Horton et al., 2021; Vipham et al., 2015; Flach et al., 2022). However, using direct-fed microbials has had mixed results (Flach et al., 2022; McAllister et al., 2011). Flach et al. (2022) found that by using probiotics, lower amounts of *Salmonella* and *E. coli* were lower in lymphatic tissues and less being

shed by cattle. Horton et al. (2020) found that vaccination had no significant effects in reducing *Salmonella* presence in lymph nodes.

While most studies evaluating lymphatic tissues mostly focus on peripheral lymph nodes due to the ease with which they can be sampled, few studies have evaluated mesenteric lymph nodes, the digestion track's primary drainage points. Currently, a macrolide antimicrobial known as Tylosin is the most common antemortem intervention for liver abscesses, with 71.2% of cattle in US feedlots receiving it (USDA, 2013). Being fed at 60-90 mg/head/day, Tylosin reduced liver abscess occurrence from 30% to 8% (Wileman et al., 2009). While Tylosin is considered a solution to limiting liver abscesses, growing concerns regarding antibiotic resistance are pushing to limit use. Organizations such as the World Health Organization strongly recommend limiting macrolide (such as Tylosin) use for livestock as the medication is important in treating *Campylobacter* infections in humans. Furthermore, a new alternative must be implemented if Tylosin is no longer allowed.

Therefore, the objective of this study was to assess the effects of adding a propionic bacteria and lactic acid bacteria mixture (DFM), Tylosin (TYL), and DFM + Tylosin (DFM+TYL) to feedlot rations to determine how they impact the presence of *Salmonella* in mesenteric lymphatic tissues of beef cattle in a feedlot environment and to determine the *Salmonella* serotypes found in those lymphatic tissues.

Methods

Cattle Procurement

The cattle for this project were finished at a commercial feedlot in the southern United States. These cattle were procured for trial placement through commercial auction markets, as is typical for beef production systems in the United States. This study utilized twelve blocks of pens

(4 pens per block/48 pens total), each containing 75 steers (for a total of 3,600 head of cattle). To be enrolled in this study, cattle arriving at the feedlot were weighed, and those cattle outside the acceptable weight range of 295-318 kg were excluded. The remaining cattle were then placed into pens that were randomly assigned one of the four treatment diets. Treatment diets consisted of a typical feedlot ration with one of the following supplements added: DFM with a dosage of 2.6 x 10^8 CFU/head, TYL, DFM+TYL, or no supplement to serve as the negative control (CON). Cattle started on the assigned treatment diet within 72-96 h of pen placement and stayed on the assigned diet for approximately 185 d. Treatments were removed from treatment diets 28 days before abattoir shipment.

Slaughter Facility Sample Collection

Upon arrival at the abattoir, cattle were carefully followed throughout the slaughter facility. When the cattle of interest reached the evisceration process, liver status was evaluated and noted for the sampling of mesenteric tissues. Mesenteric lymph nodes (MLN) were gathered from the mesentery and stored in conical tubes. With each pen of cattle entering the processing facility, 10 MLN were collected as available, with five MLN coming from cattle with no liver abscesses, while the remaining five were from cattle with abscessed livers. Overall, 503 MLNs were collected, with a breakdown on a treatment basis available in Table 5. Following collection, samples were overnight shipped from the processing facility to Colorado State University (CSU) for further processing.

Lymph Node Processing

Upon arriving at CSU, samples were held at refrigerated temperatures (for no more than 8 h) until processed. Sample processing first consisted of aseptically removing fat and fascia surrounding the MLN and then immersing it in boiling water for 3 – 6 s (depending on the size of

the lymph node). This was done to inactivate any microbial contaminants on the surface of the sample. After the immersion step, the MLN was placed in a sample bag and a rubber mallet was used to pulverize and expose the interior of the lymph nodes. Samples were then weighed, and buffered peptone water (BPW; DIFCO, Becton Dickinson [BD], Franklin Lakes, New Jersey) was added at a 1:5 ratio. Samples were mechanically pummeled for 90 s and 1-mL aliquots were plated onto duplicate Petrifilm Enterobacteriaceae Count plates (EB; 3M, Saint Paul MN). The remainder of the sample homogenate was incubated at 42˚C for 20 h and used for detection of *Salmonella* with the GENE-UP real-time polymerase chain reaction (PCR) assay.

Enterobacteriaceae and Salmonella Enumeration

The EB Petrifilm plates were incubated for 24 h at 35˚C and used to obtain Enterobacteriaceae counts. Following enumeration of colonies, EB films were press plated onto xylose lysine deoxycholate agar (XLD; Difco) to further select for *Salmonella* spp. Those XLD plates were then incubated at 35˚C for 24 h to obtain counts of *Salmonella* spp. present within the lymph node samples. The detection limit of this analysis was 6 CFU/g or 0.778 log CFU/g.

Salmonella PCR

As indicated above, the lymph node homogenate that remained after plating of aliquots onto the EB plates was incubated at 42˚C for 20 h. The enriched samples were subjected to the GENE-UP (Biomerieux, Marcy-I'Etoile, France) real-time PCR approach to determine *Salmonella* spp. presence in the samples. The GENE-UP *Salmonella* 2 kit was utilized following the protocols as recommended by Biomerieux for meat products. Sample enrichments that tested presumptive for *Salmonella* with the GENE-UP assay were subjected to a secondary enrichment (42˚C, 24 h) in tetrathionate broth base (TT; Difco) to further confirm the GENE-UP results. Following incubation, the TT broth was streak-plated onto XLD plates and incubated at 35˚C (24

h). The XLD agar plates were inspected for colonies with a black center, and if present, a representative colony was picked off, cultured in TSB (35˚C, 24 h), and subjected to the GENE-UP Salmonella 2 PCR assay as confirmation for the presence of *Salmonella*. Glycerol stocks (15%) of the confirmed *Salmonella* isolates were prepared, and these were stored at -80˚C.

Salmonella Serogrouping

Serogrouping of the *Salmonella* spp. isolated from the lymphatic tissue samples was performed using *Salmonella* antisera (Difco) following the manufacturer's instructions. *Salmonella* cultures were reactivated from the glycerol stocks by adding a small amount of the frozen culture to a test tube containing 10 mL of tryptic soy broth (TSB; Difco) and incubated at 42˚C for 24 h. While the number of *Salmonella* serogroups is vast, following a review of literature and consultation with the Diagnostics Laboratory at Colorado State University, we determined to test for serogroups B, E, D1, C1 (by Factor-7; F7) and C2. To help determine the possible serogroup, *Salmonella* O antiserum poly-A (containing somatic groups A, B, D, E1(E2, E3) E4, and L) and poly-B (containing somatic groups C1, C2, F, G, and H) were used with the culture first. If agglutination occurred with the poly-A solution, the culture was tested with the B, E, and D1 antisera. Likewise, the C2 and F7 antisera were utilized if poly-B agglutination occurred. Upon agglutination of the secondary antiserum, a serotype was identified.

Statistical Analysis of Data

Bacterial Counts

Salmonella spp. were recovered from mesenteric lymphatic tissue and expressed as least squares means for log CFU/g. For statistical analysis of the microbial data, samples with counts below the analysis detection limit (0.778 log CFU/g) were assigned a value equal to the detection limit. Data analysis of *Salmonella* spp. was conducted as a randomized block design with the pen

being the experimental unit. The variable *Salmonella* spp. counts were analyzed using R Studio (v. 4.0.0.) with treatment diet (CON, DFM, TYL, DFM+TYL), blocks (1-12), and liver status (Abscessed, Non-Abscessed) as factors of the study design with a significance at an alpha level of 0.05. Upon generating linear models of the data, the anova function was utilized to determine if significant differences were found between factors and potential interactions.

Salmonella PCR

Upon identifying and confirming *Salmonella* presence in samples using the GENE-UP system (BioMérieux**,** Marcy-l'Étoile, France), positive and negative samples were then totaled by pen. Positive odds ratios were generated on a per-pen basis before data were fit to a generalized linear model (family = Binomial) with treatment and block as factors and the weight variable set to the total number of cattle per pen. Following model creation, the anova function was used to conduct a Chi-squared analysis on the model. The emmeans function was then implemented to transcribe the results from logit to percentage.

Results

Bacterial Counts

When evaluating the mean bacterial counts of *Salmonella* spp. in the lymph node samples, no significant differences ($P = 0.27$) were identified between any of the treatments tested (Table 6). In addition, no interaction between liver status and treatment was observed for *Salmonella* counts ($P = 0.72$). Furthermore, the percent of samples below the detection limit was high for *Salmonella* spp. (range: 72.2 – 88.1).

Salmonella Prevalence

Of the 503 samples processed, *Salmonella* spp. was 22% of the samples were confirmed (91 total positive samples) by the GENE-UP system and culture confirmation methods. Upon

conducting the binomial regression analysis, no significant differences (P = 0.23) were found between treatments, along with no interaction of the liver status and treatment (P = 0.36), suggesting that neither the treatments nor liver status affected the prevalence of *Salmonella* within mesenteric lymph nodes (Table 7).

Serogrouping

Of all the *Salmonella* spp. isolates recovered from the samples, the C_1 serogroup was the most abundant and contributed to 83.3% of all the isolates. This was followed by serogroup E (14.3% of isolates tested) and serogroup B (2.4% of isolates tested; Figure 3). These results indicated that *Salmonella* serogrouping was relatively similar regardless of treatment or liver status, with most salmonellae (83.3%) belonging to the C_1 serogroup.

Discussion

Previous literature has established that feedlots in the southwest region of the United States often produce cattle with higher prevalence of *Salmonella*, with summer exacerbating the presence (Webb et al., 2017). While these studies often focus on peripheral lymphatic tissues (PLN), few have examined the mesenteric lymph nodes (MLN). One main difference between MLN and PLN tissues is their anatomical location. MLN tissues are responsible for draining lymphatic fluid from the intestine, while peripheral lymphatic nodes are located outside the body cavity and outside the central lymphoid organs. With MLN tissues serving as a first line of defense in the digestive system, it has been established that *Salmonella* can colonize cattle MLN tissues (Molossi et al., 2020). However, colonization can also occur and have no apparent negative impacts on the animal's health (Samuel et al., 1980a,b). Samuel et al. (1981) reported that 91.2 % of MLN sampled contained *Salmonella*, which was much higher than other lymphatic tissues sampled.

Furthermore, it has been established that *Salmonella* is commonly present in MLN tissues of healthy beef cattle at slaughter.

In general, the *Salmonella* presence was much lower in the current study when compared to previous studies that evaluated mesenteric lymph nodes. Samuel et al. (1980a) found greater contamination rates in jejunal nodes compared to caecal nodes within the mesentery. While jejunal nodes were primarily sampled in the current study, about 50% of the jejunal nodes sampled by Samuel et al. (1980a) were found to contain *Salmonella*. In addition, those lymph nodes contained <25 CFU/g of *Salmonella* (similar to the current investigation); however, some contained greater than 75,000 CFU/g of *Salmonella* (Samuel et al., 1980a). This wide range was not apparent in the current investigation and found the mean to be below 1 log CFU/g. One possible difference between the two studies could be the age of the cattle. While Samuel et al. (1980a) did not clearly state the age of the animals, Grispoldi et al. (2020) identified the age of the animals to be a major contributor to Enterobacteriaceae loads in lymphatic tissues. In addition, Grispolid et al. (2020) also found that the Enterobacteriaceae loads in lymphatic tissues were negatively related to distance traveled, with fewer *E. coli* strains being identified when the cattle traveled longer distances. These results are not typical, but the authors contribute them to the animal's diet beforehand (forage dominated; Griispolid et al., 2020). While *E. coli* is not the pathogen in question this still helps to establish potential factors that could contribute to such strikingly different results between Samuel et al. (1980ab) and the current investigation.

When evaluating *Salmonella* serogroups between studies, results from the current study align with previous research. The C_1 serogroup (containing species such as *S*. Newport and *S*. Montevideo) is the most common serogroup identified in the United States. Samuel et al. (1980a,b) also reported that most of the *Salmonella* identified in their study belonged to serogroup C_1 and B,

similar to the current investigation. While PLN lymph nodes share a similar spread of C_1 and E (Nickodem et al., 2023; Arthur et al., 2008), there is no way to determine genus in the current study.

Conclusions

The results of the current study indicated that incorporating DFMs into cattle diets did not affect the prevalence of *Salmonella* in the mesenteric lymph nodes. When *Salmonella* was recovered from the samples without enrichment, the mean concentration was less than 1 log CFU/g, irrespective of treatment. These salmonellae were typically made up of the C_1 serogroup at 83.3%. While diet may not have an impact, it appears that *Salmonella* can colonize mesenteric lymphatic tissues of cattle at low frequency and concentration.

CHAPTER 4

EXAMINATION OF MICROBIAL COMMUNITIES AND THEIR RESISTANCE GENES IN FECAL AND LIVER ABSCESSES FROM BEEF CATTLE FED A PROBIOTIC MIXTURE OF PROPIONIC AND LACTIC ACID BACTERIA

Introduction

Liver abscesses are a common problem in cattle, especially in those that are fed diets rich in highly fermentable carbohydrates. Lowered rumen pH, subsequent rumenitis, and bacterial translocation into the portal circulation ultimately result in liver abscess formation. Liver abscess prevalence and severity are associated with decreased production efficiency and lower carcass value. Results of the recent National Beef Quality Audit (2016) suggested that liver abscesses in feedlot cattle have increased over time (Eastwood et al., 2017). The most common management practices to control and prevent liver abscessation include dietary roughage management and antibiotics in the feed. In the U.S., tylosin phosphate (a macrolide antibiotic) and chlortetracycline are approved for inclusion in cattle diets to control liver abscesses (as well as for some additional purposes). Over 70% of cattle in large commercial beef feedlots have been reported to receive tylosin in diets for this purpose (U.S. Food and Drug Administration, 2019). While dietary antimicrobial supplementation is quite efficacious and cost-effective for controlling liver abscesses, this type of group-level treatment is receiving increasing scrutiny because of public health concerns about the potential problem of antimicrobial resistance. This could lead to joint efforts between the FDA and pharmaceutical manufacturers that may lead to label restrictions that eliminate continuous dietary supplementation of antimicrobial drugs throughout the feeding period. Further, market influences may lead to the elimination of similar restrictions even without

regulatory action. As such, identifying efficacious and cost-effective alternatives to antimicrobial drugs to control this problem would be extremely valuable to the beef industry in North America, likely leading to the rapid, widespread adoption of these management practices. Additionally, if the use of these alternative liver abscess control strategies could be shown to be beneficial in the control and prevention of antimicrobial resistance, this would be highly beneficial to the beef industry as we work to allay recent information campaigns aimed at creating concern about beef and modern production practices.

Antimicrobial resistance has traditionally been characterized using culture-based methodologies, but these methods provide a limited and likely biased perspective on the ecology of antimicrobial resistance (AMR) among entire communities of bacteria. Orally administered antimicrobial drugs are directly exposed to all the microbes in gut communities; hence, evaluation of AMR from a more global, ecological perspective has greater relevance for addressing this important public health concern.

Methods

Cattle Procurement

These are the same cattle used in Chapter 3; more details are provided in that chapter. In summary, a total of 12 blocks of pens (4 pens per block/48 pens total), each containing 75 steers (for a total of 3,600 head of cattle), were enrolled in this study. Treatment was applied at the pen level with treatment diets consisting of a typical feedlot ration with one of the following supplements added, DFM with a dosage of 2.6 x 10^8 CFU/head, TYL, DFM+TYL, or no supplement to serve as the negative control (CON) with the cattle being on the assigned diet for approximately 185 d and treatments being removed 28 d before abattoir shipment.

Fecal Sample Collection

Fecal samples were taken from all study pens 48-72 h after the initial placement of cattle into assigned pens. The next round of samples was collected on the last day that treatment diets were fed to cattle (28 d before slaughter). Sample collection consisted of gathering 2 g of feces from 10 individual fecal pats with a sterile tongue depressor and placing them into a 50 mL conical tube containing refrigerated 100% ethanol. This process was completed twice per pen, with the first 10 collected on the left side and the remainder collected from the right side of the pen (Figure 4). Samples were then transported on ice to West Texas A&M University (WTAMU) and stored at -80˚F until processing.

Slaughter Facility Sample Collection

Upon arrival at the abattoir, cattle were carefully followed throughout the whole slaughter facility. When the cattle of interest reached the evisceration process, liver status was evaluated, and if present, a liver abscess was excised from the liver and placed into a sterile bag. While the occurrence of abscesses varied between pens, 10 liver abscesses from different livers was the desired amount collected.

Sample Processing

Fecal and Liver Abscess

Shotgun metagenomics and 16S rRNA sequencing were performed on both fecal and liver abscess samples. The DNA isolation, 16S library preparation, and AMR target enriched metagenomic library steps performed similarly regardless of sample type (fecal or abscess). While fecal samples were readily available for isolation and further steps, purulent material had to be obtained from the liver abscess. The surface was first sterilized, and a sterile needle was then used to collect purulent material. In addition to the previously discussed methodologies, the purulent

material from the liver abscess was subjected to a *Salmonella* PCR that will be discussed in further detail.

DNA Isolation

DNA was isolated from 0.2 g of fecal or liver abscess samples (n = 220) using the QIAamp PowerFecal DNA extraction kit (QIAGEN). Following isolation, DNA was quantified (ng/μL) using a Qubit Flex fluorometer (ThermoFisher). It should be noted that insufficient DNA content was obtained from many samples, and multiple DNA extractions were required. If a sample was assigned for use in this study, but there was insufficient DNA content and fecal sample for methods used in this study, a USDA-APHIS representative assigned a replacement sample to the study allocation.

16S rRNA Gene Library Preparation and Sequencing

Amplicon library preparation was performed at Colorado State University for all 220 samples. The V3-V4 region of the 16S rRNA gene was amplified using the 341f (5' – CCTACGGGNGGCWGCAG – 3') and 785r (5' – GACTACHVGGGTATCTAATCC – 3') primer pair. Amplification conditions were 98°C for 3 min, followed by 12 cycles of 98°C for 30 s, 55°C for 30 s, and 72°C for 1 min. Final elongation occurred at 72°C for 5 min. Amplicons were then purified using AMPure XP beads (Beckman-Coulter, Pasadena, CA), and sequencing libraries were prepared using the Nextera IDT kit (Illumina, San Diego, CA). Libraries were purified using AMPure XP beads and pooled in equal proportions based on molarities. The resulting pooled amplicon library was sequenced on an Illumina MiSeq instrument using paired-end chemistry (2 x 250 bp) at the University of Colorado Anschutz Medical Campus' Genomics and Microarray Core.

Salmonella PCR

To determine whether *Salmonella* spp. were present in the liver abscess samples, purulent material was shipped to the Colorado State University Veterinary Diagnostic Laboratories for PCR. Samples were tested using the ThermoFisher protocol for *Salmonella* spp. detection from food and environmental samples (Revision E). Nucleic acid was extracted using the PrepSEQ Nucleic Acid Extraction kit and using the pre-clarification protocol on a Kingfisher Flex (food sample protocol). PCR was performed using the MicroSEQ *Salmonella* spp. PCR kit on the ABI 7500 platform. Results for Real-time PCR were measured by the Ct value. The Ct value correlates with the original amount of target nucleic acid in the sample and is inversely proportional (the lower the Ct value, the higher the starting amount of nucleic acid). Ct values ranging from 12-36.99 were reported as positive, 37.00-40.00 were reported as suspect/weakly positive. While samples assigned a Ct value of 0.00 were interpreted as a negative result. Those samples identified as suspect/weakly positive were noted as negative for the sake of the binomial analysis.

AMR Target-Enriched (AMR-TE) Metagenomic Library Preparation and Sequencing

The SureSelect XT HS2 DNA System Kit for Illumina Paired-End Multiplexed Sequencing Library (Agilent Technologies) was used to prepare samples for target-enriched resistome sequencing. A custom bait design targeting antimicrobial resistance genes (ARGs), 'MEGARes' (Doster et al., 2020), was used to enrich sequencing libraries for antimicrobial resistance (AMR) gene sequences. The resulting libraries were pooled equally based on their molecular weight and DNA concentrations. The pooled library was sequenced on an Illumina NovaSeq 6000 instrument using paired-end chemistry (2 x 150bp) at the North Texas Genome Center at the University of Texas - Arlington.

Bioinformatics AMR-TE

Demultiplexed AMR-TE metagenomic sequence reads were processed using the AMR++ v2 pipeline and the MEGARes v2 resistance database (Doster et al., 2020). A detailed description of MEGARes and the AMR++ pipeline can be found at http://megares.meglab.org. Briefly, reads were trimmed and filtered for quality using trimmomatic (Bolger et al., 2014), and host DNA was identified and removed by aligning trimmed reads to the *Bos taurus* genome with BWA (Li & Durbin, 2010). The remaining reads were then aligned to the MEGARes database with BW and gene sequences aligning to >80% of the reference nucleotide sequence were included for further analysis. Reads that aligned to genes requiring the presence of specific single nucleotide polymorphisms to confer resistance were identified and removed from downstream analyses. The resulting ARG count table was then imported into phyloseq (McMurdie & Holmes, 2013) using the 'import_biom' function. Metadata was imported using the 'import_qiime_sample_data' and merged with the count table into a phyloseq object. Due to low ARG counts, two samples were excluded from further analysis. Richness and Shannon diversity values were calculated for all samples with phyloseq. ARG counts for each sample were then normalized using cumulative sum scaling (Paulson et al., 2013), and beta-diversity was analyzed using Bray-Curtis dissimilarity distances. From these distances, non-metric multidimensional scaling (NMDS) was performed and plotted, and permutational multivariate analysis of variance (PERMANOVA) was used to test for significant differences in resistome structure using the vegan (Oksanen et al., 2019) and pairwiseAdonis packages. Permutational analyses of dispersion (PERMDISP) were conducted for all significant PERMANOVA outcomes using the vegan package to ensure significant differences were not the result of unequal dispersion of variability between treatment groups. Further, the relative abundance ARGs within each sample were calculated and plotted using phyloseq. The

relative abundances of classes of ARGs averaging more than 1% of the entire resistome across all samples were subset, plotted, and compared between treatment groups.

Statistical Analysis of Data

Bioinformatics

Demultiplexed 16S rRNA gene sequence reads were imported in QIIME2 version 2020.11 (Bolyen et al., 2019). Amplicon sequence variants (ASVs) were generated using DADA2 (Callahan et al., 2016), which was also used to filter reads for quality, remove chimeric sequences, and merge overlapping paired-end reads. Forward reads were trimmed at 19 bp, reverse reads at 21 bp, while both forward and reverse reads were truncated at 240 bp. Taxonomy was assigned using a Naïve Bayes classifier trained on the Greengenes version 13_8 99% OTUs database (DeSantis et al., 2006), where sequences had been trimmed to include only the base pairs from the V3-V4 region bound by the 341f/785r primer pair. Reads mapping to chloroplast and mitochondrial sequences were filtered from the representative sequences and ASV table using the 'filter-seqs' and 'filter-table' functions, and a midpoint-rooted phylogenetic tree was generated using the 'q2-phylogeny' pipeline with default settings, which was used to calculate phylogeny-based diversity metrics.

16S rRNA (ASV count table) and AMR-TE (AMR gene count table) data were then imported separately into phyloseq (McMurdie & Holems, 2013) using the 'import_biom' function. Metadata was imported using the 'import_qiime_sample_data' and merged with each count table into a phyloseq object. Richness and Shannon diversity values were calculated for all samples with phyloseq. ASV or AMR gene counts for each sample were then normalized using cumulative sum scaling (Paulson et al., 2013), and beta-diversity was analyzed using generalized UniFrac distances for ASVs (Lozupone et al., 2011, Chen et al., 2012) and Bray-Curtis dissimilarity distances for

AMR genes. From these distances, non-metric multidimensional scaling (NMDS) was performed and plotted, and a permutational multivariate analysis of variance (PERMANOVA) was used to test for significant differences in community structure using the vegan (Oksanen et al. 2019) and pairwiseAdonis packages. Permutational analyses of dispersion (PERMDISP) were conducted for all significant PERMANOVA outcomes using the vegan package to ensure significant differences were not the result of unequal dispersion of variability between groups. Further, the relative abundances of ASVs and AMR genes within each sample were calculated and plotted using phyloseq.

Salmonella PCR

Upon identifying and confirming *Salmonella* presence in samples using the GENE-UP system (BioMérieux**,** Marcy-l'Étoile, France), positive and negative samples were then totaled by pen. Positive odds ratios were generated on a per-pen basis before data was fit to a generalized linear model (family = Binomial) with treatment and block as factors and the weight variable set to the total number of cattle per pen. Following model creation, the anova function was used to conduct a Chi-squared analysis on the model. The emmeans function was then implemented to transcribe the results from logit to percentage.

Statistical Analyses

Unless specified otherwise, R version 4.1.0 (R Core Team, 2017) was used for the statistical analysis of data. Pairwise Wilcoxon rank-sum tests were performed with a Benjamini-Hochberg correction (PW+BH) for multiple comparisons. Differences in beta-diversity were tested using pairwise PERMANOVA with a Benjamini-Hochberg correction for multiple comparisons and 9,999 permutations. Additionally, pairwise PERMDISPs were carried out for all

significant PERMANOVA outcomes using 9,999 permutations to test for differences in the variability of dispersions.

Results and Discussion

16S rRNA

Fecal

When evaluating fecal samples, alpha and beta diversity between treatments was not significantly different ($P = 0.3$). However, a significant difference ($P < 0.05$) was identified between the animals upon entering and exiting the feedlot (Figures 5, 6, 7). The modulation of the cattle fecal microbiome due to feedlot rations has been previously documented (Zhang et al., 2021). Zhang et al. (2021) found that upon feeding a feedlot style ration to previously grazing cattle, an increase of Firmicutes and a decrease of Bacteroidetes occurred after 3 months. While an increase of Firmicutes and a decrease of Bacteroidetes was not identified in the current study, an increase ($P < 0.05$) of Aeromonadales and Spirochaetales was identified at the end of the feedlot duration, regardless of the treatment diet supplied. Of the orders identified, the most prevalent were the Aeromonadales, Bacteroidales, Clostridiales, Spirochaetales, Turicibacterales, Verrucomicrobiales, and YS2. Additionally, when evaluating the addition of Tylosin to the feed ration, our results reflect those of Weinroth et al. (2019), with no impacts on fecal microbiomes being identified. These results contrast with Thomas et al. (2017), where it was identified that antibiotic use in cattle modulated bacterial populations of the digestive tract. These conflicting results may originate because Thomas et al. (2017) sampled differing portions of the digestive tract rather than fecal samples. When looking at probiotic impacts in literature, they have been previously identified to modulate the fecal microbiome of cattle (Mansilla et al., 2022). While these changes were observed, those cattle were fed an ionophore, which might have had a

synergistic effect with the probiotic (Mackey et al., 2023; Mansilla et al., 2022). This contrasts the current investigation, as no changes in the fecal microbiome were found with DFM or DFM+TYL supplementation. Additionally, while not a fecal sample, modulation of the cecal microbiome portion of the digestive tract occurred using a four-species *Bacillus* supplement (Fuerniss et al., 2022). In summary, the use of probiotics and Tylosin have been previously identified to impact the fecal microbiome of cattle; however, the current investigation was unable to find differences ($P > 0.05$) between treatment diets.

Liver Abscess

Liver abscess results showed no difference ($P > 0.05$) in alpha diversity among treatment diets (Figure 8). These findings agree with previously published works, as the liver abscess is a unique environment and only caters to a select niche of microbes (Pinnell et al., 2022b; Weinroth, et al., 2018). Beta diversity results also showed no significant differences between treatment diets ($P = 0.324$) (Figure 9). These findings suggest that the treatment diets do not impact the bacteria present in liver abscesses. When evaluating the purulent material's dominant phyla, Fusobacteria comprised most of the phylum (~50%), followed by the Bacteroidetes phylum (35-45%). In addition, Actinobacteria, Firmicutes, and Proteobacteria were the next major phyla identified, making up less than 10% of the relative abundance of phyla present. When moving down to the genus level, *Fusobacterium*, *Bacteroides*, *Porphyromonas*, *Trueperella*, *Campylobacter*, and *Bacteroidales* were the most prominent genera present (Figure 10). With these genera being in relatively similar percentages between sample types, it is unsurprising that alpha and beta diversity metrics were found not to be significant ($P > 0.05$). When evaluating liver abscesses, Pinnell et al. (2022a) identified two unique communities in liver abscesses: Fusobacteria or Bacteroidetes-dominated communities. In the current study, we have communities mostly dominated by

Fusobacteria. This suggests that the source of bacteria colonizing the liver originates from the rumen compared to the hindgut microflora.

Salmonella PCR

Of the 186 samples subjected to PCR, only 7 samples were identified as positive for *Salmonella*. Regardless of the treatment diet, no differences ($P = 0.49$) were identified between treatments. The DFM treatment had 6, while the TYL diet had 1 *Salmonella*-positive sample. This resulted in the probability of receiving a positive sample being incredibly low (DFM = 0.19%; TYL = 0.03%) along with minute standard errors (DFM = 4.79 e-03; TYL = 8.38 e-04). From this, we can conclude a lack of *Salmonella* spp. present in the purulent material collected from liver abscesses regardless of the treatment. These findings are interesting as *Salmonella* spp. has been commonly identified in other studies investigating liver abscesses (Pinnell et al., 2020b). It is possible that the native presence of salmonellae in liver abscesses is below the detectable threshold of the PCR assay utilized. At the same time, PCR may not be able to detect minute levels of *Salmonella* spp. present, whereas16S and metagenomic approaches would be able to identify low levels of Salmonella OTUs within samples. However, this was not the case as Fusobacteria and Bacteroides were identified as the most common, while *Salmonella* spp. were not found at greater than 1 percent of the relative abundance in the 16S analysis conducted. In conclusion, it appears that *Salmonella* spp. were not present in purulent material from liver abscesses in our study.

Shotgun Metagenomics

Richness and Diversity of the Resistome

There were no significant differences between the richness (Figure 11A) or the diversity (Figure 11B) of fecal resistomes from cattle in the four treatment groups at either time point, and there were no significant differences between resistome richness or diversity at the beginning

versus the end of the trial for any of the treatment groups (Figure 11; pairwise Wilcoxon rank-sum, n= 47-48, $P > 0.05$).

Overall Resistome Structure

Potential differences in overall resistome composition between treatment groups were analyzed using NMDS and PERMANOVA. Based on Bray-Curtis dissimilarity distances, there were no significant differences between treatment groups at each timepoint and visualization with NMDS demonstrating no clustering based on treatment (Figure 12A; pairwise PERMANOVA, n= 47-48, $P > 0.05$). PERMANOVA detected a significant difference between resistome composition at the beginning and end of the study for two treatment groups (CON, TYL; Figure 12B pairwise PERMANOVA, n= 47-48, $P < 0.05$), but significant PERMDISPs for each of those two PERMANOVAs indicated the significant difference was the result of differing dispersions of variance opposed to actual differences in resistome composition, or potentially a combination of the two. Visualization with NMDS suggests the significant difference resulted from resistomes from individual cattle within the Tylosin group converging by the end of the trial. These results agree with the findings reported by Weinroth et al. (2019).

The Abundance of AMR Classes within the Resistome

The composition of the resistome was visualized at the level of AMR class and demonstrated that the most abundant class of ARGs were those conferring resistance to tetracyclines (Figure 13). While considerably less abundant than ARGs conferring resistance to tetracyclines, genes conferring resistance to β-lactams, MLS, and drug/biocides were also relatively abundant and common across all treatment groups and time points (Figure 13). Generally, the relative abundances of AMR classes were extremely similar across treatment groups despite a small decrease in the relative abundance of ARGs conferring resistance to tetracycline in

the Tylosin group at the beginning (Figure 13). The relative abundance of AMR classes across treatments at the end of the trial is nearly identical. To further investigate potential differences in the relative abundances of common classes of ARGs, the relative abundances of all classes averaging at least 1% of the overall resistome across all samples were plotted and compared between treatment groups at the beginning and the end of the trial (Figure 14). The slight decrease in the relative abundance of ARGs conferring resistance to tetracycline within the Tylosin group at the beginning of the trial was not significant (Figure 14), and no significant differences within any of the abundant AMR classes were detected between treatment groups at either the beginning or the end of the trial (Figure 14; pairwise Wilcoxon rank-sum, n= 47-48, $P > 0.05$). The relative abundance of ARGs conferring resistance to tetracyclines, drugs/biocides, aminoglycosides, or drug/biocides/metals did not differ between the beginning and end of the trial period for cattle in any treatment group. However, the relative abundance genes conferring resistance to MLS increased significantly in all four treatment groups over the course of the trial (Figure 14; Pairwise Wilcoxon rank-sum with Benjamini-Hochberg correction, n = 47-48, $P < 0.05$). The relative abundance of β-lactams decreased significantly in CON and TYL treatment groups from the beginning to the end of the trial, while the relative abundance of ARGs conferring multi-metal resistance and resistance to phenicol decreased (Figure 14; Pairwise Wilcoxon rank-sum with Benjamini-Hochberg correction, n = 47-48, $P < 0.05$). These results are slightly different from those reported by Weinroth et al. (2019), who found no differences in resistance class using Tylosin. Weinroth et al. (2019) reported that the biggest driver of antimicrobial resistance profiles is the geographical location of samples. While a lot of studies have evaluated the fecal content of cattle containing AMR genes, few have focused on just resistance genes identified in liver abscesses (Weinroth et al.,2018, 2019).

Conclusions

Overall, a treatment effect was not identified ($P > 0.05$) for liver abscess and fecal microbiomes, along with no effects ($P > 0.05$) on *Salmonella* spp. presence in liver abscesses. While a difference ($P < 0.05$) in alpha diversity was identified in fecal samples, this is most likely due to the cattle coming from various backgrounds at the beginning of the feeding period and being made more uniform upon completion of the feeding trial. When evaluating the resistome, no differences ($P > 0.05$) were found among treatments, suggesting treatment effects had no major effect on the antimicrobial resistance genes in fecal matter.

Table 1. Summary of findings reported by Nagaraja and Titgemeyer (2007) on differences between acute and subacute acidosis in beef cattle.

Factors	Acidosis	
	Acute	**Subacute**
Clinical Signs	Yes	No
Rumen pH	< 5.0	5.0 – 5.5
Lactic Acid Content	High	Low
Violate Fatty Acids	Below Normal	High
Gram-negative bacteria	Decreased	No change
Gram-positive bacteria	Increased	No change
Lactic Acid-utilizer bacteria	Decreased	Increased
Lactobacillus spp.	Increased	Increased

Table 2. A summary of published studies found associated with highly virulent *Salmonella* serotype outbreaks.

Paper	Country	Year	Serotype	Food Matrix	Reported Dose	Log_{10} of Dose[a]	Exposed	Ill
Abe et al.	Japan	2004	Enteritidis	Beef and Bean Sprout with Sesame Dressing	8.8 x 10^2	2.94	-	927
				Boiled Veggies with Sauce	1 x 10^3	3.00	-	107
				Omelet with Sauce	9.2 x 10^2	2.96	-	26
				Scallops with Cream Sauce	1.0 x 10^6	6.00	-	30
				Cabbage Seachicken and Harusame with Sauce	6.0 x 10^4	4.77	-	69
				Bavarois	3.33 x 10^4	4.52	-	100
Armstrong et al.**	USA	1970	Typhimurium	Chiffonade	6.17 x 10^3	3.79	60	33
Blaser & Newman **	USA	1982	Typhimurium	Suspension	2.00 x 10^9	9.30	2	2
				Suspension	4 x 10^9	9.60	3	3
Boring et al.**	USA	1971	Typhimurium	Water	2.04 x 10^2	2.31	7572	805
				Water	2.04 x 10^2	2.31	1216	230
Bollaerts et al.	USA	2008	Enteritidis	Beef	5.41 log10	5.41	5	3
				Beef	2.97 log10	2.97	3517	967
				Cake	2.65 log10	2.65	5102	1371
				Cake	5.8 log 10	5.80	13	11
				Chicken	3.63 log10	3.63	16	3
				Chicken	3.63 log10	3.63	133	53
				Egg	6.3 log 10	6.30	114	63
				Egg	3.8 log 10	3.80	884	558
Bollaerts et al.	USA	2008	Enteritidis	Egg	1.4 log 10	1.40	156	42
				Ice Cream	2.09 log 10	2.09	452	30
				Peanut	1.72 log 10	1.72	3990	644

Bollaerts et al.	USA	2008	Enteritidis	Sauce	4.74 log 10	4.74	39	39
				Soup	6.31 log 10	6.31	123	113
			Typhimurium	Ice Cream	3.79 log 10	3.79	1400	770
			Newport	Hamburger	1.23 log 10	1.23	1813	19
CDC	USA	1996	Enteritidis	Hollandaise	4.48 x 10^4	4.65	39	39
		2002	Typhimurium	Milk	-	-	211	16
Christina et al.	Germany	2007	Enteritidis	Cake	-	-	822	111
Daly et al.	USA	2010	Enteritidis	Blender	-	-	233	133
Deepanjali et al.	India	2022	Enteritidis	Chicken Shawarma	-	-	27	26
Dodhia et al.	UK	1998	Enteritidis	Egg	-	-	37	30
Draper et al.	Australia	2017	Saintpaul	Unknown	-	-	67	30
Evans et al.	UK	1995	Enteritidis	Egg	6.0 x 10^3 CFU/g	3.77	6	5
Goodman et al.	USA	1993	Enteritidis	Egg	-	-	1900	690
Grein et al.	Ireland	1997	Enteritidis	Cake	-	-	65	423
Guillier et al.	France	2013	Typhimurium	Beef	1.6-3.1 log 10	2.48*	1599	554
Hara-Kudo and Takatori	Japan	2010	Enteritidis	Puff cream	39,000 CFU/g	4.59	4	4
				Chicken Stomach	200 CFU/g	2.3	303	114
				Egg	4 MPN/g	0.6	84	19
				Rice and Beans	14,000,000 CFU/g	7.14	6	6
				Dumpling	9 MPN/g	0.95	113	75
				Vegetables	30 CFU/g	1.47	86	39
				Rice Ball with Deep Fried Tofu	1800000 CFU/g	6.25	59	29
Hara-Kudo and Takatori	Japan	2010	Agona	Soy Pulp and Egg	<30 CFU/g	1.47**	72	12
Hundy and Cameron	Australia	2002	Typhimurium	Mango Pudding	46 and 110 CFU/g	3.40*	-	28

Kapperud et al.	Norway & Finland	1990	Typhimurium	Chocolate	10 MPN/g	1.00	-	361
Kasuga et al.	Japan	2004	Enteritidis	Peanut Dressing	<100 CFU/g	2.00**	2267	418
				Peanut Dressing	4.3 MPN/g	0.63	1320	179
				Beef & Bean Sprouts	40 CFU/g	1.60	10552	967
Kasuga et al.	Japan	2004	Enteritidis	Spinach with Peanut Dressing	1.4 MPN/g	0.14	5320	644
				Macaroni Salad	1000 CFU/g	3.00	152	52
				Chicken and Eggs on Rice	27 CFU/g	1.43	133	53
				Egg Salad	0.78 MPN/g	-0.10	156	42
				Tartar Sauce	<100 CFU/g	2.00**	126	36
				Egg	1,200,000 CFU/g	6.07	191	45
				Yam with Soup	2,400 MPN/g	3.38	343	75
				Beef	2,000 CFU/g	3.30	5	3
				Egg	200 MPN/g	2.30	885	558
				4	1,000 CFU/g	3.00	11	10
				Shrimp and Egg	2,400 MPN/g	3.38	104	70
				Egg	0.135 CFU/g	-0.86	363	198
				Yams with Soup	32,000 CFU/g	4.50	123	113
				Spaghetti Salad	120,000 CFU/g	5.07	78	73
				Eggs	1,600 CFU/g	3.20	103	57
				Bavarois	1,000 CFU/g	3.00	123	100
Kasuga et al.	Japan	2004	Enteritidis	Sauce for Octopus Pancake	2,800,000 CFU/g	6.45	68	34
				Yam with Soup	50,000,000 CFU/g	7.69	11	11

Kasuga et al.	Japan	2004	Enteritidis	Cheesecake	19,000 CFU/g	4.27	5	5
				Three-layer Cake	5 MPN/g	0.69	5103	1371
				Tiramisu	1,600,000 CFU/g	6.20	7873	697
				Cake	6000 CFU/g	3.77	13	11
				Sherbet	< 3,000 CFU/g	3.47**	83	68
				Mayonnaise	460 MPN/g	2.66	2907	498
			Typhimurium	Yam with Soup	2,300 MPN/g	3.36	99	40
				Yam Soup with Quail Eggs	40,000 CFU/g	4.60	79	39
Lehmacher et al.	Germany	1995	Saintpaul	Potato Chips	4-45 for 100g	-0.61*	1000	241
Lennox et al.	UK	1954	Typhimurium	Milk	-	-	250	64
Liu et al.	China	2016	Typhimurium	Cowpea	-	-	1268	401
Murase et al.	Japan	2000	Typhimurium	Pork	2.6 x 10^5 MPN/g	5.41	140	105
Matsui et al.	Japan	2004	Enteritidis	Dessert Buns	30 cells per 100g	-0.52	22684	93
Morgan et al.	UK	1994	Enteritidis	Eggs in Ice-cream	10^5	5.00	2	2
Napoleoni et al.	EU	2021	Enteritidis	Cheese	43 MPN/L	-	-	-
Roberts-Witteveen et al.	Australia	2008	Typhimurium	Eggs	-	-	45	8
Silliker	USA	1980	Newport	Egg	1.5 x 10^5	5.17	6	1
Silliker	USA	1980	Newport	Egg	3.8 x 10^5	5.57	8	1
				Egg	1.30 x 10^5	5.11	6	3
Taylor et al.	USA	1984	Typhimurium	Eggs in Ice Cream	16^6 CFU/g	7.22	8	8
Taylor et al.**	USA	1984	Typhimurium	Ice Cream	1.0 x 10^9	9.00	1	1
				Ice Cream	7.50 x 10^8	8.87	2	2

				Ice Cream	5.00 x 10^8	8.69	2	2
				Ice Cream	5.00 x 10^8	8.69	1	1
				Ice Cream	2.50 x 10^8	8.39	1	1
				Ice Cream	1.0 x 10^8	8.00	1	1
Vought and Tatini	USA	1996	Enteritidis	Eggs in Ice-cream	2.4 CFU/ g	0.38	-	29100

[a] Log transformed dose were performed on a CFU/g or MPN/g basis.

* CFU/g reported on a range; an average of the two numbers was taken and log transformed.

**Indicated papers were identified in the Teunis et al. (2010) publication

Table 3. Summary of published studies found associated with non-highly virulent *Salmonella* serotype outbreaks.

Paper	Country	Year	Serotype	Food Matrix	Reported Dose	log10 of Dose[a]	Exposed	Ill
Al-Ghamdi et al. 1989	Saudi Arabia	1989	Minnesota	Meat	-	-	419	168
Bollaerts et al.	USA	2008	Heidelberg	Cheese	2.22 log 10	2.22	205	68
			Infantis	Ham	6.46 log 10	6.46	8	8
			Oranienburg	Soup	9.9 log 10	9.9	11	11
			Cubana	Dye	4.57 log10	4.57	17	12
CDC	USA	2002	Javiana	Roma Tomatoes	-	-	1100	141
Crowe	Ireland	1945	Aertrycke	Egg	-	-	27	23
George et al.	USA	1976	Schwarzengrund	Pancreases Extract	2.00 x 10^2	2.30	1	1
Greeenwood and Hooper	UK	1983	Napoli	Chocolate	1.71 x 10^1	1.23	2	0
				Chocolate	4.48 x 10^2	2.65	1	1
Hara-Kudo and Takatori	Japan	2010	Cerro	Bread	24 MPN/g	1.38	1577	157
			Montevideo	Radish Sprouts	363 MPN/g	2.55	96	12
			O4:H:eh,NT	Egg	1-45 MPN/g	1.65*	9	8
Kasuga et al.	Japan	2004	Oranienburg	Egg	12,000 CFU/g	4.07	9	9
			Bareilly O7	Scallop Cream Sauce	20,000 CFU/g	4.30	38	30
Meehan et al.	USA	1992	Infantis	Turkey	-	-	474	215
Nabbut et al.	Japan	1981	Muenster	Beef	5 x 10^6	6.69	12	12
Reitler et al.	USA	1960	Zanzibar	Goat Cheese	1 x 10^11	11	5	5
Rowe et al.	UK	1987	Ealing	Infant Formula	6 CFU per 450 g	-1.87	-	76
Silliker	USA	1980	Pullorum	Egg	13.0 x 10^8	9.11	12	5
				Egg	700 x 10^7	9.84	11	10

Silliker	USA	1980		Egg	1600 x 10^7	10.2	12	12
			Anatum	Egg	5.50 x 10^6	6.74	48	11
				Egg	67.0 x 10^6	7.82	24	5
			Derby	Egg	15.0 x 10^6	7.17	12	3
			Melagridis	Egg	50 x 10^5	6.69	41	16
Angelotti et al.***	USA	1961	Infantis	Ham	2.63 x 10^6	6.41	8	8
Fontaine et al.***	USA	1978	Heidelberg	Cheeses	1.66 x 10^2	2.22	205	68
Varela and Olarte	Mexico	1942	Anatum	Suspension	5 x 10^8 CFU/g	8.69	1	1
Werber et al.	Germany	2005	Oranienburg	Chocolate	1.1 - 2.8 CFU/ g	0.18*	-	439

[a] Log transformed dose on a CFU/g basis.
* CFU/g reported on a range; an average of the two numbers was taken and log transformed.
** Those listed below the state amount were log transformed at the stated amount.
***Indicated papers were identified in the Teunis et al. (2010) publication

Table 4. Odds Ratios of the effects on log dose, virulence group (High or Low), or food animal or other (Animal, Other). Significant differences ($P < 0.05$) were identified for all factors.

Factor	Odds Ratio	SD
Log Dose	1.022	1.006
Virulence Group	0.568	1.091
Food Animal	0.821	1.027

Table 5. Breakdown of treatments and abscess conditions for mesenteric lymph nodes collected (N = 503) from cattle fed either a control or treatment diet (Direct Fed Microbial, Tylosin, or Direct Fed Microbial + Tylosin).

Treatment	**Liver Status**	
	Abscessed (n*)	Non-Abscessed (n*)
Control	77	63
Direct Fed Microbial	67	65
Tylosin	55	65
Direct Fed Microbial + Tylosin	49	62

*Due to limitations in the plant and natural absence of liver abscess presence/voidness, 10 samples were not collected from each pen

Table 6. Mean *Salmonella* spp. counts (log CFU/g) of mesenteric lymphatic tissues from feedlot cattle fed either a control or treatment diet (Direct Fed Microbial, Tylosin, or Direct Fed Microbial + Tylosin). Along with diet, the cattle's liver status (abscessed or non-abscessed) was recorded upon collection of mesenteric lymphatic tissues to use as a potential indicator for *Salmonella* spp. presence.

Treatment	Liver Status	Mean ± Standard Deviation	%BDL*
Control	Abscessed	< 0.91 ± 0.22	75.3
	Non-Abscessed	< 0.87 ± 0.17	82.5
Direct Fed Microbial	Abscessed	< 0.83 ± 0.09	88.1
	Non-Abscessed	< 0.81 ± 0.06	83.1
Tylosin	Abscessed	< 0.91 ± 0.18	72.2
	Non-Abscessed	< 0.85 ± 0.13	87.7
Direct Fed Microbial + Tylosin	Abscessed	< 0.85 ± 0.13	83.7
	Non-Abscessed	< 0.87 ± 0.18	82.3

***** %BDL indicates the percent of samples, of the samples analyzed, with bacterial counts that were below the analysis detection limit (0.778 log CFU/g)
Means with a less than symbol (<) indicate that at least one sample within the treatment had a count that was below the analysis detection limit (0.778 log CFU/g)
No significant impacts ($P = 0.27$) were found between treatments or liver status on *Salmonella* spp. counts

Table 7. Results of the binomial regression of treatment diet (Control, Direct Fed Microbial, Tylosin, or Direct Fed Microbial + Tylosin) and liver status (abscessed or non-abscessed) as factors on the probability of *Salmonella* spp. occurring in mesenteric lymphatic tissues of feedlot cattle.

Treatment	Liver Status	Probability (%)	Standard Error (%)	95% Confidence Interval (%)
Control	Abscessed	38.7	6.0	27.7 – 51.1
	Non-Abscessed	47.0	6.8	34.2 – 60.3
Direct Fed Microbial	Abscessed	35.9	6.4	24.5 – 49.1
	Non-Abscessed	34.9	6.4	23.6 – 48.1
Tylosin	Abscessed	57.2	7.4	42.5 – 70.8
	Non-Abscessed	42.4	6.7	30.2 – 55.7
Direct Fed Microbial + Tylosin	Abscessed	49.4	8.0	34.4 – 64.6
	Non-Abscessed	40.5	6.8	28.1 – 54.2

No differences were identified ($P = 0.23$) between treatments or the interactions of liver status and treatment ($P = 0.36$) and their effects on the *Salmonella* prevalence within mesenteric lymph nodes.

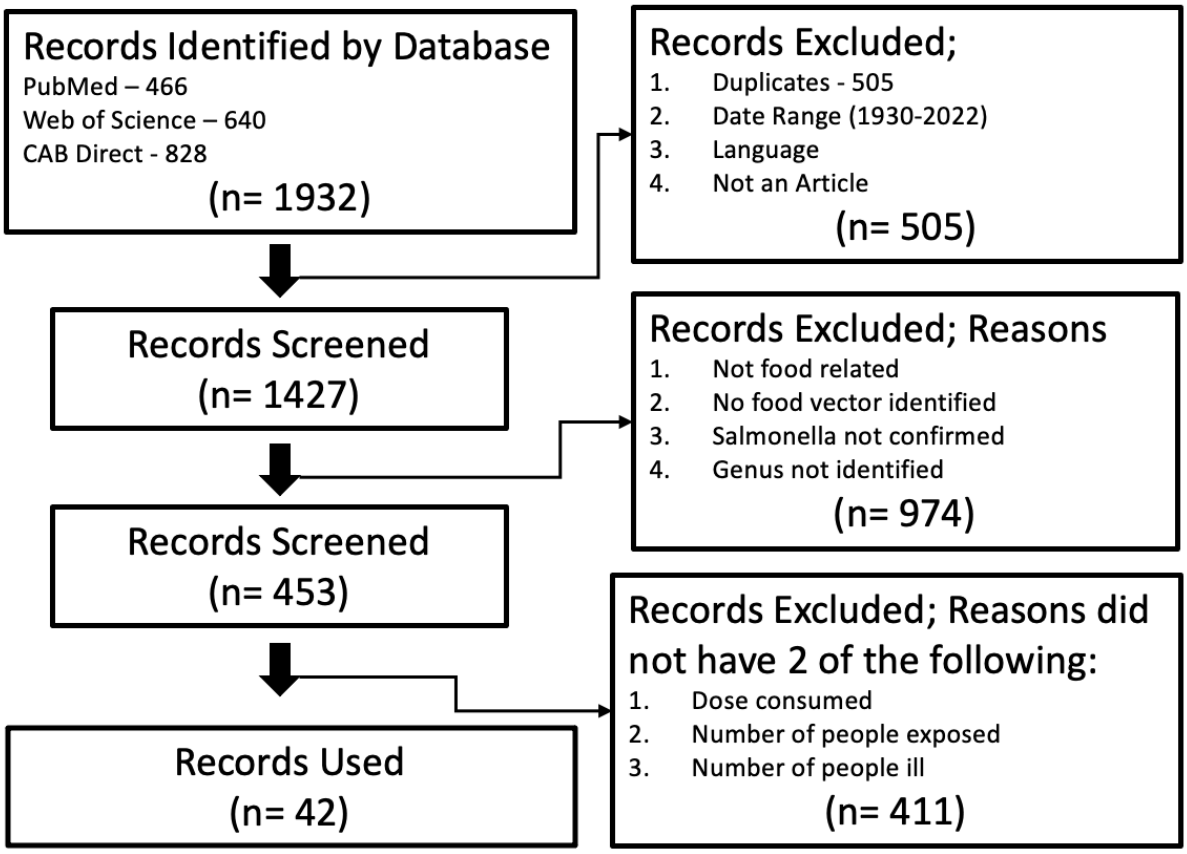

Figure 1. PRISMA flow chart of search protocols

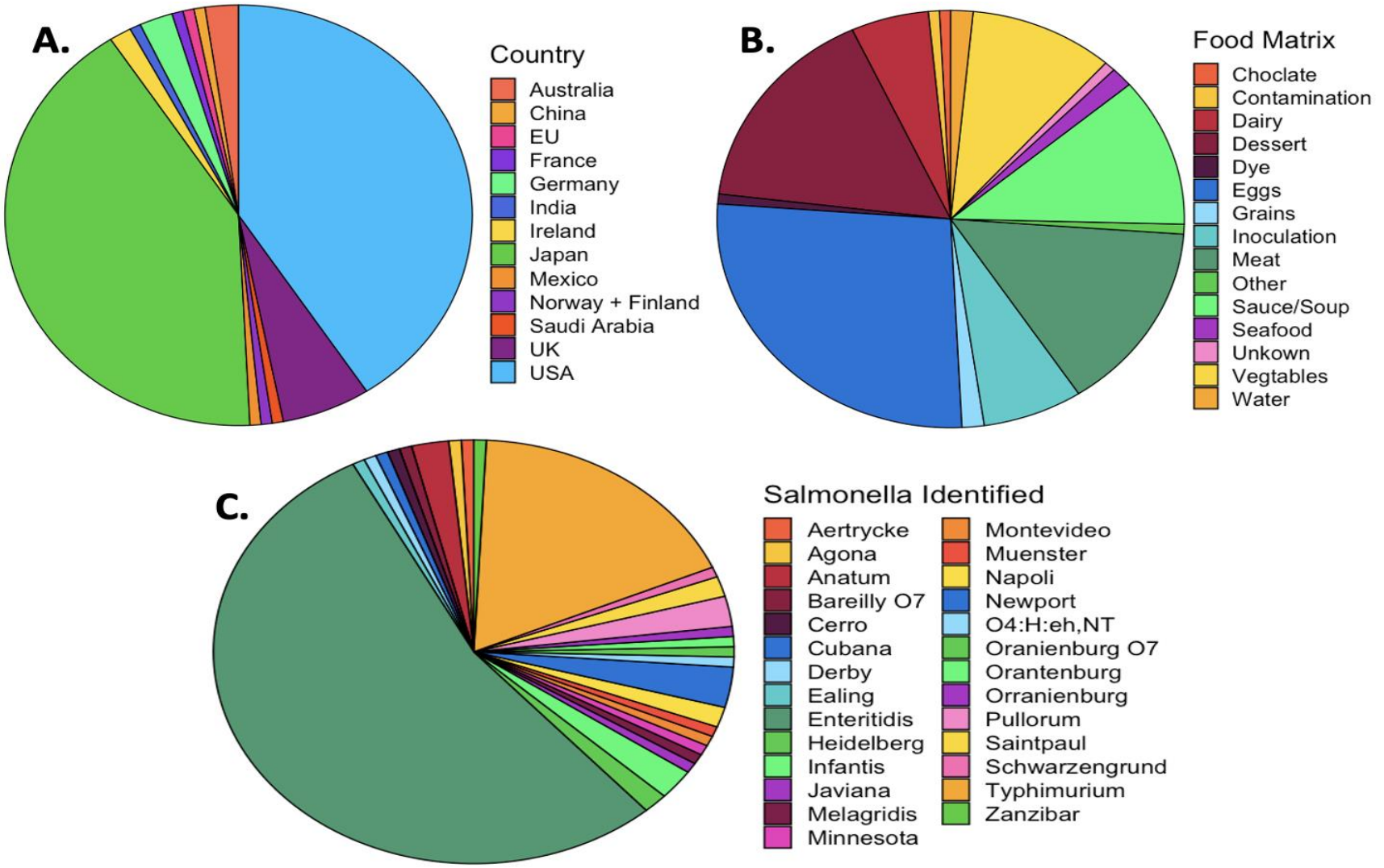

Figure 2. A summary of the countries (A.), food matrix (B.), and Salmonella serotype identified (C.) from the 42 articles that passed the screening process.

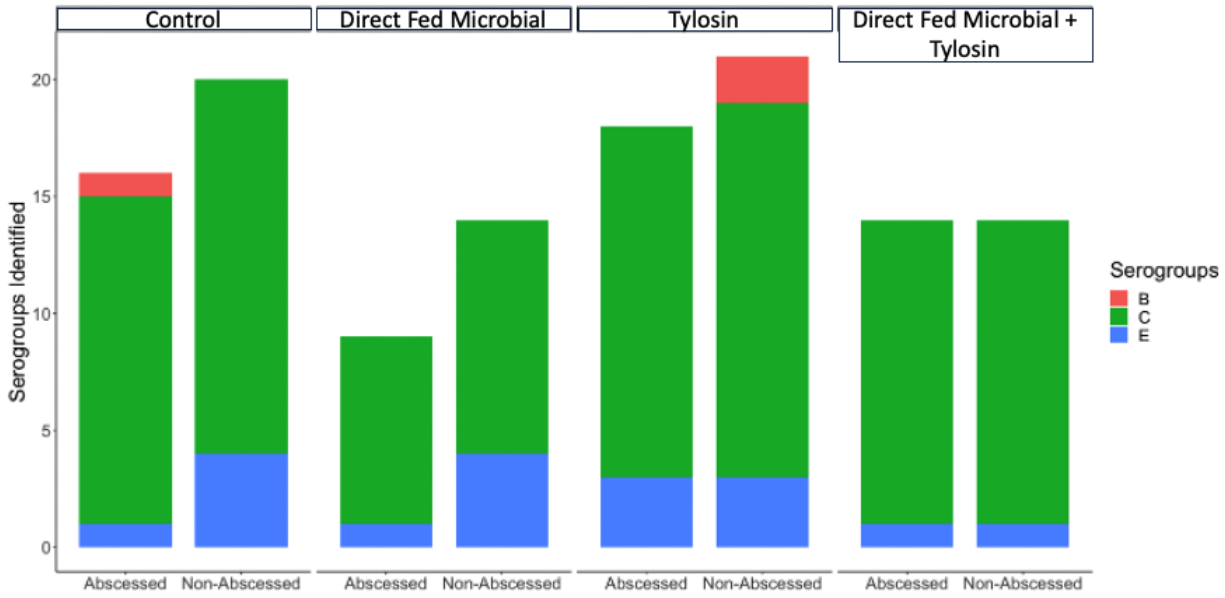

Figure 3. Salmonella spp. serogroups identified from cattle being fed a treatment diet (Control, Direct Fed Microbial, Tylosin, or Direct Fed Microbial + Tylosin) with abscessed and non-abscessed livers.

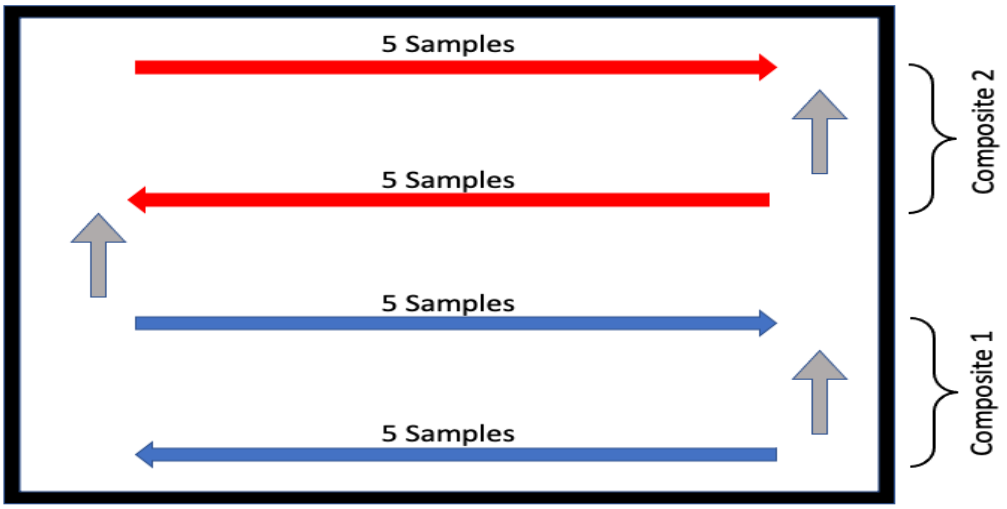

Figure 4. Fecal sampling protocol consisted of dividing the pen in half with two composites being generated for each pen. Ten fecal pats were collected for each composite throughout the area to ensure proper pen coverage.

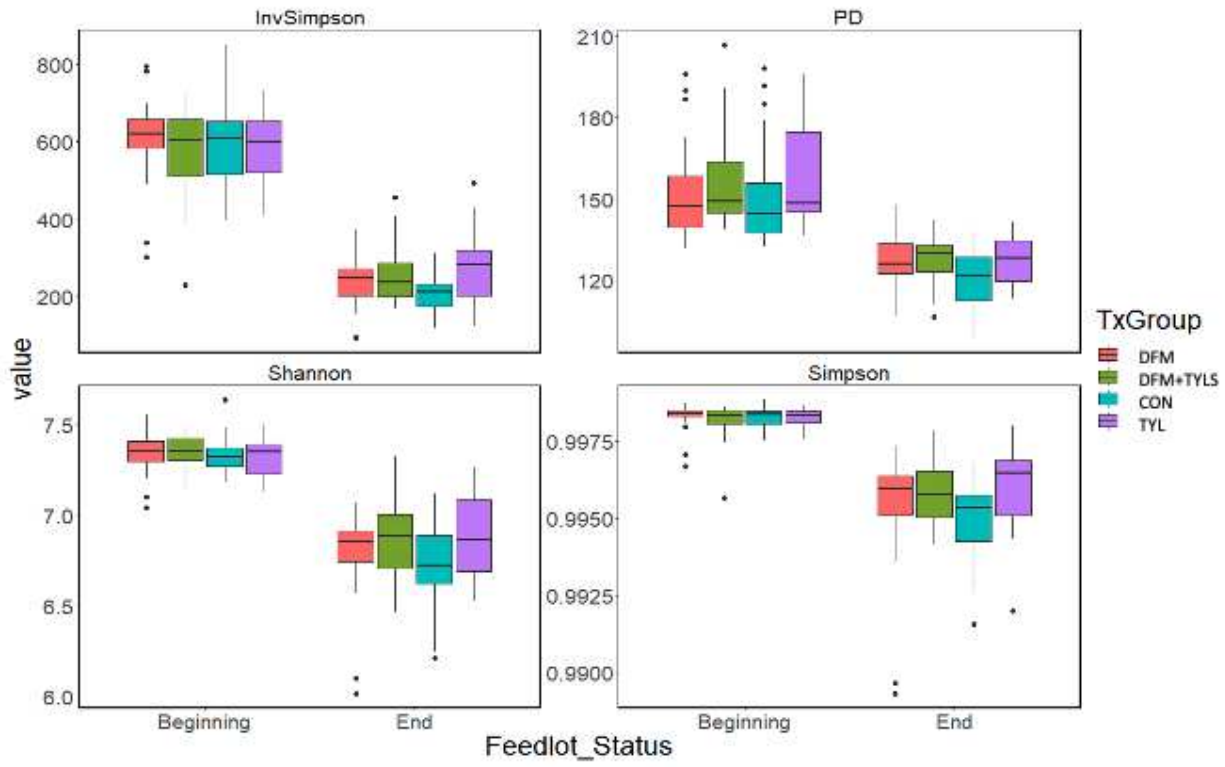

Figure 5. Alpha diversity metrics for fecal 16S rRNA samples. All metrics were found to be significantly different ($P < 0.05$) between the beginning and end of the feedlot period. However, between a direct-fed microbial (DFM), a direct-fed microbial + Tylosin (BOV+TYL), Tylosin (TYL), and control (CON), regardless of metric utilized, no significant differences ($P > 0.05$) were found.

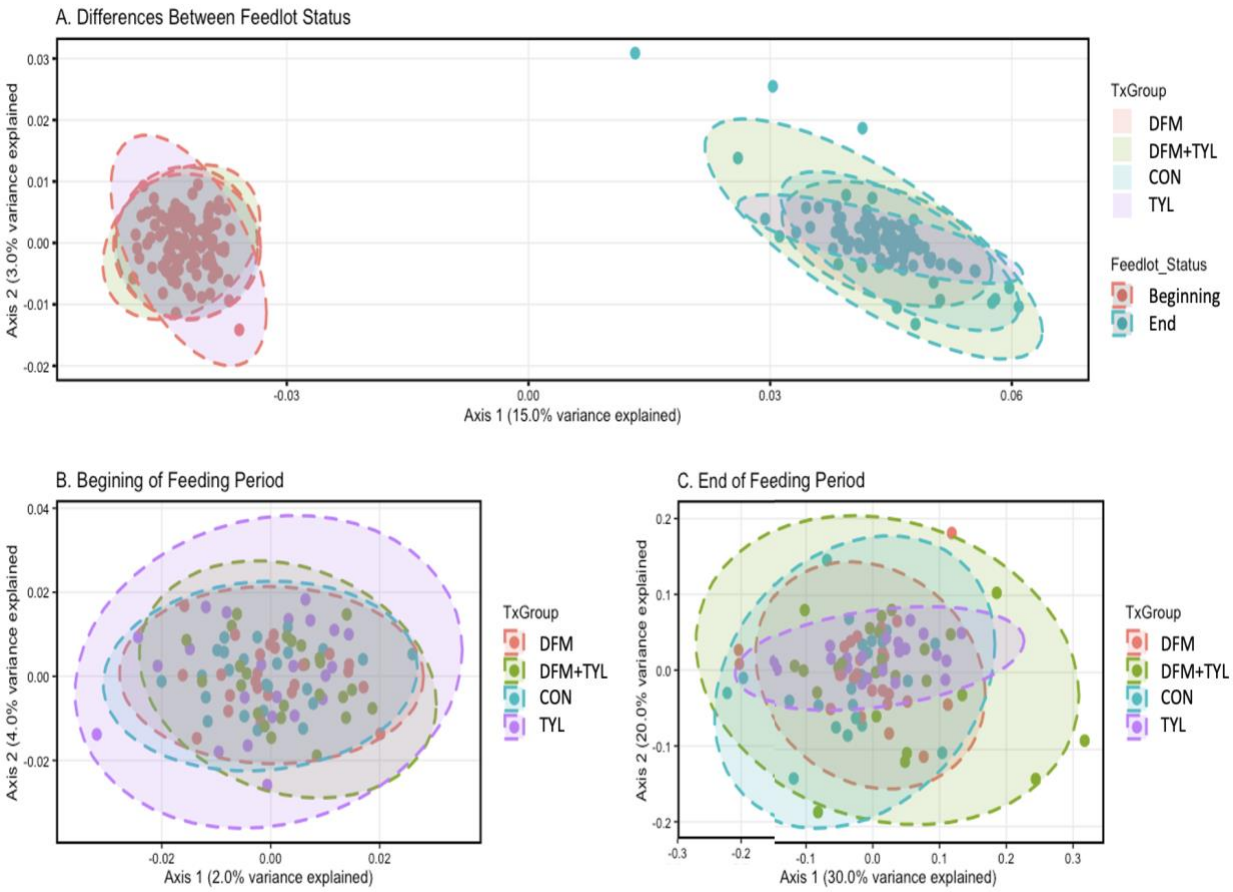

Figure 6. Beta diversity metrics for fecal 16S rRNA samples. For generalized UniFrac All significant differences ($P < 0.05$) were found between the beginning and the end of the feedlot period (A). However, between a direct-fed microbial (DFM), a direct-fed microbial + Tylosin (DFM+TYL), Tylosin (TYL), and control (CON), no significant differences ($P > 0.05$) were found regardless of feedlot period (B and C).

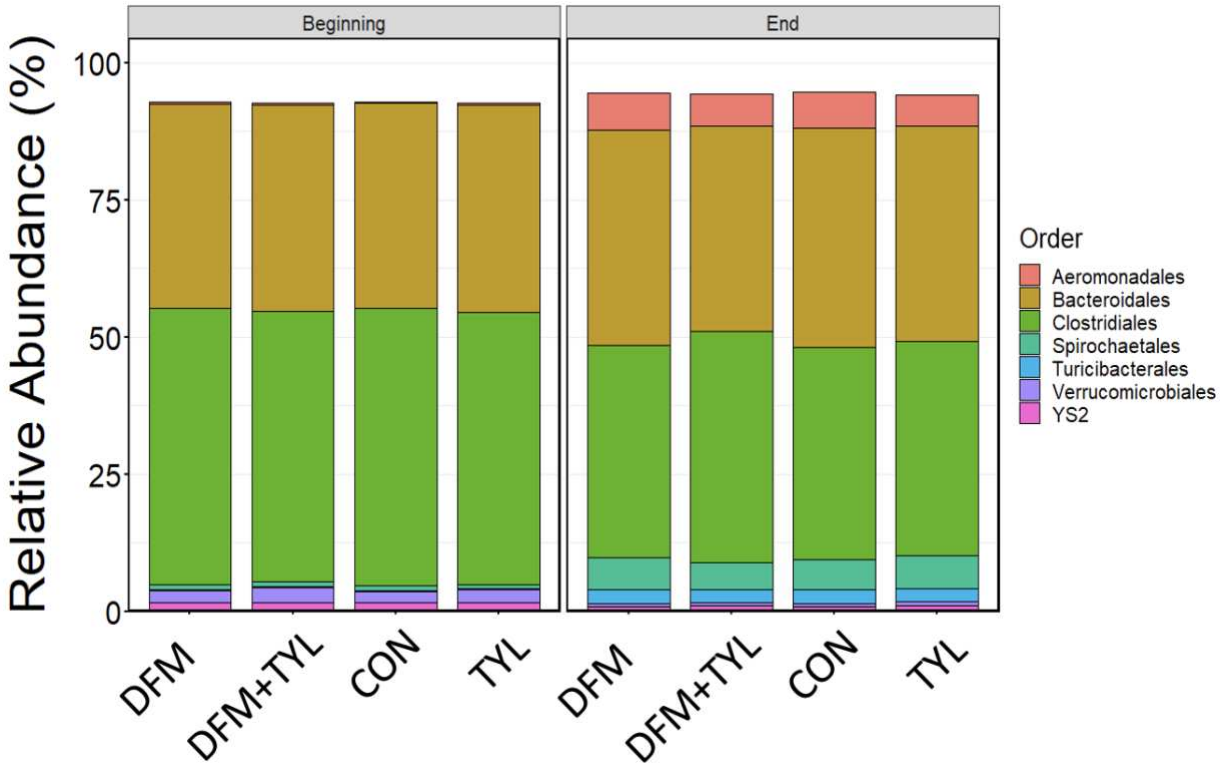

Figure 7. Relative abundance of bacterial orders present within samples for the beginning and end of the feedlot period. In addition, treatment diets consist of a direct-fed microbial (DFM), a direct-fed microbial + Tylosin (BOV+TYL), Tylosin (TYL), and control (CON).

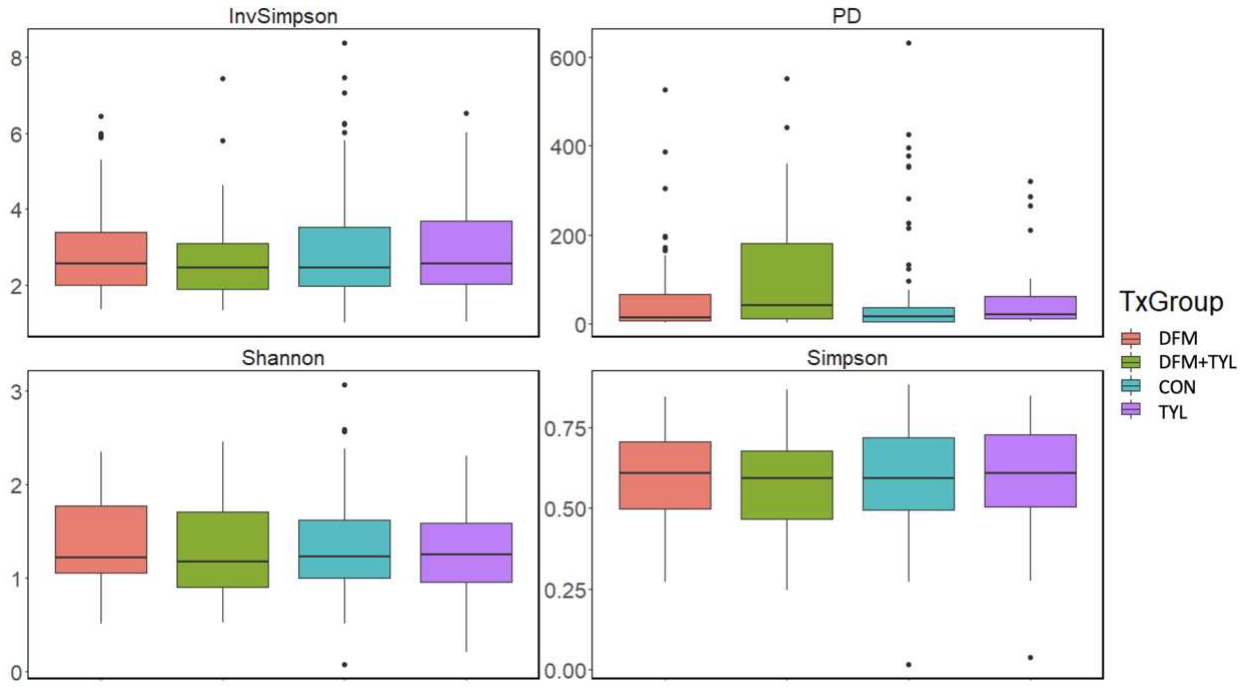

Figure 8. Alpha diversity metrics for liver abscess 16S rRNA samples. All metrics between treatment diets, a direct-fed microbial (DFM), a direct-fed microbial + Tylosin (DFM+TYL), Tylosin (TYL), and control (CON), had no significant differences ($P > 0.05$).

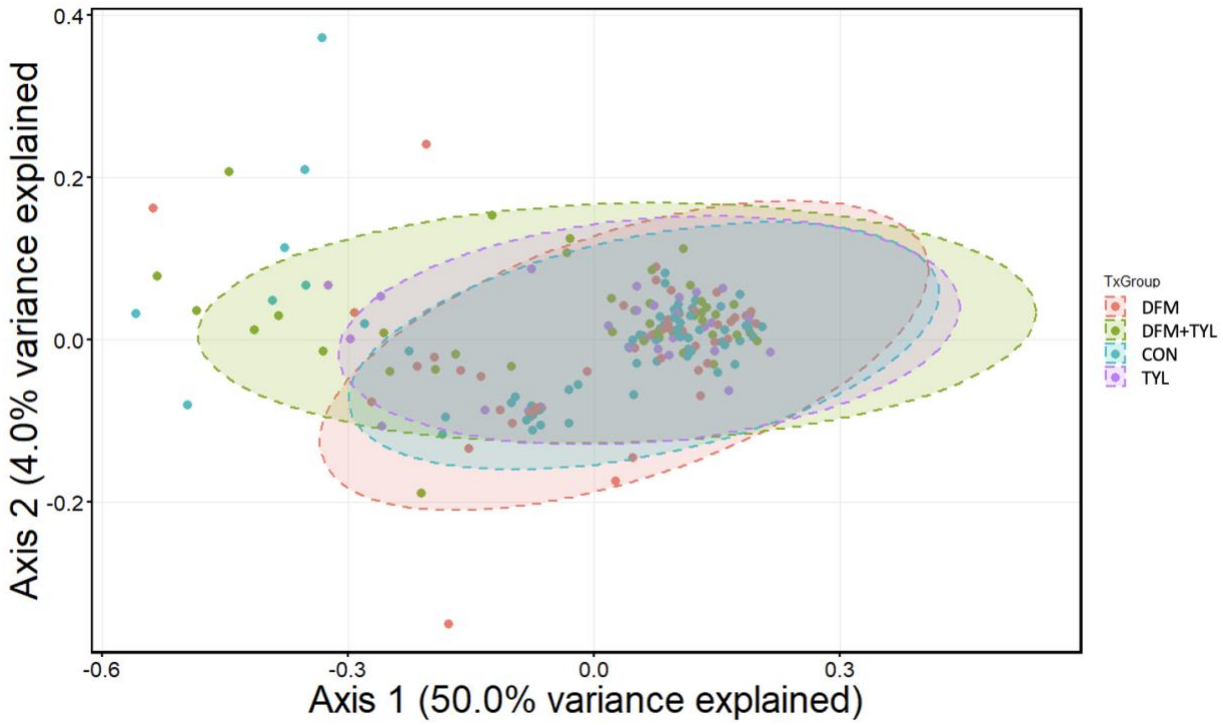

Figure 9. Beta diversity metrics for liver abscess 16S rRNA samples. Between a direct-fed microbial (DFM), a direct-fed microbial + Tylosin (DFM+TYL), Tylosin (TYL), and control (CON) diets, no significant differences ($P > 0.05$) were found.

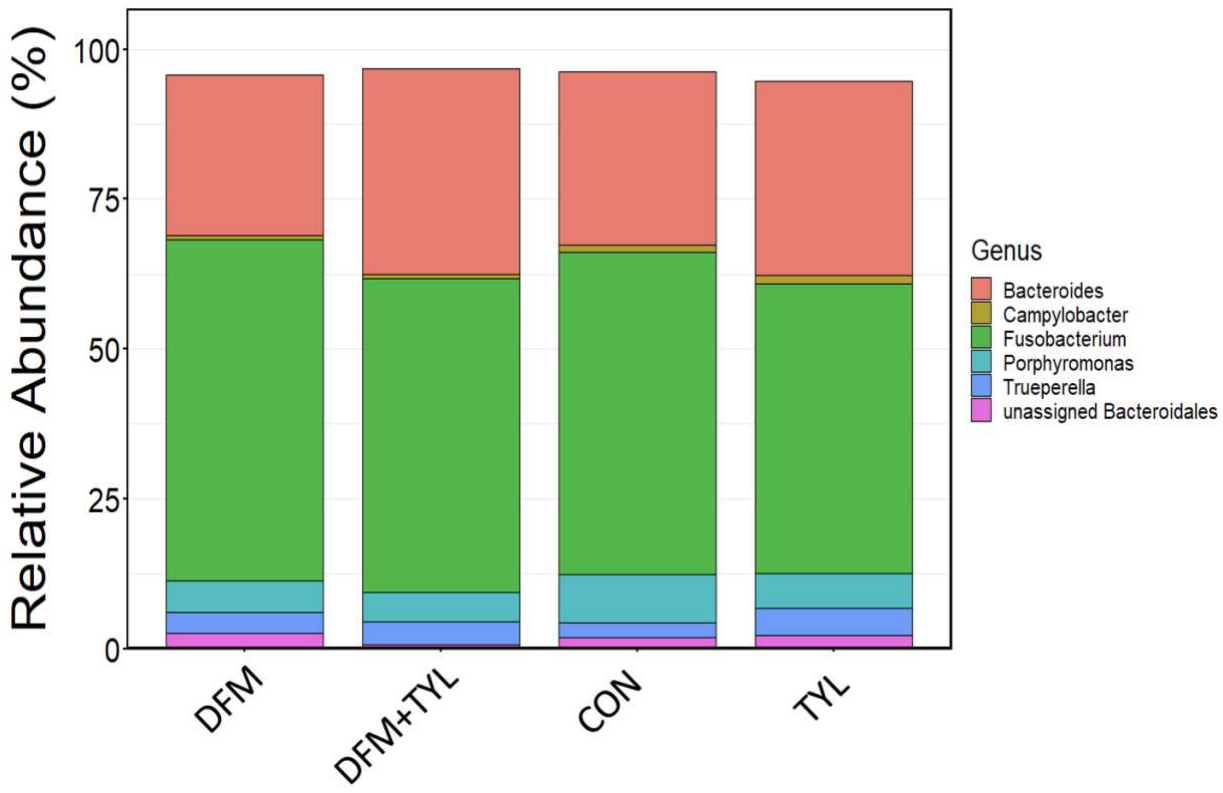

Figure 10. Relative abundance of bacterial genera present within samples for treatment diets consisting of a direct-fed microbial (DFM), a direct-fed microbial + Tylosin (DFM+TYL), Tylosin (TYL), and control (CON).

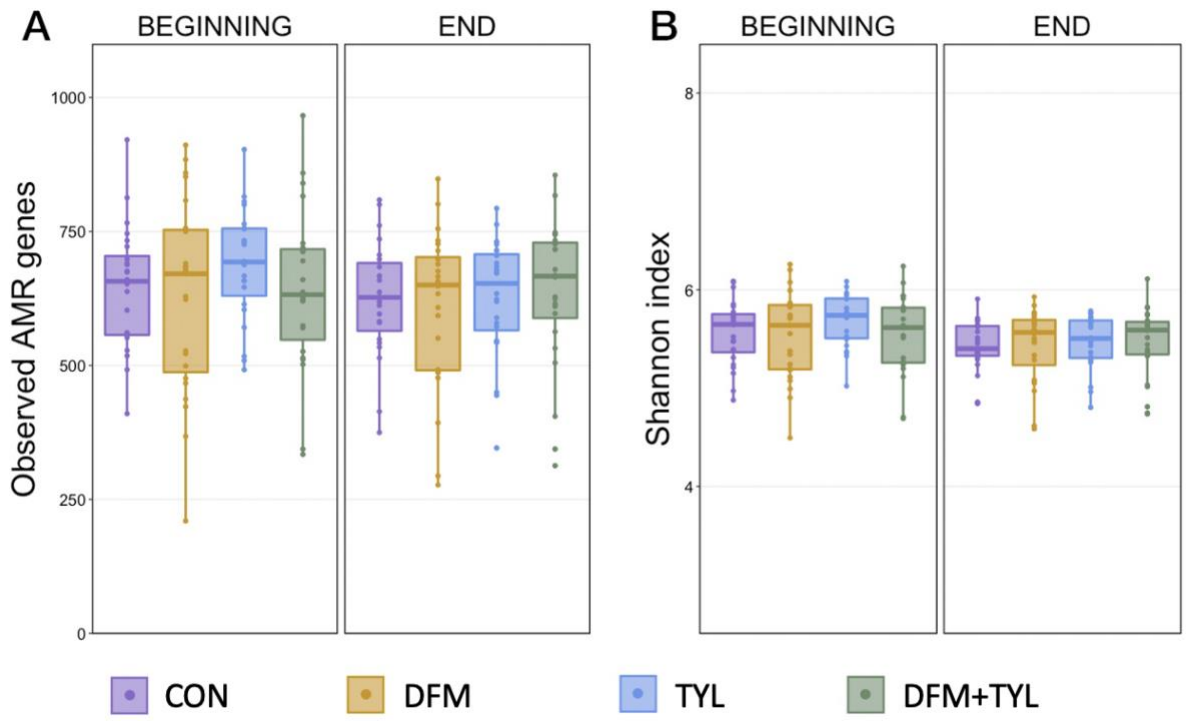

Figure 11. Boxplots displaying the number of unique ARGs and the Shannon index values demonstrating the richness (A) and diversity (B) of the resistome, respectively. No significant differences were detected between treatment groups of a direct-fed microbial (DFM), a direct-fed microbial + Tylosin (DFM+TYL), Tylosin (TYL), and control (CON) at each time point or within groups between time points (Pairwise Wilcoxon rank-sum with Benjamini-Hochberg correction, $P < 0.05$, n = 47-48).

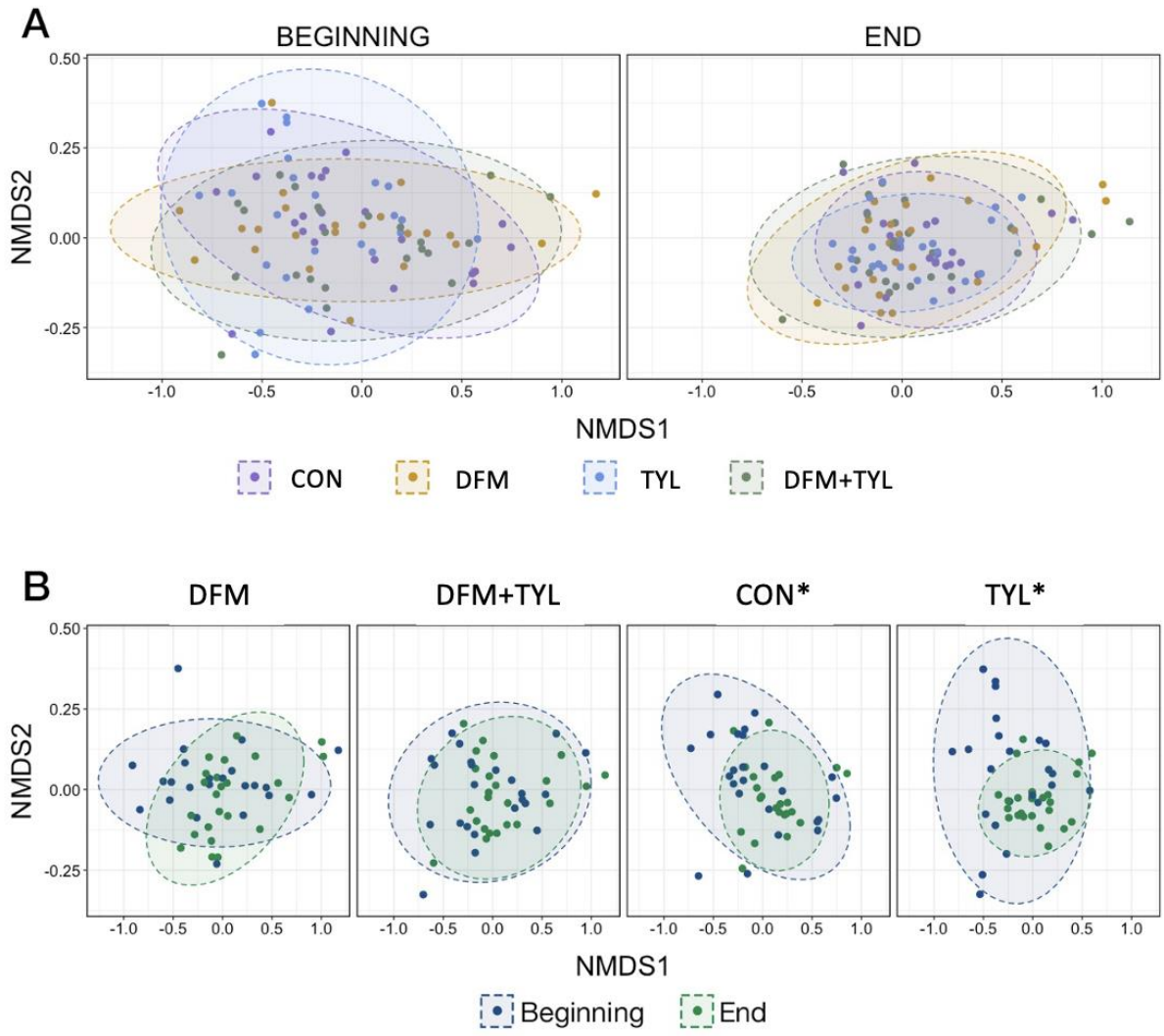

Figure 12. Non-metric multidimensional scaling (NMDS) of Bray-Curtis dissimilarity distances illustrates the variation in the resistome's structure. A) The NMDS demonstrates clustering of AMR-TE gene sequences from the four treatment groups consisting of a direct-fed microbial (DFM), a direct-fed microbial + Tylosin (DFM+TYL), Tylosin (TYL), and control (CON) at the beginning and end of the trial. B) The NMDS demonstrates the clustering of ARGs from the beginning and end of the trial within each treatment group. Each point represents a resistome from an individual animal colored by treatment group (A) or timepoint (B). Dashed lines and shaded areas represent 95% confidence ellipses for each treatment group or time point. An asterisk denotes significant differences in community structure between time points (PERMANOVA, n= 47-48, $P < 0.05$).

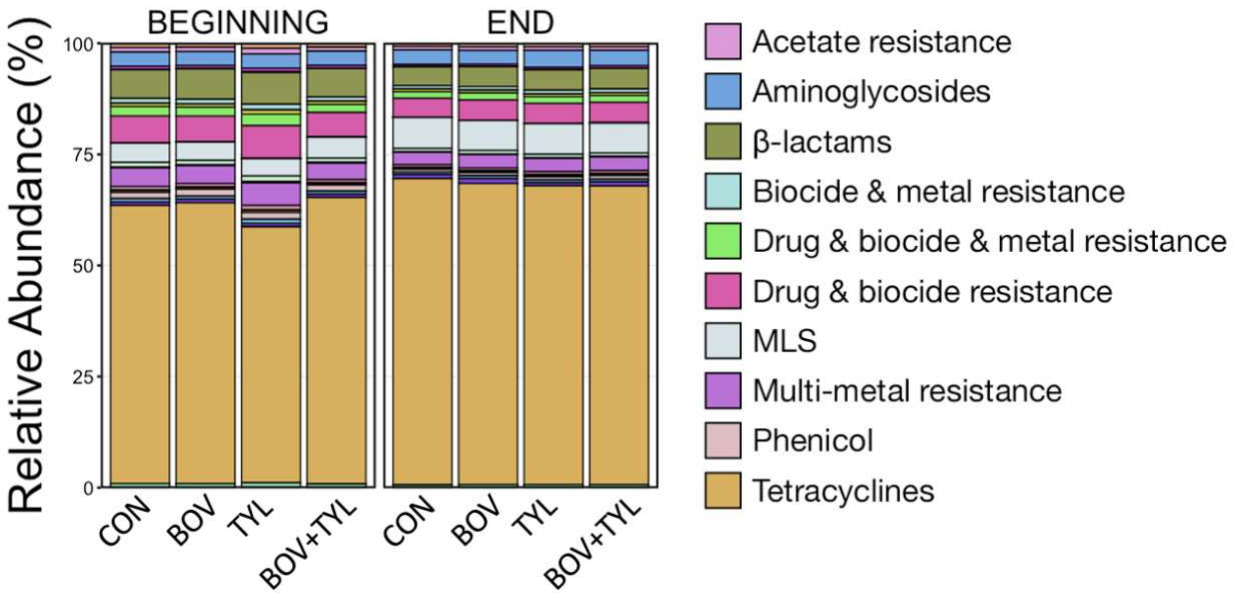

Figure 13. Bar plot illustrating the mean relative abundance of AMR classes within treatment a direct-fed microbial (DFM), a direct-fed microbial + Tylosin (DFM+TYL), Tylosin (TYL), and control (CON) groups from the beginning and end of the trial. Abundances were normalized to the total number of CSS-normalized ARGs within each sample. The ten most abundant classes across all samples are displayed in the legend.

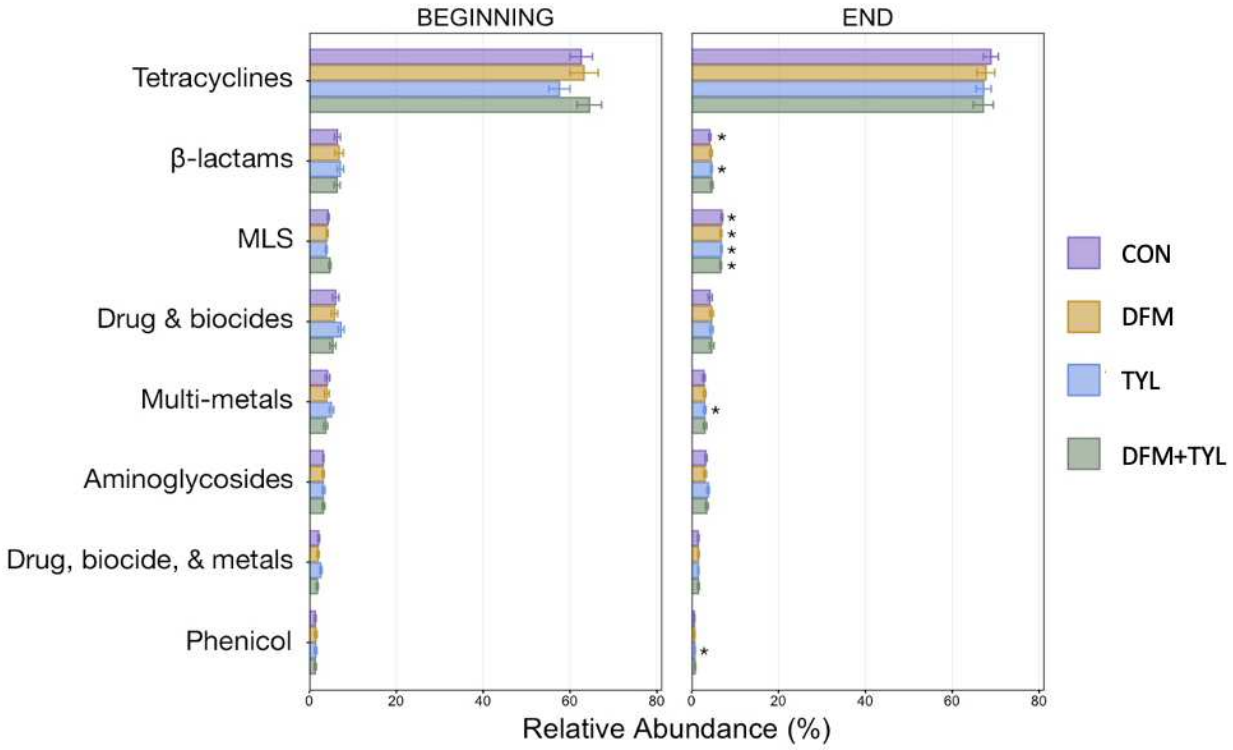

Figure 14. Bar plot demonstrating potential differences in the relative abundance of AMR classes comprising an average of at least 1% of the overall resistome across all samples. Error bars display the standard error of the mean. No significant differences were detected between treatments consisting of a direct-fed microbial (DFM), a direct-fed microbial + Tylosin (DFM+TYL), Tylosin (TYL), and control (CON) groups within any of the 8 classes of ARGs at either timepoint (Pairwise Wilcoxon rank-sum with Benjamini-Hochberg correction, $P > 0.05$, $n = 47\text{-}48$). Significant differences in the relative abundance of AMR classes between the beginning and end of the trial within each treatment group are illustrated with an asterisk (Pairwise Wilcoxon rank-sum with Benjamini-Hochberg correction, $n = 47\text{-}48$, $P < 0.05$).

WORKS CITED

Abdela, N. 2016. Sub-acute Ruminal Acidosis (SARA) and its Consequence in Dairy Cattle: A Review of Past and Recent Research at Global Prospective. Achievements in the Life Sciences. 10:187–196. doi:10.1016/j.als.2016.11.006.

Abe, K., N. Saito, F. Kasuga, and S. Yamamoto. 2004. Prolonged incubation period of salmonellosis associated with low bacterial doses. Journal of Food Protection. 67:2735–2740. doi:10.4315/0362-028X-67.12.2735.

Allen, M.S., Bradford, B.J., Harvatine, K.J., 2005. The cow as a model to study food intake regulation. Ann. Rev. Nutr. 25, 523–547. Andersen, P.H., Bergelin, B., Christensent, K.A., 1994. Effect of feeding regimen on concentration of free endotoxin in ruminal fluid of cattle. J. Anim. Sci. 72, 487–491.

Amachawadi, R. G., and T. G. Nagaraja. 2016. Liver abscesses in cattle: A review of incidence in Holsteins and of bacteriology and vaccine approaches to control in feedlot cattle12. Journal of Animal Science. 94:1620–1632. doi:10.2527/jas.2015-0261.

Amachawadi, R. G., H. M. Scott, C. Aperce, J. Vinasco, J. S. Drouillard, and T. G. Nagaraja. 2015. Effects of in-feed copper and tylosin supplementations on copper and antimicrobial resistance in faecal enterococci of feedlot cattle. J Appl Microbiol. 118:1287–1297. doi:10.1111/jam.12790.

Arowolo, M. A., and J. He. 2018. Use of probiotics and botanical extracts to improve ruminant production in the tropics: A review. Animal Nutrition. 4:241–249. doi:10.1016/j.aninu.2018.04.010.

Arthur, T. M., D. M. Brichta-Harhay, J. M. Bosilevac, M. N. Guerini, N. Kalchayanand, J. E. Wells, S. D. Shackelford, T. L. Wheeler, and M. Koohmaraie. 2008. Prevalence and Characterization of Salmonella in Bovine Lymph Nodes Potentially Destined for Use in Ground Beef†. Journal of Food Protection. 71:1685–1688. doi:10.4315/0362-028X-71.8.1685.

ASPA, A. S. for P. 2019. People at Risk of Food Poisoning. FoodSafety.gov. Available from: https://www.foodsafety.gov/people-at-risk.

Bailey, C. B., and C. C. Balch. 1961. Saliva secretion and its relation to feeding in cattle: 1. The composition and rate of secretion of parotid saliva in a small steer. British Journal of Nutrition. 15:371–382. doi:10.1079/BJN19610047.

Bakowski, M. A., V. Braun, and J. H. Brumell. 2008. Salmonella-Containing Vacuoles: Directing Traffic and Nesting to Grow. Traffic. 9:2022–2031. doi:10.1111/j.1600-0854.2008.00827.x.

Bates, D., M. Mächler, B. Bolker, and S. Walker. 2015. Fitting Linear Mixed-Effects Models Using lme4. Journal of Statistical Software. 67:1–48. doi:10.18637/jss.v067.i01.
Beukers, A. G., R. Zaheer, S. R. Cook, K. Stanford, A. V. Chaves, M. P. Ward, and T. A. McAllister. 2015. Effect of in-feed administration and withdrawal of tylosin phosphate on antibiotic resistance in enterococci isolated from feedlot steers. Frontiers in Microbiology. 6. Available from: https://www.frontiersin.org/articles/10.3389/fmicb.2015.00483.

Bolger, A. M., M. Lohse, and B. Usadel. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics. 30:2114–2120. doi:10.1093/bioinformatics/btu170.

Bollaerts, K., M. Aerts, C. Faes, K. Grijspeerdt, J. Dewulf, and K. Mintiens. 2008. Human salmonellosis: Estimation of dose-illness from outbreak data. RISK ANALYSIS. 28:427–440. doi:10.1111/j.1539-6924.2008.01038.x.

Bolyen, E., J. R. Rideout, M. R. Dillon, N. A. Bokulich, C. C. Abnet, G. A. Al-Ghalith, H. Alexander, E. J. Alm, M. Arumugam, F. Asnicar, Y. Bai, J. E. Bisanz, K. Bittinger, A. Brejnrod, C. J. Brislawn, C. T. Brown, B. J. Callahan, A. M. Caraballo-Rodríguez, J. Chase, E. K. Cope, R. Da Silva, C. Diener, P. C. Dorrestein, G. M. Douglas, D. M. Durall, C. Duvallet, C. F. Edwardson, M. Ernst, M. Estaki, J. Fouquier, J. M. Gauglitz, S. M. Gibbons, D. L. Gibson, A. Gonzalez, K. Gorlick, J. Guo, B. Hillmann, S. Holmes, H. Holste, C. Huttenhower, G. A. Huttley, S. Janssen, A. K. Jarmusch, L. Jiang, B. D. Kaehler, K. B. Kang, C. R. Keefe, P. Keim, S. T. Kelley, D. Knights, I. Koester, T. Kosciolek, J. Kreps, M. G. I. Langille, J. Lee, R. Ley, Y.-X. Liu, E. Loftfield, C. Lozupone, M. Maher, C. Marotz, B. D. Martin, D. McDonald, L. J. McIver, A. V. Melnik, J. L. Metcalf, S. C. Morgan, J. T. Morton, A. T. Naimey, J. A. Navas-Molina, L. F. Nothias, S. B. Orchanian, T. Pearson, S. L. Peoples, D. Petras, M. L. Preuss, E. Pruesse, L. B. Rasmussen, A. Rivers, M. S. Robeson, P. Rosenthal, N. Segata, M. Shaffer, A. Shiffer, R. Sinha, S. J. Song, J. R. Spear, A. D. Swafford, L. R. Thompson, P. J. Torres, P. Trinh, A. Tripathi, P. J. Turnbaugh, S. Ul-Hasan, J. J. J. van der Hooft, F. Vargas, Y. Vázquez-Baeza, E. Vogtmann, et al. 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol. 37:852–857. doi:10.1038/s41587-019-0209-9.

Brown, T. R., and T. E. Lawrence. 2010. Association of liver abnormalities with carcass grading performance and value. Journal of Animal Science. 88:4037–4043. doi:10.2527/jas.2010-3219.

Callahan, B. J., P. J. McMurdie, M. J. Rosen, A. W. Han, A. J. A. Johnson, and S. P. Holmes. 2016. DADA2: High-resolution sample inference from Illumina amplicon data. Nat Methods. 13:581–583. doi:10.1038/nmeth.3869.

Carneiro, M. R. P., P. H. Cabello, R. L. C. Albuquerque-Junior, S. Jain, and A. L. Candido. 2015. Characterization of a foodborne outbreak caused by Salmonella Enteritidis in Aracaju, State of Sergipe, Brazil. Rev Soc Bras Med Trop. 48:334–337. doi:10.1590/0037-8682-0260-2014.

Cazer, C. L., E. R. B. Eldermire, G. Lhermie, S. A. Murray, H. M. Scott, and Y. T. Grohn. 2020. The effect of tylosin on antimicrobial resistance in beef cattle enteric bacteria: a systematic review and meta-analysis. Prev Vet Med. 176:104934. doi:10.1016/j.prevetmed.2020.104934.

CDC. 2023. Salmonellosis and Ground Beef | DFWED | CDC. Available from: https://www.cdc.gov/ncezid/dfwed/prevention-priorities/salmonella-and-ground-beef.html.

CDC. 2022. Centers for Disease Control and Prevention. Illnesses and Germs. Available from: https://www.cdc.gov/foodsafety/foodborne-germs.html.

Checkley, S. L., E. D. Janzen, J. R. Campbell, and J. J. McKinnon. 2005. Efficacy of vaccination against Fusobacterium necrophorum infection for control of liver abscesses and footrot in feedlot cattle in western Canada. Can Vet J. 46:1002–1007.

Chen, H., R. H. L. Chiang, and V. C. Storey. 2012. Business Intelligence and Analytics: From Big Data to Big Impact. MIS Quarterly. 36:1165–1188. doi:10.2307/41703503.

Davedow, T., C. Narvaez-Bravo, R. Zaheer, H. Sanderson, A. Rodas-Gonzalez, C. Klima, C. W. Booker, S. J. Hannon, A. L. Bras, S. Gow, and T. McAllister. 2020. Investigation of a Reduction in Tylosin on the Prevalence of Liver Abscesses and Antimicrobial Resistance in Enterococci in Feedlot Cattle. Frontiers in Veterinary Science. 7. Available from: https://www.frontiersin.org/articles/10.3389/fvets.2020.00090.

DeSantis, T. Z., P. Hugenholtz, N. Larsen, M. Rojas, E. L. Brodie, K. Keller, T. Huber, D. Dalevi, P. Hu, and G. L. Andersen. 2006. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Appl Environ Microbiol. 72:5069–5072. doi:10.1128/AEM.03006-05.

Dewell, G. A., C. J. Rademacher, and Y. Sato. 2022. Review of regulations and indications for the use of in-feed antimicrobials for production animals. Journal of the American Veterinary Medical Association. 260:S129–S132. doi:10.2460/javma.22.07.0300.

Dodd, C. C., D. G. Renter, X. Shi, M. J. Alam, T. g. Nagaraja, and M. W. Sanderson. 2011. Prevalence and Persistence of Salmonella in Cohorts of Feedlot Cattle. Foodborne Pathogens and Disease. 8:781–789. doi:10.1089/fpd.2010.0777.

Doster, E., S. M. Lakin, C. J. Dean, C. Wolfe, J. G. Young, C. Boucher, K. E. Belk, N. R. Noyes, and P. S. Morley. 2020. MEGARes 2.0: a database for classification of antimicrobial drug, biocide and metal resistance determinants in metagenomic sequence data. Nucleic Acids Res. 48:D561–D569. doi:10.1093/nar/gkz1010.

Eastwood, L. C., C. A. Boykin, M. K. Harris, A. N. Arnold, D. S. Hale, C. R. Kerth, D. B. Griffin, J. W. Savell, K. E. Belk, D. R. Woerner, J. D. Hasty, R. J. Delmore Jr., J. N. Martin, T. E. Lawrence, T. J. McEvers, D. L. VanOverbeke, G. G. Mafi, M. M. Pfeiffer, T. B. Schmidt, R. J. Maddock, D. D. Johnson, C. C. Carr, J. M. Scheffler, T. D. Pringle, and A. M. Stelzleni. 2017. National Beef Quality Audit-2016: Transportation, mobility, and harvest-floor assessments of targeted characteristics that affect quality and value of cattle, carcasses, and by-products1. Translational Animal Science. 1:229–238. doi:10.2527/tas2017.0029.

Edrington, T. S., T. M. Arthur, G. H. Loneragan, K. J. Genovese, D. L. Hanson, R. C. Anderson, and D. J. Nisbet. 2020. Evaluation of two commercially-available Salmonella vaccines on Salmonella in the peripheral lymph nodes of experimentally-infected cattle. Therapeutic Advances in Vaccines and Immunotherapy. 8:2515135520957760. doi:10.1177/2515135520957760.

Eng, S.-K., P. Pusparajah, N.-S. Ab Mutalib, H.-L. Ser, K.-G. Chan, and L.-H. Lee. 2015. Salmonella: A review on pathogenesis, epidemiology and antibiotic resistance. Frontiers in Life Science. 8:284–293. doi:10.1080/21553769.2015.1051243.

Evans, M. R., S. M. Parry, and C. D. Ribeiro. 1995. Salmonella outbreak from microwave cooked food. Epidemiol Infect. 115:227–230. doi:10.1017/s0950268800058350.

Fenske, G., J. Pouzou, R. Pouillot, D. Taylor, S. Costard, and F. Zagmutt. 2023.The genomic and epidemiological virulence patterns of *Salmonella enterica* serovars. medRxiv. doi: https://doi.org/10.1101/2022.12.13.22283417.

Flach, M. G., O. B. Dogan, W. M. Kreikemeier, K. K. Nightingale, and M. M. Brashears. 2022. Reduction of Pathogens in Feces and Lymph Nodes Collected from Beef Cattle Fed Lactobacillus salivarius (L28), Lactobacillus acidophilus (NP51) and Propionibacterium freudenreichii (NP28), Commercially Available Direct-Fed Microbials. Foods. 11:3834. doi:10.3390/foods11233834.

Forrester, L. J., B. J. Campbell, J. N. Berg, and J. T. Barrett. 1985. Aggregation of platelets by Fusobacterium necrophorum. J Clin Microbiol. 22:245–249.

FSIS. 2021. Performance Standards Salmonella Verification Program for Raw Poultry Products | Food Safety and Inspection Service. Available from: http://www.fsis.usda.gov/policy/fsis-directives/10250.2.

FSIS. 2019. Changes to the Salmonella Verification Testing Program: Proposed Performance Standards for Salmonella in Raw Ground Beef and Beef Manufacturing Trimmings and Related Agency Verification Procedures. 2019. Federal Register. Available from: https://www.federalregister.gov/documents/2019/10/28/2019-23473/changes-to-the-salmonella-verification-testing-program-proposed-performance-standards-for-salmonella.

Fuerniss, L. K., K. K. Kreikemeier, L. D. Reed, M. D. Cravey, and B. J. Johnson. 2022. Cecal microbiota of feedlot cattle fed a four-species Bacillus supplement. Journal of Animal Science. 100:skac258. doi:10.1093/jas/skac258.

Getabalew, M., T. Alemneh, and D. Zewdie. 2020. Types and Uses of Growth Promoters in Beef Cattle. 3:1–5.

González, L. A., X. Manteca, S. Calsamiglia, K. S. Schwartzkopf-Genswein, and A. Ferret. 2012. Ruminal acidosis in feedlot cattle: Interplay between feed ingredients, rumen function and feeding behavior (a review). Animal Feed Science and Technology. 172:66–79. doi:10.1016/j.anifeedsci.2011.12.009.

Goto, H., A. Q. Qadis, Y. H. Kim, K. Ikuta, T. Ichijo, and S. Sato. 2016. Effects of a bacterial probiotic on ruminal pH and volatile fatty acids during subacute ruminal acidosis (SARA) in cattle. J Vet Med Sci. 78:1595–1600. doi:10.1292/jvms.16-0211.

Grant, R.J., Albright, J.L., 1995. Feeding behaviour and management factors during the transition period in dairy cattle. J. Anim. Sci. 73, 2791–2803. Harb, M.Y., Reynolds, V.S., Campling, R.C., 1985. Eating behavior, social dominance and voluntary intake of silage in group-fed milking cattle. Grass Forage Sci. 40, 113–118.

Grispoldi, L., M. Karama, C. Hadjicharalambous, F. de Stefani, G. Ventura, M. Ceccarelli, M. Revoltella, P. Sechi, C. Crotti, A. D'Innocenzo, G. Couto-Contreras, and B. Cenci-Goga. 2020. Bovine lymph nodes as a source of *Escherichia coli* contamination of the meat. International Journal of Food Microbiology. 331:108715. doi:10.1016/j.ijfoodmicro.2020.108715.

Guillier, L., C. Danan, H. Bergis, M. Delignette-Muller, S. Granier, S. Rudelle, A. Beaufort, and A. Brisabois. 2013. Use of quantitative microbial risk assessment when investigating foodborne illness outbreaks: The example of a monophasic Salmonella Typhimurium 4,5,12:i:- outbreak implicating beef burgers. International Journal of Food Microbiology. 166:471–478. doi:10.1016/j.ijfoodmicro.2013.08.006.

Hara-Kudo, Y., H. Konuma, Y. Kamata, M. Miyahara, K. Takatori, Y. Onoue, Y. Sugita-Konishi, and T. Ohnishi. 2013. Prevalence of the main food-borne pathogens in retail food under the national food surveillance system in Japan. Food Additives and Contaminants Part A- Chemistry Analysis Control Exposure & Risk Assesment. 30:1450–1458. doi:10.1080/19440049.2012.745097.

Hara-Kudo, Y., and K. Takatori. 2011. Contamination level and ingestion dose of foodborne pathogens associated with infections. Epidemiology and Infection. 139:1505–1510. doi:10.1017/S095026881000292X.

Hennessy, T. W., C. W. Hedberg, L. Slutsker, K. E. White, J. M. Besser-Wiek, M. E. Moen, J. Feldman, W. W. Coleman, L. M. Edmonson, K. L. MacDonald, and M. T. Osterholm. 1996. A national outbreak of Salmonella enteritidis infections from ice cream. The Investigation Team. N Engl J Med. 334:1281–1286. doi:10.1056/NEJM199605163342001.

Herrick, R. T., C. L. Rogers, T. J. McEvers, R. G. Amachawadi, T. G. Nagaraja, C. L. Maxwell, J. B. Reinbold, and T. E. Lawrence. 2022. Exploratory observational quantification of liver abscess incidence, specific to region and cattle type, and their associations to viscera value and bacterial flora. Applied Animal Science. 38:170–182. doi:10.15232/aas.2021-02228.

Horton, B. C., K. B. Gehring, J. E. Sawyer, and A. N. Arnold. 2021. Evaluation of Autogenous Vaccine Use in Mitigating Salmonella in Lymph Nodes from Feedlot Cattle in Texas. J Food Prot. 84:80–86. doi:10.4315/JFP-20-171.

Hundy, R. L., and S. Cameron. 2002. An outbreak of infections with a new Salmonella phage type linked to a symptomatic food handler. Commun Dis Intell Q Rep. 26:562–567.

Jaborek, J., 2023. Industry concerns with liver abscesses in finishing cattle. Michigan State University Extension. Available from: https://www.canr.msu.edu/news/industry-concerns-with-liver-abscesses-in-finishing-cattle.

Kasuga, Fumiko, M. Hirota, M. Wada, T. Yunokawa, H. Toyofuku, M. Shibatsuji, H. Michino, T. Kuwasaki, S. Yamamoto, and S. Kumagai. 2004. Archiving of Food Samples from Restaurants and Caterers—Quantitative Profiling of Outbreaks of Foodborne Salmonellosis in Japan. Journal of Food Protection. 67:2024–2032. doi:10.4315/0362-028X-67.9.2024.

Latimer, H. K., L.-A. Jaykus, R. A. Morales, P. Cowen, and D. Crawford-Brown. 2001. A Weighted Composite Dose – Response Model For Human Salmonellosis. Risk Analysis. 21:295–306. doi:10.1111/0272-4332.212112.

Laufer, A. S., J. Grass, K. Holt, J. M. Whichard, P. M. Griffin, and L. H. Gould. 2015. Outbreaks of Salmonella infections attributed to beef --United States, 1973-2011. Epidemiol Infect. 143:2003–2013. doi:10.1017/S0950268814003112.

Lecuyer, P., J. Diego, D. Murphy, E. Trovillion, M. Jones, D. Sahm, and V. Fraser. 1996. Nosocomial outbreak of gastroenteritis due to Salmonella senftenberg. Clinical Infectious Diseases. 23:734–742. doi:10.1093/clinids/23.4.734.

Lehmacher, A., J. Bockemuhl, and S. Aleksic. 1995. Nationwide outbreak of human salmonellosis in Germany due to contaminated paprika and paprika-powdered potato chips. Epidemiology and Infection. 115:501–511. doi:10.1017/S0950268800058660.

Li, H., and R. Durbin. 2010. Fast and accurate long-read alignment with Burrows-Wheeler transform. Bioinformatics. 26:589–595. doi:10.1093/bioinformatics/btp698.

Lozupone, C., M. E. Lladser, D. Knights, J. Stombaugh, and R. Knight. 2011. UniFrac: an effective distance metric for microbial community comparison. ISME J. 5:169–172. doi:10.1038/ismej.2010.133.

Mackey, S. J., R. F. Cooke, E. A. Colombo, A. T. Pickett, L. F. D. Batista, E. Block, and A. P. Brandão. 2023. Supplementing pre- and probiotic ingredients to feedlot steers: effects on health, growth performance, and physiological responses. animal. 17:100770. doi:10.1016/j.animal.2023.100770.

Mansilla, F. I., C. A. Ficoseco, M. H. Miranda, E. Puglisi, M. E. F. Nader-Macías, G. M. Vignolo, and C. A. Fontana. 2022. Administration of probiotic lactic acid bacteria to modulate fecal microbiome in feedlot cattle. Sci Rep. 12:12957. doi:10.1038/s41598-022-16786-z.

Matsui, T., S. Suzuki, H. Takahashi, T. Ohyama, J. Kobayashi, H. Izumiya, H. Watanabe, F. Kasuga, H. Kijima, K. Shibata, and N. Okabe. 2004. Salmonella Enteritidis outbreak associated with a school-lunch dessert: cross-contamination and a long incubation period, Japan, 2001. Epidemiology and Infection. 132:873–879. doi:10.1017/S0950268804002778.

McCullough, N. B., and C. W. Eisele. 1951a. Experimental Human Salmonellosis: III. Pathogenicity of Strains of Salmonella Newport, Salmonella Derby, and Salmonella Bareilly Obtained from Spray-Dried Whole Egg. The Journal of Infectious Diseases. 89:209–213. doi:10.1093/infdis/89.3.209.

McCullough, N., and C. W. Eisele. 1951b. Experimental human salmonellosis. IV. Pathogenicity of strains of Salmonella pullorum obtained from spray-dried whole egg. J Infect Dis. 89:259–265. doi:10.1093/infdis/89.3.259.

McMurdie, P. J., & Holmes, S. (2013). phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PloS one, 8(4), e61217.

Meehan, P., T. Atkeson, D. Kepner, and M. Melton. 1992. A Foodborne Outbreak of Gastroenteritis Involving 2 Different Pathogens. American Journal of Epidemiology. 136:611–616. doi:10.1093/oxfordjournals.aje.a116539.

Molossi, F. A., B. S. de Cecco, L. C. Henker, T. P. Vargas, M. P. Lorenzett, M. V. Bianchi, C. D. Lorenzo, L. Sonne, D. Driemeier, and S. P. Pavarini. 2021. Epidemiological and pathological aspects of salmonellosis in cattle in southern Brazil. Cienc. Rural. 51:e20200459. doi:10.1590/0103-8478cr20200459.

Morgan, D., S. Mawer, and P. Harman. 1994. The Role of Home-made Ice-cream as a Vehicle of Salmonella-enteritidis Phage Type-4 Infection from Fresh Shell Egg. Epidemiology and Infection. 113:21–29. doi:10.1017/S0950268800051426.

Müller, H. C., C. L. Van Bibber-Krueger, O. J. Ogunrinu, R. G. Amachawadi, H. M. Scott, and J. S. Drouillard. 2018. Effects of intermittent feeding of tylosin phosphate during the finishing period on feedlot performance, carcass characteristics, antimicrobial resistance, and incidence and severity of liver abscesses in steers1. Journal of Animal Science. 96:2877–2885. doi:10.1093/jas/sky166.

Murase, T., M. Yamada, T. Muto, A. Matsushima, and S. Yamai. 2000. Fecal excretion of Salmonella enterica serovar Typhimurium following a food-borne outbreak. Journal of Clinical. 38:3495–3497. doi:10.1128/JCM.38.9.3495-3497.2000.

Nabbut, N. H., E. K. Barbour, H. M. Al-Nakhli, and S. I. Zamel. 1982. A Food Illness Outbreak Caused by Salmonella muenster. J Food Prot. 45:23–25. doi:10.4315/0362-028X-45.1.23.

Nagaraja, T. G., and M. M. Chengappa. 1998. Liver abscesses in feedlot cattle: a review. Journal of Animal Science. 76:287–298. doi:10.2527/1998.761287x.

Nagaraja, T. G., and E. C. Titgemeyer. 2007. Ruminal Acidosis in Beef Cattle: The Current Microbiological and Nutritional Outlook1, 2. Journal of Dairy Science. 90:E17–E38. doi:10.3168/jds.2006-478.

Napoleoni, M, L. Villa, L. Barco, L. Busani, V. Cibin, C. Lucarelli, A. Tiengo, A. Dionisi, F. Conti, F. Nunes, L. Tantucci, M. Staffolani, V. Silenzi, R. Fraticelli, B. Morandi, G. Blasi, E. Rocchegiani, and S. Fisichella. 2021. A Strong Evidence Outbreak of Salmonella Enteritidis in Central Italy Linked to the Consumption of Contaminated Raw Sheep Milk Cheese. Microorganisms. 9. doi:10.3390/microorganisms9122464.

Nickodem, C., A. N. Arnold, K. B. Gehring, J. J. Gill, J. T. Richeson, K. L. Samuelson, H. M. Scott, J. K. Smith, T. M. Taylor, J. Vinasco, and K. N. Norman. 2023. A Longitudinal Study on the Dynamics of Salmonella enterica Prevalence and Serovar Composition in Beef Cattle Feces and Lymph Nodes and Potential Contributing Sources from the Feedlot Environment. Applied and Environmental Microbiology. 89:e00033-23. doi:10.1128/aem.00033-23.

Oscar, T. 2004. Dose-Response Model for 13 Strains of Salmonella. Risk Analysis. 24:41–49. doi:10.1111/j.0272-4332.2004.00410.x.

Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Szoecs E, Wagner H (2018) vegan: Community Ecology Package. R package version 2.5-2.

Paulson, J. N., O. C. Stine, H. C. Bravo, and M. Pop. 2013. Robust methods for differential abundance analysis in marker gene surveys. Nat Methods. 10:1200–1202. doi:10.1038/nmeth.2658.

Pinnell, L. J., G. Kuiper, K. L. Huebner, E. Doster, J. K. Parker, N. Alekozai, J. G. Powers, R. L. Wallen, K. E. Belk, and P. S. Morley. 2023. More than an anthropogenic phenomenon: Antimicrobial resistance in ungulates from natural and agricultural environments. Science of The Total Environment. 858:159789. doi:10.1016/j.scitotenv.2022.159789.

Pinnell, L. J., C. W. Whitlow, K. L. Huebner, T. C. Bryant, J. Martin, K. E. Belk, and P. S. Morley. 2022a. Not All Liver Abscesses Are Created Equal: The Impact of Tylosin and Antibiotic Alternatives on Bovine Liver Abscess Microbial Communities and a First Look at Bacteroidetes-Dominated Communities. Front Microbiol. 13:882419. doi:10.3389/fmicb.2022.882419.

Pinnell, L. J., and P. S. Morley. 2022b. The Microbial Ecology of Liver Abscesses in Cattle. Veterinary Clinics of North America: Food Animal Practice. 38:367–381. doi:10.1016/j.cvfa.2022.08.004.

Reinhardt, C. D., and M. E. Hubbert. 2015. Control of liver abscesses in feedlot cattle: A review11Contribution no. 10-205-J from the Kansas Agric. Exp. Stn., Manhattan 66506. The Professional Animal Scientist. 31:101–108. doi:10.15232/pas.2014-01364.

Rezac, D. J., D. U. Thomson, S. J. Bartle, J. B. Osterstock, F. L. Prouty, and C. D. Reinhardt. 2014. Prevalence, severity, and relationships of lung lesions, liver abnormalities, and rumen health scores measured at slaughter in beef cattle1. Journal of Animal Science. 92:2595–2602. doi:10.2527/jas.2013-7222.

Roberts, M. C. 2008. Update on macrolide-lincosamide-streptogramin, ketolide, and oxazolidinone resistance genes. FEMS Microbiol Lett. 282:147–159. doi:10.1111/j.1574-6968.2008.01145.x.

Saginala, S., T. G. Nagaraja, K. F. Lechtenberg, M. M. Chengappa, K. E. Kemp, and P. M. Hine. 1997. Effect of Fusobacterium necrophorum leukotoxoid vaccine on susceptibility to experimentally induced liver abscesses in cattle1. Journal of Animal Science. 75:1160–1166. doi:10.2527/1997.7541160x.

Samuel, J. L., J. A. Eccles, and J. Francis. 1981. Salmonella in the intestinal tract and associated lymph nodes of sheep and cattle. Epidemiology & Infection. 87:225–232. doi:10.1017/S0022172400069448.

Samuel, J. L., D. A. O'Boyle, W. J. Mathers, and A. J. Frost. 1980a. The Contamination with Salmonella of Bovine Livers in an Abattoir. Australian Veterinary Journal. 56:526–528. doi:10.1111/j.1751-0813.1980.tb02579.x.

Samuel, J. L., D. A. O'Boyle, W. J. Mathers, and A. J. Frost. 1980b. Distribution of Salmonella in the carcases of normal cattle at slaughter. Research in Veterinary Science. 28:368–372. doi:10.1016/S0034-5288(18)32724-3.

Silliker, J. H. 1980. Status of Salmonella - Ten Years Later. J Food Prot. 43:307–313. doi:10.4315/0362-028X-43.4.307.

Soto-Navarro, S.A., Duff, G.C., Krehbiel, C.-R., Galyean, M.L., Malcom-Callis, K.J., 2000. Influence of feed intake fluctuation, feeding frequency, time of feeding and rate gain on performance by limit-fed steers. Prof. Anim. Sci. 16, 13–20.

Tan, Z. L., T. G. Nagaraja, and M. M. Chengappa. 1994. Selective enumeration of Fusobacterium necrophorum from the bovine rumen. Appl Environ Microbiol. 60:1387–1389.

Taylor, D. N., I. K. Wachsmuth, Y. H. Shangkuan, E. V. Schmidt, T. J. Barrett, J. S. Schrader, C. S. Scherach, H. B. McGee, R. A. Feldman, and D. J. Brenner. 1982. Salmonellosis associated with marijuana: a multistate outbreak traced by plasmid fingerprinting. N Engl J Med. 306:1249–1253. doi:10.1056/NEJM198205273062101.

Teunis, P. F. M., F. Kasuga, A. Fazil, I. D. Ogden, O. Rotariu, and N. J. C. Strachan. 2010. Dose-response modeling of Salmonella using outbreak data. Int J Food Microbiol. 144:243–249. doi:10.1016/j.ijfoodmicro.2010.09.026.

Thomas, M., M. Webb, S. Ghimire, A. Blair, K. Olson, G. J. Fenske, A. T. Fonder, J. Christopher-Hennings, D. Brake, and J. Scaria. 2017. Metagenomic characterization of the effect of feed additives on the gut microbiome and antibiotic resistome of feedlot cattle. Sci Rep. 7:12257. doi:10.1038/s41598-017-12481-6.

USDA. 2022. Announces Action to Declare Salmonella an Adulterant in Breaded Stuffed Raw Chicken Products. Available from: https://www.usda.gov/media/press-releases/2022/08/01/usda-announces-action-declare-salmonella-adulterant-breaded-stuffed.

USDA ERS. 2021. Economic Cost of Major Foodborne Illnesses Increased $2 Billion From 2013 to 2018. Available from: https://www.ers.usda.gov/amber-waves/2021/april/economic-cost-of-major-foodborne-illnesses-increased-2-billion-from-2013-to-2018/.

U.S. Food and Drug Administration. 2019. CVM GFI #213 New Animal Drugs and New Animal Drug Combination Products Administered in or on Medicated Feed or Drinking Water of Food-Producing Animals: Recommendations for Drug Sponsors for Voluntarily Aligning Product Use Conditions with GFI #209. U.S. Food and Drug Administration. Available from: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/cvm-gfi-213-new-animal-drugs-and-new-animal-drug-combination-products-administered-or-medicated-feed.

Uyeno, Y., S. Shigemori, and T. Shimosato. 2015. Effect of Probiotics/Prebiotics on Cattle Health and Productivity. Microbes Environ. 30:126–132. doi:10.1264/jsme2.ME14176.

Vipham, J. L., G. H. Loneragan, L. M. Guillen, J. C. Brooks, B. J. Johnson, A. Pond, N. Pond, and M. M. Brashears. 2015. Reduced Burden of Salmonella enterica in Bovine Subiliac Lymph Nodes Associated with Administration of a Direct-fed Microbial. Zoonoses Public Health. 62:599–608. doi:10.1111/zph.12187.

Vought, K. J., and S. R. Tatini. 1998. Salmonella enteritidis contamination of ice cream associated with a 1994 multistate outbreak. J Food Prot. 61:5–10. doi:10.4315/0362-028x-61.1.5.

Webb, H. E., D. M. Brichta-Harhay, M. M. Brashears, K. K. Nightingale, T. M. Arthur, J. M. Bosilevac, N. Kalchayanand, J. W. Schmidt, R. Wang, S. A. Granier, T. R. Brown, T. S. Edrington, S. D. Shackelford, T. L. Wheeler, and G. H. Loneragan. 2017. Salmonella in Peripheral Lymph Nodes of Healthy Cattle at Slaughter. Frontiers in Microbiology. 8. Available from: https://www.frontiersin.org/articles/10.3389/fmicb.2017.02214.

Werber, D., J. Dreesman, F. Feil, U. van Treeck, G. Fell, S. Ethelberg, A. M. Hauri, P. Roggentin, R. Prager, I. S. T. Fisher, S. C. Behnke, E. Bartelt, E. Weise, A. Ellis, A. Siitonen, Y. Andersson, H. Tschäpe, M. H. Kramer, and A. Ammon. 2005. International outbreak of Salmonella Oranienburg due to German chocolate. BMC Infect Dis. 5:7. doi:10.1186/1471-2334-5-7.

Weinroth, M. D., J. N. Martin, E. Doster, I. Geornaras, J. K. Parker, C. R. Carlson, J. L. Metcalf, P. S. Morley, and K. E. Belk. 2019. Investigation of tylosin in feed of feedlot cattle and effects on liver abscess prevalence, and fecal and soil microbiomes and resistomes. J Anim Sci. 97:4567–4578. doi:10.1093/jas/skz306.

Weinroth, M. D., H. M. Scott, B. Norby, G. H. Loneragan, N. R. Noyes, P. Rovira, E. Doster, X. Yang, D. R. Woerner, P. S. Morley, and K. E. Belk. 2018. Effects of Ceftiofur and Chlortetracycline on the Resistomes of Feedlot Cattle. Applied and Environmental Microbiology. 84:e00610-18. doi:10.1128/AEM.00610-18.

Wickham H (2016). ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York. ISBN 978-3-319-24277-4, https://ggplot2.tidyverse.org.

Wileman, B. W., D. U. Thomson, C. D. Reinhardt, and D. G. Renter. 2009. Analysis of modern technologies commonly used in beef cattle production: Conventional beef production versus nonconventional production using meta-analysis. Journal of Animal Science. 87:3418–3426. doi:10.2527/jas.2009-1778.

Zhang, Z., L. Yang, Y. He, X. Luo, S. Zhao, and X. Jia. 2021. Composition of Fecal Microbiota in Grazing and Feedlot Angus Beef Cattle. Animals (Basel). 11:3167. doi:10.3390/ani11113167.

APPENDIX

PUB MED Search String:
https://pubmed.ncbi.nlm.nih.gov/?term=%28%22Salmonella%22%29+AND+%28%22outbreak%22+OR+%22foodborne+illness%22%29+AND+%28%22exposure%22+OR+%22infected%22+OR+%22dose%22+OR+%22ill%22%29+NOT+%28%22pet%22+OR+%22zoonoses%22+OR+%22resistance%22%29&filter=lang.english&size=200

Web of Science Search String:
https://www.webofscience.com/wos/woscc/summary/83a89770-398f-492f-8d3e-c624a39203ce-54e09714/relevance/1

CAB Direct Search String:
https://www.cabdirect.org/cabdirect/search/?q=(%22Salmonella%22)%20AND%20(%22outbreak%22%20OR%20%22foodborne%20illness%22)%20AND%20(%22exposure%22%20OR%20%22infected%22%20OR%20%22dose%22%20OR%20%22ill%22)%20NOT%20(%22pet%22%20OR%20%22zoonoses%22%20OR%20%22resistance%22)