# ASSOCIATION OF LIVER ABSCESS PRESENCE AND EPITHELIAL INTEGRITY OF THE HINDGUT IN FEEDLOT CATTLE TO *SALMONELLA* CARRIAGE IN SUBILIAC LYMPH NODES

by

Chandler Ann Dockray

A Thesis Submitted in Partial Fulfillment

of the Requirements for the Degree

MASTER OF SCIENCE

Major Subject: Animal Science

West Texas A&M University

Canyon, Texas

December 2022

## ABSTRACT

Salmonellosis is the leading cause of bacteria foodborne illness in the United States with over 1 million illnesses annually (Scallan et al., 2015). *Salmonella* colonizes in the intestines and stress can cause disruptions of the tight junctions (Boyle et al., 2006) allowing for infection of other tissues such as the lymph nodes and liver (Ring, 1985). The objective of this study was to quantify the prevalence and concentration of *Salmonella* in liver, colon, and subiliac lymph nodes (SLN). Feedlots (*n*=6) were surveyed, from the Texas Panhandle, quarterly across one year for differences in management. Liver (*n*=8), colon (*n*=8), and SLN (*n*=16) were sampled from cattle from each feedlot, quarterly. Liver samples were organized into severely abscessed (*n*=4) and edible (n=4). Colon samples were organized into #1 score (*n*=4) and #2 score (*n*=4) colons; categorization was based on the integrity of the epithelial cell layer, #1 colon layers resembled a very ridged surface and #2 colon surfaces were free of ridges and smooth. Weather data was collected from the Texas Tech University Mesonet. *Salmonella* was uncommon in the winter months of quarter 1 (1.70% prevalence) and quarter 2 (0% prevalence). In the warmer months, *Salmonella* prevalence increased dramatically with quarter 3 having 90.63% prevalence and quarter 4 having 20.45% prevalence. Overall, the greatest prevalence of *Salmonella* was in SLN from carcasses with a #2 colon (27.59%); the lowest prevalence of *Salmonella* was in the tissue from edible livers (18.75%). The greatest concentration of *Salmonella* was detected in #2

colons (2.16 logCFU/g); the lowest prevalence of *Salmonella* was detected in edible liver tissue (0.1logCFU/g). Feedlot B had the highest average prevalence of *Salmonella* (29.35%) whereas Feedlot A had the lowest average prevalence (17.19%) among all quarters and sample types. Of the liver and colon samples, roughly 19% of samples were positive for *Salmonella* whereas 25% of the SLN samples were positive for *Salmonella.* The relative risk of a SLN being positive for *Salmonella* when associated with a #1 colon or #2 colon was 0.77 *(P* = 0.37) and 0.71 *(P* = 0.22), respectively. The relative risk of a SLN being positive for *Salmonella* when associated with an edible or abscessed liver was 0.75 (*P* = 0.30) and 0.83 (*P* = 0.49), respectively. We hypothesize, samples taken during quarter 3 demonstrated the greatest prevalence of *Salmonella* likely due to an increase in precipitation and temperature. These data suggest that *Salmonella* proliferation is strongly associated to local climatic conditions. This would suggest that as temperature and precipitation increase during warmer months strategies need to be developed to minimize *Salmonella*. Though *Salmonella* is of notable risk in lymph nodes, these data illustrate other edible products such as the liver are also of concern.

**APPROVAL**

Approved:

_____

Dr. Travis Tennant, Chairman, Thesis Committee Date

_____

Dr. Ty Lawrence, Member, Thesis Committee Date

_____

Dr. Loni Lucherk, Member, Thesis Committee Date

_____

Dr. Tyson Brown, Member, Thesis Committee Date

_____

Dr. Kevin Pond, Dean Department of Agricultural Science Date

_____

Dr. Lance Keith, Head Department of Agricultural Science Date

_____

Dr. Angela Spaulding, Dean, Graduate School Date

## ACKNOWLEDGEMENTS

I first would like to thank my parents for supporting me and all the many prayers they have sent up on my behalf. I wouldn't be where I am or the person I am without both of you.

I would like to thank my committee members Dr. Travis Tennant, Dr. Ty Lawrence, Dr. Loni Lucherk, and Dr. Tyson Brown for their guidance and knowledge during my time at WTAMU. Thank you for allowing me to find my true passion.

Thank you to my BCRC family for being by my side and my helping hand throughout my entire study. I truly wouldn't have been able to complete it without you all.

This thesis is dedicated to my Papa David. His genes run deep and I wouldn't have the passion for meat science without the dedication he had to this family. I'm thankful for the memories and love our family has been able to share about him.

## Table of Contents

LIST OF TABLES ..... viii
LIST OF FIGURES ..... ix

Chapter 1 ..... 1
Introduction ..... 1

Chapter 2 ..... 5
Review of Literature ..... 5
2.1 *Salmonella* ..... 5
2.1.2 Non-Typhoid Salmonella ..... 6
2.1.3 Typhoid Salmonella ..... 7
2.1.4 Serotypes ..... 8
2.2 CRISPR ..... 9
2.3 Contamination ..... 11
2.3.2 Feed Contamination ..... 11
2.3.3 Fecal Contamination ..... 12
2.3.4 Shedding Factors ..... 13
2.3.5 Fly Contamination ..... 14
2.3.6 Hide Contamination ..... 15
2.3.7 Bird Contamination ..... 16
2.4 Routes of Infection ..... 17
2.4.2 Liver Prevalence ..... 18
2.4.3 Intestinal Prevalence ..... 19
2.4.4 Lymph Node Contamination ..... 20
2.5 Time of Year Influence on *Salmonella* ..... 21
2.6. Location Influence on *Salmonella* ..... 22
2.7 Intervention Strategies ..... 23
2.8 Conclusion ..... 24

2.9 Literature Cited ..... 26

Chapter 3 ..... 39

3.1 Introduction ..... 39

3.2 Materials and Methods ..... 42

3.2.2 Feedlot survey ..... 42

3.2.3 Sample collection ..... 43

3.3 *Salmonella* Prevalence and Enumeration ..... 44

3.3.2 Sample processing ..... 44

3.3.2 Salmonella culture ..... 45

3.4 Results ..... 46

3.4.2 Receiving cattle management and type of cattle received ..... 46

3.4.3 Capacity and pen management ..... 48

3.4.4 Ration ingredients and adjustments ..... 48

3.4.5 Pest management ..... 49

3.4.6 Weather ..... 50

3.4.7 Feedlot prevalence ..... 50

3.4.8 Sample prevalence ..... 51

3.4.9 Concentration ..... 52

3.5 Discussion ..... 52

3.6 Conclusion ..... 56

3.7 Literature Cited ..... 58

## LIST OF TABLES

**Table 3.1.** Different observations and outcomes among different feedlots …..……..65

**Table 3.2.** Type of cattle fed by each feedlot...……...……..…66

**Table 3.3.** Prevalence of *Salmonella* by feedlot and quarter with the inclusion of all sample types...……...…….……67

## LIST OF FIGURES

**Figure 3.1.** Capacity of each feedlot by head of cattle in comparison to the total prevalence of *Salmonella* of each feedlot..……...…….…...68

**Figure 3.2.** Prevalence of sample type by day of collection……...69

**Figure 3.3.** Average temperature taken six days prior to harvest and day of harvest for each feedlot by quarter……...70

**Figure 3.4.** Total precipitation taken six days prior to harvest and day of harvest for each feedlot by quarter……...71

**Figure 3.5.** Concentration and prevalence of *Salmonella* across different samples type for inclusion of the entire study……...……72

**Figure 3.6.** Sample type prevalence variability based on average temperature taken six days prior to sampling and day of sampling……..……73

**Figure 3.7.** Sample type prevalence variability based on average precipitation taken six days prior to sampling and day of sampling……..……74

**Figure 3.8.** Sample type prevalence variability based on average relative humidity taken six days prior to sampling and day of sampling.……...75

# Chapter 1

## Introduction

Foodborne illnesses are a societal burden that have been reported to cause 600 million cases and 420,000 deaths, globally. In the United States, *Salmonella* is responsible for roughly 1 million of the 1.2 million illnesses that are attributed to food (Scallan et al., 2015; Forgey et al., 2020). Symptoms associated with salmonellosis in humans are commonly fever, diarrhea, abdominal cramps, nausea, vomiting, and headaches. After infection, symptoms usually initiate between six hours to six days and last for four to seven days (CDC-NORS, 2018). Beef is responsible for approximately 4% of *Salmonella* outbreaks each year in the United States (Laufer et al., 2015; CDC-NORS, 2018). *Salmonella* is a gram negative, rod-shaped bacteria that is most commonly found growing anaerobically (Andino and Hanning, 2014), but has also been reported to cultivate aerobically. Temperatures of 7 to 48 ºC, and pH levels of 4 to 8 are environments that *Salmonella* can proliferate in (Baird-Parker et al., 1990). *Salmonella* belongs to the Enterobacteriaceae family and is divided into two species, enterica and bonogori (Brenner et al., 2000; Gutema et al., 2019). The two species are divided because *S.* bonogori is primarily found in cold-blooded vertebrates (Fierer and Guiney, 2001). *S.*

enterica has six subspecies that include enterica, salamea, arisonae, diarizonae, houtenae, and indica (Uzzau et al., 2000; Gal-More et al., 2014). Within those six subspecies, approximately 2,600 serotypes exist (Andino and Hanning, 2014; Gal-More et al., 2014). These serotypes are distinguished from one another by carbohydrates, lipopolysaccharides, and flagella structures (Fierer and Guiney, 2001; Coburn et al., 2006). *S.* enterica is a pathogen known to cause two forms of disease: typhoid and non-typhoid (NT) *salmonellosis*. There are approximately 80.3 million cases of foodborne illnesses caused by NT *Salmonella* (Gutema et al., 2019). Primarily, NT *Salmonella* has a broad host range and frequently infects immune-compromised individuals. All NT serotypes, aside from a few, have the ability to infect humans and cause bacterial diarrhea (Gutema et al., 2019). The large range of hosts is a contributing factor to non-typhoid *Salmonella* serovars ability to regularly cause illness in individuals. The most common NT *Salmonella* seryotypes, *S.* Enteritidis and *S.* Typhimurium infects 2.6 per 100,000 and 1.5 per 100,000 individuals, respectively (Tack et al., 2019). Typhoid *Salmonella* is human-specific and the origin of enteric fever by infection of S. Typhi or Paratyphi (Gal-More et al., 2014). Enteric fever, a result of typhoid *Salmonella*, has been reported to cause 27 million cases globally that resulted in over 200,000 deaths of humans (Crump et al., 2004). *Salmonella* commonly colonizes in the intestines of humans and animals, which is why foods that are sourced from animals can be the origin of *Salmonella* illnesses in humans. Cattle can become infected with *Salmonella* from other cattle, the environment, and other animals such as birds, insects, and rodents (Chomel et al., 2007), and the most common route of infection is via fecal-oral (Ly and Casanoca, 2007). Once infected cattle have the ability to continue that cycle of contamination by shedding,

*Salmonella* and infecting the environment (Ring, 1985; Xu et al., 2017; Gutema et al., 2019). Shedding of *Salmonella* into the environment can taint fertilized crops that are used in rations (Himathongkham et al., 1999) and hides of cattle which can then lead to carcass contamination during harvest (Hanlon et al., 2018). *Salmonella* that is infecting soil, water, pests, and feedstuffs are another source from which cattle can become infected (Jones et al., 1982; Ring et al., 1985). Though infection of *Salmonella* via fecal-oral route is the most common, but infection can occur transdermally (Xu et al., 2017) and intranasally (Fedorka-Cray et al., 1995). When *Salmonella* is present in the environment many vectors can become the source from which can lead to prevalence in cattle. The source can come from the environment, other cattle, birds, and pests (Hanson et al., 2020). The presence of *Salmonella* is most often seen in environments that have a dryer (Sofos et al., 1999) and warmer climate (Fedorka-Cray et al., 1998). When *Salmonella* is present in the environment many vectors can become the source that can lead to prevalence in cattle. Infected cattle have shown positive prevalence in edible liver and liver abscesses (Amachawadi and Nagaraja, 2015), along with intestinal (Boyle et al., 2006) and lymph node prevalence (Hanlon et al., 2016). The prevalence of *Salmonella* within the body is able to avoid defense mechanisms by the host because of the presence of pathogenicity islands that are responsible for encoding virulence factors (Galan, 1999; Wang et al., 2020). *Salmonella* prevalence in the lymph nodes is of importance because they are encased in fat tissue which has the potential to go into beef trimmings that are used to produce ground beef products (Arthur et al., 2008). In 2021, FSIS reported that 1.53% of all ground beef samples test positive for quantification of *Salmonella* at retail. Preventative measures such as vaccinations, antimicrobials, and trying to control source

of entry have been some of the strategies implemented to try and control *Salmonella* at the feedlot level (Buncic and Sofos, 2012). Current in-plant interventions focus on surface contamination, because *Salmonella* can be harbored in lymph nodes, there isn't success of decreasing the burden of *Salmonella* only the burden of *E. coli* 0157:H7 (Aurther et al., 2008; Haneklaus et al., 2012).

# Chapter 2

## Review of Literature

### *Salmonella*

Salmonella is a member of the Enterobacteriaceae family and is a non-spore forming, rod shaped, and gram-negative bacteria. Growth of Salmonella is optimal in environments at 37 ºC, pH 7, and $a_w$ of a minimum of 0.93. Survival of Salmonella can be observed in environments of 5 ºC to 47 ºC, pH as low as .05, and survival increases as $a_w$ decreases (Adams and Moss, 2007). Salmonella is most commonly spread via the fecal-oral route and is recognized as an infection when the bacteria have been ingested and are located within the distal small intestine (Ly and Casanoca, 2007). Once an animal is infected, the bacteria are able to penetrate the epithelium of the intestine and then spread to the spleen and liver. Salmonella has a unique pathogenic property that allows entry into cells that normally have phagocytic functions. This particular ability enables Salmonella bacteria to engage with deeper tissues or avoid defense mechanism of the host organism. This is achieved by genetic loci that are clustered in centisome 63 of the chromosome (Galan, 1996). Genomes of Salmonella have many clusters of genes that are referred to as Salmonella pathogenicity islands (SPIs) which are responsible for encoding virulence factors, SPI-1 and SPI-2 (Galan, 1999; Ly and Casanoca, 2007; Wang et. al., 2020;). Both SPIs are responsible for encoding of type III secretion systems (T3SS) which are made up of many proteins and allow for the proteins to be secreted through a multi-membrane envelope, this permits bacterial virulence factors to be transmitted into

the plasma of the host cell (Galan, 1999; Galan and Wolf-Watz, 2006). It has also been reported that Peyer's patches and M cells are a site of escape for the bacteria (Carter and Collins, 1974; Vazquez-Toress and Fang, 2000). Peyer's patches are anatomical features in the mucosa of the small intestines and are secondary immune organs the contain follicles of B lymphocytes and T lymphocyte areas. M cells are located within the follicle-associated epithelium of Peyer's patches (De Lau et. al., 2012). Carter and Collins (1974) reported that mice which were intragastrically infected that Salmonella reached the lower intestinal tract more quickly than the small intestines or the stomach and remained in larger numbers. The bacteria was also viable which they contributed to the systemic infection that was a result from penetration through the intestinal mucosa.

*Non-Typhoid Salmonella*

When compared to six other leading foodborne pathogens, non- typhoidal (NT) *Salmonella* was the primary cause of hospitalization and death (Scallan et al., 2015). Typhimurium and Enteritidis are the ubiquitous NT serovars of S. enterica (Gal-More et al., 2014) and are tied to 50% of cases that are reported globally. There have been 9.4 million infections caused by NT *Salmonella,* and 80.3 million of those cases are of foodborne origin (Gutema et al., 2019). Both S. Enteritidis and Typhimurium are unrestricted serotypes that are able to cause disease in a wide range of host species (Uzzua et al., 2000). NT *Salmonella* serotypes are observed to more vigorously and resiliently colonize in the intestines than typhoidal serotypes. Because NT *Salmonella* is more durable in the intestinal tract, inflammation develops and this prolongs infection (Hohmann, 2001). Gastroenteritis is commonly the result of NT *Salmonella*, but in Sub-

Saharan Africa, NT *Salmonella* invasive disease is being associated with patients (Gal-More et al., 2014). Gastroenteritis is seen in 95% of NT *Salmonella* cases and invasive disease is seen up to 5%. S. Typhimurium is the primary cause of invasive disease cases (20-25%) (Gal-More et al., 2014) and is the most frequent cause of gastroenteritis in humans (Ibarra and Steel-Mortimer, 2009). Symptoms of NT *Salmonella* usually appear 6 to 12 hours after ingestion and these symptoms may last for 10 days (Glynn and Palmer, 1992). S. Enteritidis is the most common serotype that is associated with foodborne illnesses and outbreaks and is primarily associated with poultry products. S. Enteritidis is known to infect the ovaries of hens asymptomatically and contaminate eggs (Guard-Petter, 2001).

*Typhoid Salmonella*

Common typhoid serovars of S. enterica are serotypes Typhi, Sendai, and Paratyphi A, B, or C (de Jong et al., 2012).Typhoid *Salmonella* is the cause of enteric fever and has been reported to cause 27 million cases globally that result in over 200,000 deaths in humans (Crump et al., 2004). S. Typhi causes typhoid fever and paratyphoid fever is caused by S. Paratyphi. These strains of *Salmonella* only live in humans and are commonly observed in developing countries (Gal-More et al., 2014). This serotype of *Salmonella* is host restrictive and is associated with a particular species (Uzzau et al., 2000). In the United States, about 350 people are diagnosed with typhoid fever and 90 people are diagnosed with paratyphoid fever every year, however, worldwide typhoid fever affects 11 to 21 million people and paratyphoid fever affects about 5 million people per year. Both diseases cause the same types of symptoms with an addition of a cough and rash. The effects of typhoid *Salmonella* are frequently seen in the developing

countries from inadequate sanitation techniques, lack of clean water, and it is spread via the fecal-oral route (Gal-More et al., 2014). *Salmonella* Typhi is the most common contributing agent of enteric fever (Hohmann, 2001).

*Serotypes*

Differentiating of *Salmonella* is based on the serotype's somatic (O), flagellar antigens (H), and capsular antigens (Vi) (Adams and Moss, 2007). Because, *Salmonella* occurs naturally in the intestinal tract of ruminant animals and is more commonly NT *Salmonella* (Forgey et al., 2020). In the literature, there are discrepancies about what *Salmonella* serotype is the most common in cattle. Wray and Davies (2000) state that S. Typhimurium and Dublin are the most isolated serovars from cattle, whereas Fedorka-Cray et al., (1998) reported in feedlot cattle that S. Anatum was most frequently found. Most serotypes that are in cattle are non-hosted adapted (Rings, 1985). Richardson (1975) reported that S. Newport was the most common disease when looking at other serotypes other than S. Typhimurium and Dublin, however, when considering S. Typhimurium and Dublin, S. Dublin was reported to be the predominant *Salmonella* serotype. When cattle are infected with S. Typhimurium, it frequently causes enteritis in young calves that results in diarrhea. S. Dublin can result in abortions in cows and shedding is also seen in healthy cattle that are both young and adult in age (Costa et al., 2012). Young calves are more likely to become infected with *Salmonella* (Gutema et al., 2019) because of the low pH levels of the abomasum (Ring, 1985).

## CRISPR

Because *Salmonella* enterica has more than 2,600 serovars that have different routes of transmission, host colonization and different abilities of resistance to antimicrobials (Thompson et al., 2018), and S. enterica subspecies enterica accounts for the majority of salmonellosis clinical cases, it is important to be able to identify the different serovars that are associated within a single outbreak or clinical case (Shariat et al., 2015). The S. enterica subspecies enterica comprises more than 1,500 serovars that can lead to human and animal illnesses. It is important to know which serovars pose a risk to human health such as, serovars Enteritidis and Typhimurium, and those that are only restricted to certain species such as, serovar Gallinmarum that is avian-restricted and poses little risk to human health (Thompson et al., 2018). *Salmonella* serotyping begins with determining two surface antigens, O-polysaccharide and flagellin proteins, which can recognize beyond 2,500 serotypes (Fabre et al., 2012). *Salmonella* is pre-enriched; once enriched in a medium, the following cultures are steaked onto an agar and 1 to 2 $H_2S$-positive *Salmonella* colonies are selected for confirmation of *Salmonella*. This process is able to identify the most abundant strain. (Thompson et al., 2018). Repeated elements in bacteria are called clustered regularly interspaced short palindromic repeat (CRISPR)-Cas systems and are detected in bacterial genomes. CRISPR is a family of DNA sequences and consist of a succession of 24 to 47 bp repeated sequences (or direct repeats), however, Jansen et al. (2002) reported that direct repeats can vary in size from 21 to 37 bp, that are separated by sequences of spacers. The length and position of the sequences vary and can be unique for a single strain (Jansen et al., 2002). The unique component of the CRISPR-Cas system is the variability of the spacer sequences that are

separated by direct repeat sequences (Thompson et al., 2018). CRISPR spacer content was strongly correlated with serotype and subtype and is an alternative for both serotyping and pulsed-field gel electrophoresis methods (Fabre et al., 2012). Serotyping *Salmonella* is done by sequencing of the CRISPR loci, two distinct CRISPR were found in chromosomes of *Salmonella*, CRISPR1 and CRISPR2 (Shariat et al., 2015; Thompson et al., 2018; Fabre et al., 2012). CRISPR1 locus is located between genes *cysD-cysJ*, down from *iapF* gene, and CRISPR 2 is located up from the *cysJ-ygcF* gene, *ygcF* gene, in genomes of *Salmonella* (Touchon and Rocha, 2010; Fabre et al., 2012). Cas genes are functional domains that are responsible for the propagation and functioning of CRISPR. CRISPR is assessed by identifying different locations and characteristics (Touchon and Rocha, 2010). Thompson et al. (2018) combined two different *Salmonella* serovars, S. Enteritidis and Kentucky, at different proportions and serovar Kentucky was able to be detected when it only accounted for 0.01% of the sample. These serovars are able to be distinguished from one another by using CRISPRFinder to identify the CRISPR spacers. Fabre et al. (2012) observed a difference in insertion sequence, the orientation of the cas3 gene, and deletion at the direct repeats of CRISPR1 when comparing different structures that corresponded in *Salmonella* genomes. CRISPR1 and CRISPR2, in the same study, were separated by less than 20 kb in the genomes of S. enterica and S. bongori. CRISPR is able to detect multiple serovars in a single sample, this is an important factor due to the fact the 94% of samples contain more than one *Salmonella* serotype (Thompson et al., 2018).

## Contamination

### *Feed Contamination*

Two routes that cattle can potentially become infected, experimentally, are oral ingestion of the pathogen and inoculation of the ileal ligated loop (Watson et al., 1995; Costa et al., 2012). Naturally, cattle can become infected with *Salmonella* orally through the environment because *Salmonella* can be detected in the soil, water, fecal matter, other animals, pests, and feedstuffs (Jones et al., 1982; Ring, 1985). Jones et al. (1982) isolated S. Mbandaka from cattle in three dairy farms and it was determined that feed containing vegetable fat supplement was the source of the contamination from isolating the organism from unopened bags of the supplement. It is also important in making sure that an adequate diet is fed to cattle so there isn't an unbalanced intestinal flora that favors the growth of *Salmonella*. Chambers and Lysons (1979) tested the effects of volatile fatty acids, lactate concentrations, and pH level on the growth of *Salmonella*. It was observed that high concentrations of volatile fatty acids and low pH levels decreased the prevalence of *Salmonella*. Reports also showed that after a period of starvation the conditions in the rumen were high pH levels and low volatile fatty acid levels which would favor *Salmonella*. Indication of the importance of ration and bunk management and the role the rumen plays in the fate of *Salmonella*. If the balance in the rumen is off this allows for organisms to pass to the intestine, causing clinical disease and shedding that infects other animals.

*Fecal Contamination*

Feces are a common source from which *Salmonella* serotypes are isolated (Ring, 1985; Xu et al., 2017; Gutema et al., 2019). Cattle have the capability of shedding 102 to 107 CFU of *Salmonella* per gram of feces. This is a human risk because of the use of manure as crops (Himathongkham et al., 1999) and fecal contamination of the environment onto the hides of animals, and contamination of the carcass if there is poor sanitation during harvest (Forgey et al., 2020). *Salmonella* has the ability to survive in fecal matter of cattle anywhere from 131 days to 30 months (Ring, 1985). Himathongkham et al. (1999) did a survival and prevalence test of *Salmonella* in cow manure at different temperature ranges (4ºC, 20ºC, and 37ºC) and from different levels of the fecal sample (top, middle, and bottom). In the top layer, *Salmonella* increased until day three and then decreased by six log CFU on day 48 and *Salmonella* had better survival rates at 20ºC. In goats and lambs, fecal samples tested positive 10.3% of the time for *Salmonella* in goats and 11.4% in lambs. Hide samples for lambs were detected at 25.5% whereas only 3.3% of goat hides were positive for *Salmonella* (Hanlon et al., 2018).

The prevalence of *Salmonella* was tested and compared between the two. Lot-fed cattle had a 6.8% detection rate whereas cattle that were grass-fed had 4.5% detection, but this was not significant to detect a difference (Fegan et al., 2004). It is difficult to tell if cattle are shedding *Salmonella* in their feces because healthy asymptomatic cattle can be actively shedding *Salmonella* serotypes and contaminating the environment (Fitzgerald et al., 2003; Gutema et al., 2019).

*Shedding Factors*

There are many factors that can affect the amount of shedding of *Salmonella* that is observed in fecal samples. Fitzgerald et al. (2003) observed that different stressors that cattle go through, depending upon the stage of production, can influence the amount of fecal shedding of *Salmonella* that is isolated, specifically in dairy cattle. Multiparous cows that were lactating shed more *Salmonella* than cattle that were lactating but were primiparous and cows that were less than 60 days in milk of their lactation status shed more *Salmonella* than cows that were greater than 60 days. Fitzgerald et al. (2003) did not observe that heat stress influences the amount of shedding that occurs but Fedorka-Cray et al. (1997) observed that samples that were collected during August had prevalence rates that were higher than samples collected in October through December. Transportation of cattle can also increase *Salmonella* prevalence rates (Dewell et al., 2008). Also, days on feed influences shedding of *Salmonella*; cattle had 3.5% of samples test positive when on feed for a shorter amount of time (8 days) and 7.4% of samples came back positive for cattle that were fed for a longer period of time (180 days). In contrast, Frost et al. (1988) observed a decrease in *Salmonella* that were feed for longer than 80 days. Cattle that were fed for a shorter amount of time may have had a lower percentage of samples come back positive for *Salmonella* than those fed for a longer period of time. Cattle that were transported from farms in Tennessee to feedlots in Texas, had *Salmonella* prevalence increase from 0.0%-1.5% to 8.0% after 30 days at the feedlot (Corrier et al., 1990). The increase of *Salmonella* shedding would indicate that the cattle were already infected with *Salmonella* prior to transportation, however, the transport stress-induced *Salmonella* growth. During transportation, cattle can contaminate hides

and the trailer with infected feces and also the beef processor once unloaded (Dewell et al., 2008). Traveling and holding animals increases the chance of infection and the risk for other animals to become infected (Samuel et al., 1980).

*Fly Contamination*

Environmental factors such as flies and other pests contributes to the spread of *Salmonella*. Xu et al. (2017) captured flies from 33 farms that had cattle (50 flies per farm). Of all flies that were captured (n = 1650), *Salmonella* was isolated 11% of the time. Farms had prevalence rates as low as 0% to 78% and 79% of the farms were known to have carried *Salmonella*. It has been well published that fecal matter of cattle is a rich source of *Salmonella* and most have observed flies in fecal matter. Thomson et al. (2019) detected an increase of *Salmonella* in house flies when they were exposed to inoculated manure, the increase of *Salmonella* on the house flies was observed on the external surface of their bodies, and within the anterior and mid regions of their guts at 4 h after exposure. Because flies feed on the manure of cattle, flies carry off *Salmonella* from manure, it then attaches to the exterior portion of the fly's body. This leads to contamination, by the infected flies, of food or water sources that cattle might consume. Flies can not only contaminate the environment around the cattle but also directly into the cattle by biting. Flies that harbor *Salmonella* introduce contamination transdermally from biting on the ventral abdomen (Mian et al., 2002). Lesions, from fly bites, can be found on hides of cattle. Black spots, pits, and scars are some of the damage observed from cattle hides from cattle that have never been treated for fly infestation. Black spots appear to contain abnormal amounts of pigment in those areas, there can be not only hide damage, but the underlying tissue may be spongy and damaged. Pits are indentions in the

hide and can leave open lesions. Scars are evidence of past damage that have now healed but in some cases, there is spongy tissue beneath showing further damage to the animal than just at hide level (Guglielmone et al., 1999).

*Hide Contamination*

Hides can also become infected with *Salmonella* through fecal matter, the same as flies are. Hide contamination is of concern because it leads to contamination of the carcass as the animal is processed (Hanlon et al., 2018). Hides of cattle can become contaminated by infected pen mates during transportation and in holding pens due to the close proximity of those animals. Cattle that have to be transported for longer distances (>160.9 km) have twice the risk of having a positive *Salmonella* hide sample at slaughter compared to cattle that are transported shorter distances (Dewell et al., 2008). Koohmaraie et al. (2012) obtained hide samples that were 96% positive for *Salmonella* while the hide was still on the carcass; after the hide was removed positive carcass *Salmonella* samples dropped to 47%. The 47% prevalence was before antimicrobial interventions had taken place, and after interventions, 7.14% of trim samples from the same carcasses, tested positive for *Salmonella*. This indicates that hide contamination is a contributor to *Salmonella* in ground beef. Interventions for carcasses, which have been contaminated at the beef processing plant or before, can include multiple carcass washes that are established before and after hide removal including hot water and lactic acid. Hides can be contaminated at the plant from the knock box and holding pens. Small et al. (2006) reported 10% of samples from the holding pens from 5 different abattoirs tested positive for *Salmonella*, and 7.8% of samples taken at the knock box tested positive for *Salmonella*. Forgey et al. (2020) took samples from multiple processors that harvested

sheep and reported that 2.5% lactic acid application or 200 ppm of chlorinated hide wash influenced the reduction of *Salmonella* and Shiga toxin-producing *Escherichia coli*. *Salmonella* decreased fivefold on carcasses with proper sanitary practices.

*Bird Contamination*

Birds are also a source of *Salmonella* because they are able to harbor zoonotic infectious diseases (Chomel et al., 2007). Many different factors contribute to the ways that birds become infected with *Salmonella* and the way that they infect humans and livestock. Humans are able to directly acquire *Salmonella* from birds or indirectly by livestock that have been infected through bird contamination (Alley et al., 2002). Andres et al. (2012) observed different factors that might influence prevalence rates of *Salmonella* in birds that were housed near pigs that were known to have high *Salmonella* prevalence. It was observed from fecal samples (n = 810) that birds found close to the pigs had a higher rate of positive samples than those that were found off the location. In addition, migratory birds had fewer positive *Salmonella* samples than non-migratory birds. Results also showed that the bird's diet influenced the probability of finding positive samples. Birds that would be considered herbivorous had lower positive *Salmonella* samples than birds with diets that would be more carnivorous. This correlates with Molina-Lopez et al. (2011) in which raptor species of birds had a higher probability of having *Salmonella* because they feed upon and consume animals that have already died and may have had a *Salmonella* infection. Birds that are infected with *Salmonella* excrete the pathogen through feces (Connolly et al., 2006; Molina-Lopez et al., 2011). This fecal material can contaminate the environment including feed and water sources and *Salmonella* is frequently transmitted through the fecal-oral route (Luque et al., 2009).

Pennycott et al. (2006) and Molina-Lopez et al. (2011) reported that S. Typhimurium was the most common serotype that was cultured from different species of birds. In the raptor birds, that fecal samples were collected from, there was a higher prevalence of multidrug-resistant *Salmonella* isolates (Molina-Lopez et al., 2011). Pregnant ewes were studied when 16% of the ewes had complications within the last third of their gestation and experienced abortions, stillbirths, and premature lambs. The outbreak of abortions was associated with the *Salmonella* serotype Indiana. *S*. Indiana that was isolated from the liver and intestinal tract of pigeons was found to have a genetic relationship to the *Salmonella* that was found to cause complications in the ewes. The ewes became infected by the birds through fecal contamination. The ewes were treated with enrofloxacin; enrofloxacin is a used to treat bacterial infections which controlled the outbreak and was also used as a preventative in the ewes. Along with antibiotic use, covered water and feeding troughs were also put into place (Luque et al., 2009).

**Routes of Infection**

Cattle can become infected with *Salmonella* transdermally through fly bites (Mian et al., 2002), by the oral-fecal route through ingestion (Gal-More et al., 2014), and airborne transmission (Kallapura et al., 2014). Airborne transmission of *Salmonella* is able to infect animals via dust or bacterial aerosols. Bacterial aerosols and dust contamination has been linked to the litter in broiler sheds. Management practices did not seem to have an impact on *Salmonella* in dust, litter, or aerosols (Chinivasagam et al., 2009). However, Berrang et al. (1995) observed that fans in hatching incubators and cabinets can be the cause of *Salmonella* spread. In chicks that were intratracheally challenged with *Salmonella*, they showed colonization of the ceca-cecal tonsils within 24

hours of challenge, and the liver, spleen, and trachea all became compromised with *Salmonella*, as well. It also took very low doses (100 cells) of *Salmonella* for the chickens to become infected with *Salmonella* when chickens were dosed via the respiratory route. Pigs were used to study the different routes of *Salmonella* infection via esophagotomy, intranasal, transthoracic, and tonsil explant inoculation. The study showed that animals are able to become infected with *Salmonella* through the tonsils, the lungs, and nasal cavities. Pigs that were esophagotomized and transthoracically challenged had at least one positive sample of *Salmonella* in each of the tissues that were tested. Even though *Salmonella* is regularly thought to enter the animal via the fecal-oral route, this study provides evidence that infection can occur by other routes (Fedorka-Cray et al., 1995). Of cattle that were intradermally inoculated with *Salmonella* in the legs of the animals, all of the peripheral lymph nodes resulted in positive *Salmonella* results. *Salmonella* was detected in the superficial cervical and popliteal lymph nodes after 2, 4, 6, and 8 days following inoculation from cattle that the right foreleg and right hind leg had been the sources of inoculation, respectively, as well as the popliteal lymph node from the inoculation of the left hind leg. However, no subiliac superficial cervical lymph nodes were detected to be *Salmonella*-positive (Edrington et al., 2013).

### *Liver Prevalence*

*Salmonella* can be detected in edible liver and liver abscesses (Vieira-Pinto et al., 2011; Amachawadi and Nagaraja, 2015; Im et al., 2015). *Salmonella* is able to enter into the liver after it has been colonized in the small intestines and spreads to the lymphatics and blood stream by phagocytes, then ultimately to the liver and other organs (Wang et. al., 2020). Liver abscesses, in beef cattle, are most commonly a result of feeding higher

starch and lower roughage diets than cattle would naturally consume. Bacteria from the ruminal flora is the primary agent, with *Fusobacterium necrophorum* and *Actinomyces pyogenes* being the most isolated, respectively (Nagaraja and Chengappa, 1998). Amachawadi and Nagaraja, (2015) reported the second most isolated bacteria isolated, *Actinomyces,* was renamed *Truperella*. In the same study, *Salmonella* was first reported to be isolated from 100% of the 10 liver abscesses that were collected. This might have contributed to *Salmonella* being present in the gut and crossing over the epithelial barrier where it then could enter portal circulation and then lead to liver infection by the portal capillary system (Nagaraja and Lechtenberg, 2007). In another study, *Salmonella* was isolated in 20 to 25% of liver abscesses and serotype *S.* Lubbock was the most commonly cultured (Amachawadi et al., 2016). Herrick et al. (2022) isolated *Salmonella enterica* from liver abscess samples. Abscess samples collected from processors of fed beef and cull cow had *Salmonella enterica* present in 27.5% and 16.5% of samples, respectively. Liver abscess prevalence also differed among different processor types. Incidence of liver abscess was greater in fed beef processing facilities (20.3%) than for cull for cull beef processing facilities (17.6%). However, the greatest abscess incidence was observed in fed Holsteins (25.0%).

### *Intestinal Prevalence*

*Salmonella* can infect the intestines and have the potential to escape by disrupting the tight junctions that are located at the interface between epithelial cells (Boyle et al., 2006). When individuals become infected via fecal-oral route, it has the ability to contaminate the intestines and in turn breaks down the tight junctions. Tight junctions are where cells join together to form a semi-permeable barrier and are most often found at

epithelial cells. This function can breakdown and allow for *Salmonella* infection and symptoms such as diarrhea (Sousa et al., 2005). Type 3 secretion system of the SPI-1 is what the tight junction disruptions are dependent on. More specifically, SPI-1 secreted effectors SopB, SopE, SopE2, and SipA are responsible for membrane invasion during infections of *Salmonella* (Boyle et al., 2006). The disruption of the tight junction is of importance because it seals the epithelial cell layers and regulates the passage of different cell types (Wang et al., 2020)

### *Lymph Node Contamination*

*Salmonella* is transmitted to animals in multiple routes but also found in many different tissues. The most concerning internal tissue that *Salmonella* is found is in the lymph node due to the number of lymph nodes that are in the body and the amount that goes into ground beef (Haneklaus et al., 2012; Hanlon et al., 2016). Lymph nodes of both cull and fed cattle have been observed to harbor *Salmonella*. These lymph nodes were found in the flank, chuck and flank. Chuck and flank region lymph nodes are of more importance because they are encased in fat and often go into beef trimmings that become ground beef, unlike mesenteric lymph nodes that are taken out during evisceration (Arthur et al., 2008). Samuel et al. (1980) isolated *Salmonella* in 76 out of 100 cattle. In the animals, *Salmonella* was isolated from 54 of the mesenteric nodes and 62 samples of rumen contents but ruminal lymph nodes only came back with 2 positive samples, suggesting that the intestines are a source of entry for *Salmonella*. Tonsils are thought to be the primary route of infection for *Salmonella* to travel to the superpharyngeal lymph nodes (Fedorka-Cray et al., 1995). *Salmonella* that is ingested orally has to pass through the four stomachs before it enters the intestines where the ileum and cecum allow for

penetration of the pathogen to infect other body tissues, including liver, lymph nodes, kidney, spleen, and saliva (Ring, 1985). Penetration of *Salmonella* may due to the fact that stress decreases the integrity of the intestines (Wray and Davies, 2000).

**Time of Year Influence on *Salmonella***

Different times of the year can influence the prevalence rates of *Salmonella*. Alley et al. (2002) reported the months that S. Typhimurium was isolated from sparrow bird mortalities. The highest prevalence rates were observed to be in the months of September to December, which are the summer and spring months for New Zealand. Dairy cattle were observed to shed *Salmonella* more in the months of May to July than February (Wells et al., 2001). In a feedlots located in three different regions of the United States, all regions had samples with higher *Salmonella* prevalence in August than in October through December (Fedorka-Cray et al., 1998). Sofos et al. (1999) observed an increase of *Salmonella*-positive samples collected from feedlot steer and heifers when collected during the dry season (May to June), and a decrease in prevalence during the wet season (November to January). For fed cattle, the percentage of *Salmonella*-positive samples before evisceration was higher during the dry season when compared to the wet season. A majority of the time the contamination was highest during the warmer months and lower in the colder months but there was an increase in some of the colder months (January) and a decrease in the middle of the warmer months (July) (Hanson et al., 2020). Nickelson et al. (2019) did not observe a difference between cattle from Mexico or U.S. origin, but there was a difference in prevalence rates of *Salmonella* in cattle that were sampled in cool and warm seasons. Cool season samples were 46.5% positive and warm

season samples were 57.5% positive for *Salmonella*. These trends for *Salmonella*-positive samples show the impact of the environment on prevalence rates.

### Location Influence on *Salmonella*

Location of cattle and the prevalence rates of *Salmonella* have also been correlated. Wells et al. (2001) reported that within the United States, dairy cattle are highest in prevalence in the south region, with 45% of dairies having a positive *Salmonella* sample. The lowest prevalence was from the Northeast region of the United States. Fedorka-Cray et al. (1998) sampled different regions within the United States, region 1 (AZ, CA, ID, WA), region 2 (IA, IL, MN, SD), and region 3 (CO, KS, OK, NE, TX). The highest prevalence of *Salmonella* was recovered from region 3 with 6.9% of the samples positive, whereas 0.3% and 2.2% of the samples were positive from regions 2 and 3, respectively. The prevalence of *Salmonella* can also differ among continents. Gutema et al. (2019) compiled data from every continent, except Antarctica. Prevalence was the lowest in Europe (2%) and highest in North America (16%). These varied results are due to the number of publications from which cattle samples were analyzed. North America had 26 publications and 33,577 cattle samples, while Europe had 8 publications and 6,470 cattle samples. Nickelson et al. (2019) sampled lymph nodes from cattle of Mexico and United States origin, to see if the prevalence of *Salmonella* differed from cattle of different origin. Prevalence rate of lymph nodes in Mexican origin cattle did not differ from those of U.S. origin cattle, with prevalence rates of 54.0% and 50.0%, respectively.

## Intervention Strategies

With *Salmonella* contamination of the carcass and in lymph nodes, which may transfer into the meat, many interventions have been put into place to try and control *Salmonella*. Some of these interventions are utilized before the animals get to the beef processor. Feedlots use antimicrobials, vaccines, controlling environmental factors that are a source of entry for *Salmonella*, and other strategies. Interventions at the beef processor level include separation of clean and dirty areas, disinfecting regimes of equipment and workers, and decontamination treatments of the carcass (Buncic and Sofos, 2012). Horton et al. (2021) observed the effects of an autogenous vaccination given at the stocker phase only, feedlot only, and given to cattle at both phases. Cattle that received the autogenous vaccination did not have any lymph nodes that tested positive for *Salmonella* and the cattle that didn't receive the vaccination had 5% of the lymph nodes come back positive for *Salmonella*. However, *Salmonella* vaccinations are used in less than 6% of feedlots (USDA-APHIS, 2010) and only 10% of dairies (USDA-APHIS, 2009). Cutting out lymph nodes could be an intervention, however, there are numerous lymph nodes encased in fat and to remove them, especially at a commercial level, is cost prohibitive (Arthur et al., 2008; Haneklaus et al., 2012; Gragg et al., 2013). Cross-contamination prevention in processing plants is the main reason for the contamination of carcasses from the hide. A 4.5 fold reduction has been observed for hide decontamination treatments of warm NaOH in commercial processing plants (Arthur et al., 2008). Carcasses can become infected at the processing plant in the holding pens, stunning box, and roll-out ramp (Small et al., 2006). Implementing proper practices during the harvest process of sheep was seen to decrease cross-contamination by fivefold,

these results were seen when the plant was tested before and after employees went through proper training. In the same processing plant, a 16-fold decrease of *Salmonella* prevalence was observed on hides when a 2.5% lactic acid spray was applied when compared to those before the 2.5% lactic acid spray (Forgey et al., 2020). Chilling has various factors that can contribute to controlling *Salmonella*. Low temperature, relative humidity, airspeed, and water activity changes during chilling can cause stress and mortality. Chilling of ruminant carcasses to a temperature of at least 7ºC can suppress the growth of the pathogen (Buncic and Sofos, et al., 2012). Direct-fed microbials have an impact on cattle that shed *Salmonella*. This pre-harvest intervention is easier to implement into regimens because it can be incorporated into the diet of the cattle. Stephens et al. (2007) reported a reduction of *Salmonella* concentration shedding when Lactobacillus acidophilus NP51 was fed in high doses to cattle before slaughter. This direct-fed microbial not only decreased *Salmonella* but also feedlot performance characteristics such as weight gain and feed-to-gain ratio. These results show that at every stage of production there can be intervention strategies to help reduce the isolation rate of *Salmonella* in meat. Typhoid infections of *Salmonella* in human patients are usually treated with ampicillin, chloramphenicol, and fluoroquinolones. Fluoroquinolones, chloramphenicol, and oxytetracycline are commonly used to treat non-typhoid infections. However, both types of infection are able to develop drug resistance by destroying the activity of the drug via plasmids (Wang et al., 2020).

## Conclusion

*Salmonella* is one of the leading foodborne pathogens in the world today. *Salmonella* is an important pathogen because of its ability to infect many different

species and the different methods of infection. *Salmonella* is detected in many different environments and has the ability to affect cattle in multiple stages of production. Ingestion of the bacteria is the most common way that individuals become infected. S. Typhimurium is a common serotype that can cause illnesses in humans and also is the common serotype found in cattle. Cattle and other animals can shed *Salmonella* through their feces and can shed without symptoms. Feces can contaminate the water, feed, and hide. If cattle ingest these, infections of the intestines can escape and enter the lymph nodes. These lymph nodes are encased in fat and may go into trimmings for ground beef which can lead to illnesses in humans. Feces aren't the only source of *Salmonella*; flies, birds, feed ingredients, and water sources are a few ways that *Salmonella* can transfer into the body. Intervention strategies have been put into place to help lower the rate of *Salmonella* in cattle before harvest and during harvest. Intervention practices have been shown to reduce *Salmonella*. This shows that pre-and post-harvest intervention technologies are critical to human health.

## Literature Cited


Adams, M. R., and Moss, M. O. (2007). *Food Microbiology.* (3rd ed.).

Alley, M. R. , Connolly, J. H., Fenwick, S. G., Mackereth, G. F., Leyland, M. J., Rogers, L. E., Haycock, M., Nicol, C., & Reed, C. E. M. (2002). An epidemic of salmonellosis caused by *Salmonella* Typhimurium DT160 in wild birds and humans in New Zealand. *New Zealand Veterinary Journal*, *50*(5), 170–176. https://doi.org/10.1080/00480169.2002.36306

Amachawadi, R. G., and Nagaraja, T. G. (2015). First Report of Anaerobic Isolation of *Salmonella* enterica from Liver Abscesses of Feedlot Cattle. *Journal of Clinical Microbiology*, *53*(9), 3100–3101. https://doi.org/10.1128/JCM.01111-15

Amachawadi, R. G., Thomas, M., Nagaraja, T. G., & Scaria, J. (2016). Genome Sequences of *Salmonella* enterica subsp. enterica Serovar Lubbock Strains Isolated from Liver Abscesses of Feedlot Cattle. *Genome Announcements (Washington, DC)*, *4*(3). https://doi.org/10.1128/genomeA.00319-16

Andrés, S., Vico, J. P., Garrido, V., Grilló, M. J., Samper, S., Gavín, P., Herrera-León, S., & Mainar-Jaime, R. C. (2013). Epidemiology of Subclinical Salmonellosis in Wild Birds from an Area of High Prevalence of Pig Salmonellosis: Phenotypic and Genetic Profiles of *Salmonella* Isolates. *Zoonoses and Public Health*, *60*(5), 355-365. https://doi.org/10.1111/j.1863-2378.2012.01542.x

Arthur, T. M., Brichta-Harhay, D. M., Bosilevac, J. M., Guerini, M. N., Kalchayanand, N., Wells, J. E., Shackelford, S. D., Wheeler, T. L., & Koohmaraie, M. (2008). Prevalence and Characterization of *Salmonella* in Bovine Lymph Nodes Potentially

Destined for Use in Ground Beef. *Journal of Food Protection*, *71*(8), 1685–1688. https://doi.org/10.4315/0362-028X-71.8.1685

Berrang, M. E., Cox, N. A., and Bailey, J. S. (1995). Measuring air-borne microbial contamination of broiler hatching cabinets. *Journal of Applied Poultry Research*, *4*(1), 83–87. https://doi.org/10.1093/japr/4.1.83

Boyle, E. C., Brown, N. F., and Finlay, B. B. (2006). *Salmonella* enterica serovar Typhimurium effectors SopB, SopE, SopE2 and SipA disrupt tight junction structure and function. *Cellular Microbiology*, *8*(12), 1946–1957. https://doi.org/10.1111/j.1462-5822.2006.00762.x

Buncic, S. and Sofos, J. (2012). Interventions to control *Salmonella* contamination during poultry, cattle and pig slaughter. *Food Research International*, *45*(2), 641–655. doi:10.1016/j.foodres.2011.10.018

Carter, P. B., and Collins, F. M. (1974). The route of enteric infection in normal mice. *The Journal of Experimental Medicine*, *139*(5), 1189–1203. https://doi.org/10.1084/jem.139.5.1189

Chambers, P. G., and Lysons, R. J. (1979). The inhibitory effect of bovine rumen fluid on *Salmonella* typhimurium. *Research in Veterinary Science*, *26*(3), 273–276. https://doi.org/10.1016/S0034-5288(18)32879-0

Chinivasagam, H. N., Tran, T., Maddock, L., Gale, A., & Blackall, P. J. (2009). Mechanically Ventilated Broiler Sheds: a Possible Source of Aerosolized *Salmonella*, Campylobacter, and Escherichia coli. *Applied and Environmental Microbiology*, *75*(23), 7417–7425. https://doi.org/10.1128/AEM.01380-09

Chomel, B. B., Belotto, A., and Meslin, F.-X. (2007). Wildlife, exotic pets, and emerging zoonoses. *Emerging Infectious Diseases*, *13*(1), 6–11. https://doi.org/10.3201/eid1301.060480

Connolly, J. H., Alley, M. R., Dutton, G. J., and Rogers, L. E. (2006). Infectivity and persistence of an outbreak strain of *Salmonella* enterica serotype Typhimurium DT160 for house sparrows (Passer domesticus) in New Zealand. *New Zealand Veterinary Journal*, *54*(6), 329–332. https://doi.org/10.1080/00480169.2006.36719

Corrier, D. E., Purdy, C. W., and DeLoach, J. R. (1990). Effects of marketing stress on fecal excretion of *Salmonella* spp in feeder calves. *American Journal of Veterinary Research*, *51*(6), 866–869.

Costa, L. F., Paixão, T. A., Tsolis, R. M, Bäumler, A. J., and Santos, R. L. (2012). Salmonellosis in cattle: Advantages of being an experimental model. *Research in Veterinary Science*, *93*(1), 1–6. https://doi.org/10.1016/j.rvsc.2012.03.002

Crump, J. A., Luby, S. P., Mintz, E. D. (2004). The global burden of typhoid fever. *Bulletin of the World Health Organization*, *82*(5), 346–353. https://doi.org/10.1590/S0042-96862004000500008

de Jong, H. K, Parry, C. M., van der Poll, T., and Wiersinga, W. J. (2012). Host-Pathogen Interaction in Invasive Salmonellosis. *PLoS Pathogens*, *8*(10), e1002933–e1002933. https://doi.org/10.1371/journal.ppat.1002933

de Lau, W., Kujala, P., Schneeberger, K., Middendorp, S., Li, V. S. W., Barker, N., Martens, A., Hofhuis, F., DeKoter, R. P., Peters, P. J., Nieuwenhuis, E., and Clevers, H. (2012). Peyer's Patch M Cells Derived from Lgr5+ Stem Cells Require SpiB and Are

Induced by RankL in Cultured “Miniguts” *Molecular and Cellular Biology*, *32*(18), 3639–3647. https://doi.org/10.1128/MCB.00434-12

Dewell, G. A., Simpson, C. A., Dewell, R. D., Hyatt, D. R., Belk, K. E., Scanga, J. A., Morley, P. S., Grandin, T., Smith, G. C., Dargatz, D. A., Wagner, B. A., and Salman, M. D. (2008). Risk Associated with Transportation and Lairage on Hide Contamination with *Salmonella* enterica in Finished Beef Cattle at Slaughter. *Journal of Food Protection*, *71*(11), 2228–2232. https://doi.org/10.4315/0362-028X-71.11.2228

Edrington, T. S., Loneragan, G. H., Hill, J., Genovese, K. J., He, H., Callaway., T. R., Anderson, R. C., Brichta-Harhay, D. M., & Nisbet, D. J. (2013). Development of a Transdermal *Salmonella* Challenge Model in Calves. *Journal of Food Protection*, *76*(7), 1259–1263. https://doi.org/10.4315/0362-028X.JFP-12-319

Fabre, L., Zhang, J., Guigon, G., Le Hello, S., Guibert, V. (2012) CRISPR Typing and Subtyping for Improved Laboratory Surveillance of *Salmonella* Infections. PLOS ONE 7(5): e36995 https://doi.org/10.1371/journal.pone.0036995

Fedorka-Cray, P. J., Kelley, L. C., Stabel, T. J., Gray, J. T., and Laufer, J. A. (1995). Alternate routes of invasion may affect pathogenesis of *Salmonella* typhimurium in swine. *Infection and Immunity*, *63*(7), 2658–2664. https://doi.org/10.1128/IAI.63.7.2658-2664.1995

Fedorka-Cray, P. J., Dargatz, D. A., Thomas, L. A., and Gray, J. T. (1998). Survey of *Salmonella* serotypes in feedlot cattle. *Journal of Food Protection*, *61*(5), 525–530. https://doi.org/10.4315/0362-028X-61.5.525

Fegan, N., Vanderlinde, P., Higgs, G., and Desmarchelier, P. (2004). Quantification and prevalence of *Salmonella* in beef cattle presenting at slaughter. *Journal of Applied Microbiology*, *97*(5), 892–898. https://doi.org/10.1111/j.1365-2672.2004.02380.x

Fitzgerald, A. C., Edrington, T. S., Looper, M .L., Callaway, T. R., Genovese, K. J., Bischoff, K. M., McReynolds, J. L., Thomas, J. D., Anderson, R. C., and Nisbet, D. J. (2003). Antimicrobial susceptibility and factors affecting the shedding of E. coli O157:H7 and *Salmonella* in dairy cattle. *Letters in Applied Microbiology*, *37*(5), 392–398. https://doi.org/10.1046/j.1472-765X.2003.01417.x

Forgey, S. J., Englishbey, A. K., Casas, D. E., Jackson, S. P., Miller, M. F., Echeverry, A., and Brashears, M. M. (2020). Presence of Presumptive Shiga Toxin–Producing Escherichia coli and *Salmonella* on Sheep during Harvest in Honduras. *Journal of Food Protection*, *83*(11), 2008–2013. https://doi.org/10.4315/0362-028X.JFP-19-424

Frost, A. J., O'Boyle, D., and Samuel, J. L. (1988). The isolation of *Salmonella* spp from feed lot cattle managed under different conditions before slaughter. *Australian Veterinary Journal*, *65*(7), 224–225. https://doi.org/10.1111/j.1751-0813.1988.tb14464.x

Galán, J. E. (1999). Interaction of *Salmonella* with host cells through the centisome 63 type III secretion system. *Current Opinion in Microbiology*, *2*(1), 46–50. https://doi.org/10.1016/S1369-5274(99)80008-3

Galán, J. E., and Wolf-Watz, H. (2006). Protein delivery into eukaryotic cells by type III secretion machines. *Nature (London)*, *444*(7119), 567–573. https://doi.org/10.1038/nature05272

Gal-Mor, O., Boyle, E. C., and Grassl, G. A. (2014). Same species, different diseases: how and why typhoidal and non-typhoidal *Salmonella* enterica serovars differ. *Frontiers in Microbiology*, *5*, 391–391. https://doi.org/10.3389/fmicb.2014.00391

Glynn, J. R., and Palmer, S. R. (1992). Incubation period, severity of disease, and infecting dose: evidence from a *Salmonella* outbreak. Am. J. Epidemiol. 136, 1369–1377. https://doi.org/10.1093/oxfordjournals.aje.a116449

Gragg, S. E., Loneragan, G. H., Brashears, M. M., Nightingale, K. K., Brichta-Harhay, D. M., Ruiz, H., Elder, J. R., Garcia, L. G., Miller, M. F., Echeverry, A., and Ramirez Porras, R. G. (2013). Substantial Within-Animal Diversity of *Salmonella* Isolates From Lymph Nodes, Feces, and Hides of Cattle at Slaughter. *Applied and Environmental Microbiology*, *79*(15), 4744–4750. https://doi.org/10.1128/AEM.01020-13

Guard-Petter, J. (2001). The chicken, the egg and *Salmonella* Enteritidis. Environmental Microbiology. 3:421-430. https://doi.org/10.1046/j.1462-2920.2001.00213.x

Guglielmone, A. A, Gimeno, E., Idiart, J., Fisher, W. F., Volpogni, M. M., Quaino, O., Anziani, O. S., Flores, S. G., and Warnke, O. (1999). Skin lesions and cattle hide damage from Haematobia irritans infestations. *Medical and Veterinary Entomology*, *13*(3), 324–329. https://doi.org/10.1046/j.1365-2915.1999.00167.x

Gutema, F. D., Agga, G. E., Abdi, R. D., De Zutter, L., Duchateau, L., and Gabriël, S. (2019). Corrigendum: Prevalence and Serotype Diversity of *Salmonella* in Apparently Healthy Cattle: Systematic Review and Meta-Analysis of Published Studies, 2000-

2017. *Frontiers in Veterinary Science*, *6*, 184–184. https://doi.org/10.3389/fvets.2019.00184

Haneklaus, A. N., Harris, K. B., Griffin, D. B., Edrington, T. S., Lucia, L. M., and Savell, J. W. (2012). *Salmonella* Prevalence in Bovine Lymph Nodes Differs among Feedyards. *Journal of Food Protection*, *75*(6), 1131–1133. https://doi.org/10.4315/0362-028X.JFP-11-530

Hanlon, K. E , Miller, M. F., Guillen, L. M., and Brashears, M. M. (2016). *Salmonella* Presence in Mandibular, Mesenteric, and Subiliac Lymph Nodes Collected from Sheep and Goats in the United States. *Journal of Food Protection*, *79*(11), 1977–1981. https://doi.org/10.4315/0362-028X.JFP-16-193

Hanlon, K. E., Miller, M. F., Guillen, L. M., Echeverry, A., Dormedy, E., Cemo, B., Branham, L. A., Sanders, S., and Brashears, M. M. (2018). Presence of *Salmonella* and Escherichia coli O157 on the hide, and presence of *Salmonella*, Escherichia coli O157 and Campylobacter in feces from small-ruminant (goat and lamb) samples collected in the United States, Bahamas and Mexico. *Meat Science*, *135*, 1–5. https://doi.org/10.1016/j.meatsci.2017.08.003

Hanson, D. L., Ison, J. J., Malin, K. S., Webb, H. E., (2020). *Salmonella* White Paper. Beef Industry Food Safety Council (BIFSCo)

Herrick, R., Rogers, C., McEvers, T., Amachawadi, R., Nagaraja, T., Maxwell, C., and Lawrence, T. (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*(2), 170–182. https://doi.org/10.15232/aas.2021-02228

Himathongkham, S., Bahari, S., Riemann, H., and Cliver, D. (1999). Survival of Escherichia coli O157:H7 and *Salmonella* typhimurium in cow manure and cow manure slurry. *FEMS Microbiology Letters*, *178*(2), 251–257. https://doi.org/10.2307/1593057

Hohmann, E. L. (2001). Nontyphoidal Salmonellosis. *Oxford Journals*, 32(2),263-269. https://doi.org/10.1086/318457

Horton, B. C,. Gehring, K. B., Sawyer, J. E., and Arnold, A. N. (2021). Evaluation of Autogenous Vaccine Use in Mitigating *Salmonella* in Lymph Nodes from Feedlot Cattle in Texas. *Journal of Food Protection*, *84*(1), 80–86. https://doi.org/10.4315/JFP-20-171

Ibarra, J. A., and Steele-Mortimer, O. (2009). *Salmonella* the ultimate insider. *Salmonella* virulence factors that modulate intracellular survival. *Cellular Microbiology*, *11*(11), 1579–1586. https://doi.org/10.1111/j.1462-5822.2009.01368.x

Im, M. C., Seo, K. W., Bae, D. H., and Lee, Y. J. (2016). Bacterial Quality and Prevalence of Foodborne Pathogens in Edible Offal from Slaughterhouses in Korea. *Journal of Food Protection*, *79*(1), 163–168. https://doi.org/10.4315/0362-028X.JFP-15-251

Jansen, R., van Embden, J. D. A., Gaastra, W., and Schouls, L. M. (2002). Identification of genes that are associated with DNA repeats in prokaryotes. *Molecular Microbiology*, *43*(6), 1565–1575. https://doi.org/10.1046/j.1365-2958.2002.02839.x

Jones, P., Collins, P., Brown, G., and Aitken, M. (1982). Transmission of *Salmonella* mbandaka to cattle from contaminated feed. *Journal of Hygiene*, *88*(2), 255-263. https://doi.org/10.1017/S002217240007011X

Kallapura, G., Morgan, M. J., Pumford, N. R., Bielke, L. R., Woldfenden, A. D., Faulkner, O. B., Latorre, J. D., Menconi, A., Hernandez-Velasco, X., Kuttappan, V. A., Hargis, B. M., and Tellez, G. (2014). Evaluation of the respiratory route as a viable portal of entry for *Salmonella* in poultry via intratracheal challenge of *Salmonella* Enteritidis and *Salmonella* Typhimurium. *Poultry Science*, *93*(2), 340–346. https://doi.org/10.3382/ps.2013-03602

Koohmaraie, M., Scanga, J. A., De La Zerda, M. J., Koohmaraie, B., Tapay, L., Beskhlebnaya, V., Mai, T., Greeson, K., and Samadpour, M. (2012). Tracking The Sources Of *Salmonella* In Ground Beef Produced From Nonfed Cattle. *Journal of Food Protection*, *75*(8), 1464–1468. https://doi.org/10.4315/0362-028X.JFP-11-540

Luque, I., Echeita, A., León, J., Herrera-León, S., Tarradas, C., González-Sanz, R., Huerta, B., and Astorga, R. J. (2009). *Salmonella* Indiana as a cause of abortion in ewes: Genetic diversity and resistance patterns. *Veterinary Microbiology*, *134*(3), 396–399. https://doi.org/10.1016/j.vetmic.2008.08.015

Ly, K. T., and Casanova, J. E. (2007). Mechanisms of *Salmonella* entry into host cells. *Cellular Microbiology*, *9*(9), 2103–2111. https://doi.org/10.1111/j.1462-5822.2007.00992.x

Mian, L. S., Maag, H., Tacal, J. V. (2002). Isolation of *Salmonella* from muscoid flies at commercial animal establishments in San Bernardino County, California. J. Vector Ecol. 27:82–85

Molina-Lopez, R. A., Valverdú, N., Martin, M., Mateu, E., Obon, E., Cerdà-Cuéllar, M., and Darwich, L. (2011). Wild raptors as carriers of antimicrobial-resistant *Salmonella* and

Campylobacter strains. *Veterinary Record*, *168*(21), 565–565. https://doi.org/10.1136/vr.c7123

Nagaraja, T. G., and Chengappa, M. M. (1998). Liver abscesses in feedlot cattle: a review. *Journal of Animal Science*, *76*(1), 287–298. https://doi.org/10.2527/1998.761287x

Nagaraja, T. G., and Lechtenberg, K. F. (2007). Liver Abscesses in Feedlot Cattle. *The Veterinary Clinics of North America. Food Animal Practice*, *23*(2), 351–369. https://doi.org/10.1016/j.cvfa.2007.05.002

Nickelson, K. J., Taylor, T. M., Griffen, D. B., Savell, J. W., Gehring, K. B., and Arnold, A. N. (2019). Assessment of *Salmonella* Prevalence in Lymph Nodes of U.S. and Mexican Cattle Presented for Slaughter in Texas. *Journal of Food Protection*, *82*(2), 310–315. https://doi.org/10.4315/0362-028X.JFP-18-288

Pennycott, T. W., Park, A., and Mather, H. A. (2006). Isolation of different serovars of *Salmonella* enterica from wild birds in Great Britain between 1995 and 2003. *Veterinary Record*, *158*(24), 817–820. https://doi.org/10.1136/vr.158.24.817

Richardson, A. (1975). Outbreaks of bovine salmonellosis caused by serotypes other than S. dublin and S. typhimurium. *The Journal of Hygiene*, *74*(2), 195–203. https://doi.org/10.1017/S0022172400024256

Rings, M. D. (1985). Salmonellosis in Calves. *Veterinary Clinics of North America: Food Practice*, 1(3), 529-539. https://doi.org/10.1016/S0749-0720(15)31301-3

Samuel, J. L., O'Boyle, D. A., Mathers, W. J., and Frost, A. J. (1980). Isolation of *Salmonella* from mesenteric lymph nodes of healthy cattle at slaughter. *Research in*

*Veterinary Science*, *28*(2), 238–241. https://doi.org/10.1016/S0034-5288(18)32754-1

Scallan, E., Hoekstra, R. M., Mahon, B. E., Jones, T. F., and Griffin, P. M. (2015). An assessment of the human health impact of seven leading foodborne pathogens in the United States using disability adjusted life years. *Epidemiology and Infection*, *143*(13), 2795–2804. https://doi.org/10.1017/S0950268814003185

Shariat, N., Timme, R. E., Pettengill, J. B., Barrangou, R., and Dudley, E. G. (2015). Characterization and evolution of *Salmonella* CRISPR-Cas systems. *Microbiology (Society for General Microbiology)*, *161*(Pt 2), 374–386. https://doi.org/10.1099/mic.0.000005

Small, A., James, C., James, S., Davies, R., Liebana, E., Howell, M., Hutchison, M., and Buncic, S. (2006). Presence of *Salmonella* in the Red Meat Abattoir Lairage after Routine Cleansing and Disinfection and on Carcasses. *Journal of Food Protection*, *69*(10), 2342–2351. https://doi.org/10.4315/0362-028X-69.10.2342

Sofos, J. N., Kochevar, S. L., Reagan, J. O., and Smith, A. C. (1999). Incidence of *Salmonella* on Beef Carcasses Relating to the U.S. Meat and Poultry Inspection Regulations. *Journal of Food Protection*, *62*(5), 467–473. https://doi.org/10.4315/0362-028X-62.5.467

Sousa, S., Lecuit, M., and Cossart, P. (2005). Microbial strategies to target, cross or disrupt epithelia. *Current Opinion in Cell Biology*, *17*(5), 489–498. https://doi.org/10.1016/j.ceb.2005.08.013

Stephens, T. P., Loneragan, G. H., Karunasena, E. and Brashears. M. M. (2007). Reduction of Escherichia coli O157 and *Salmonella* in feces and on hides of feedlot cattle using

various doses of a direct-fed microbial. Journal of Food Protection. 70:2386-2391. https://doi.org/10.4315/0362-028X-70.10.2386

Thompson, C. P., Doak, A. N., Amirani, N., Schroeder, E. A., Wright, J., Kariyawasam, S., Lamendella, R., Shariat, N. W. (2018). High-Resolution Identification of Multiple *Salmonella* Serovars in a Single Sample by Using CRISPER-SeroSeq. *Applied and Environmental Microbiology, 84*(32), eD1859-18. https://doi.org/10.1128/AEM .01859-18.

Thomson, J., Yeater, K., Zurek, L., and Nayduch, D. (2019). Abundance and Accumulation of Escherichia coli and *Salmonella* Typhimurium Procured by Male and Female House Flies (Diptera: Muscidae) Exposed to Cattle Manure. *Annals of the Entomological Society of America, 110*(1), 37-44. https://doi.org/10.1093/aesa/saw082

Touchon, M., and Rocha, E. P. C. (2010). The small, slow and specialized CRISPR and anti-CRISPR of Escherichia and *Salmonella*. *PloS One*, *5*(6), e11126–e11126. https://doi.org/10.1371/journal.pone.0011126

USDA-APHIS. Date, 2009, Highlights of Dairy 2007 Part V: Changes in Dairy Cattle Health and Management Practices in the United States, 1996-2007

USDA-APHIS. Date, 2010, Vaccination of cattle and calves on U.S. Beef Cow-Calf Operations. Accessed 22DEC, 2013.

Uzzau, S., Brown, D. J., Wallis, T., Rubino, S., Leori, G., Bernard, S., Casadesús, J., Platt, D. J., and Olsen, J. E. (2000). Host adapted serotypes of *Salmonella* enterica. *Epidemiology and Infection*, *125*(2), 229–255. https://doi.org/10.1017/S0950268899004379

Vazquez-Torres, A., and Fang, F. C. (2000). Cellular routes of invasion by enteropathogens. *Current Opinion in Microbiology*, *3*(1), 54–59. https://doi.org/10.1016/S1369-5274(99)00051-X

Vieira-Pinto, M., Vieira, M. J., Oliveria, M., Fontes, M. C., Themudo, P., and Semedo-Lemsaddek, T. (2011). *Salmonella* sp. in edible offal (liver and tongue) from pigs slaughtered for consumption. Iowa State University Digital Repository.

Wang, M., Qazi, I. H., Wang, L., Zhou, G., and Han, H. (2020). *Salmonella* Virulence and Immune Escape. *Microorganisms (Basel)*, *8*(3), 407. https://doi.org/10.3390/microorganisms8030407

Watson, P. R., Paulin, S. M., Bland, A. P., Jones, P. W., and Wallis, T. S. (1995). Characterization of intestinal invasion by *Salmonella* typhimurium and *Salmonella* dublin and effect of a mutation in the invH gene. *Infection and Immunity*, *63*(7), 2743–2754. https://doi.org/10.1128/IAI.63.7.2743-2754.1995

Wells, S. J., Fedorka-Cray, P. J., Dargatz, D. A., Ferris, K., and Green, A. (2001). Fecal Shedding of *Salmonella* spp. by Dairy Cows on Farm and at Cull Cow Markets. *Journal of Food Protection*, *64*(1), 3–11. https://doi.org/10.4315/0362-028X-64.1.3

Wray, C., and Davies, R. H. (2000). *Salmonella* Infections in Cattle. *CAB International*

Xu, Y., Tao, S., Hinkle, N., Harrison, M., and Chen, J. (2018). *Salmonella*, including antibiotic-resistant *Salmonella*, from flies captured from cattle farms in Georgia, U.S.A. *The Science of the Total Environment*, *616-617*, 90–96. https://doi.org/10.1016/j.scitotenv.2017.10.3

# Chapter 3

## Association of liver abscess presence and epithelial integrity of the hindgut in feedlot cattle to *Salmonella* carriage in the peripheral lymph

### Introduction

*Salmonella* prevalence is most commonly correlated to certain season and regions (Fedorka-Cray et al., 1998; Sofos et al., 1999; Nickelson et al., 2019; Wottlin et al., 2022). *Salmonella* detection and enumeration is often reflective of the temperature within the season rather than the season itself (Wells et al., 2001). Most frequently *Salmonella* prevalence is the highest during summer and fall, and lowest during the spring and winter months. This is due to *Salmonella* proliferation during warmer weather and suppression during colder weather (Wottlin et al., 2022). There is a suggestion that *Salmonella* is more successful in surviving in dry conditions (Wray and Davies, 2000; Sofos et al., 1999).

The manner and sources from which cattle can become infected with *Salmonella* can be multi-factorial. Cattle become infected most commonly orally (Ly and Casanoca, 2007; Wang et al., 2020) but also transdermally (Mian et al., 2002) and via airborne transmission (Kallapura et al., 2014). Infections that occur orally can be from a multitude of sources for example, fecal matter (Gutema et al., 2019), feed contamination (Costa et al., 2012), and pests (Chomel et al., 2007; Xu et al., 2017). Transdermal infections can be a result of fly bites (Mian et al., 2002) and airborne transmission from bacteria in dust

(Chinivasagam et al., 2009). These likely causes of *Salmonella* are all harbored in a feedlot setting and the magnitude of their influence can be based on different management practices implemented.

Because *Salmonella* is most commonly obtained via fecal-oral route, *Salmonella* colonizes within the distal small intestine (Ly and Casanoca, 2007; Wang et al., 2020). The intestines are lined with a monolayer of epithelial cells. Tight junctions are responsible for attaching epithelial cells to one another thus are in charge of holding the epithelial barrier together. M-cells are of epithelial foundation and are present within Peyer's patches and are responsible for capturing intestinal lining antigens and presenting the antigens to the body's immune system (Rescigno et al., 2001; Sousa et al., 2005). The most frequent point of penetration for bacteria is through M cells when *Salmonella* uses invasive genes encoded by *Salmonella* pathogenicity islands. The ability of *Salmonella* to escape through the gastrointestinal tract results in other organ tissues becoming infected (Resigno et al., 2001).

*Salmonella* has been recovered from liver tissue that is both edible and abscessed (Im et al., 2015; Amachawadi et al., 2016; Herrick et al., 2022). Liver abscesses are pus-filled capsules that are most commonly a result of aggressive feeding practices. In a feedlot setting, cattle are normally fed a lower roughage and higher dietary energy diets which can result in cattle having fluctuations of pH within the rumen that can be the foundation of acidosis, rumentitis, and consequently liver abscesses (Nagaraja and Chengappa, 1998). Because *Fusobacterium necrophorum* is a bacteria most commonly found in the ruminal flora it has also been deemed as the primary agent found in liver, however, Nagaraja and Chengapa (1998) isolated *Salmonella* from 10 liver abscesses.

The presence of certain bacteria in liver abscesses is the ability of the bacteria to escape the epithelial barrier and enter the portal circulation and by the portal capillary system and this results in an infected liver. Translocation of *Salmonella* from the bloodstream, rumen, or intestines can not only be observed in liver abscesses but also in edible liver tissue (Vieira-Pinot et al., 2011; Im et al., 2015; Herrick et al., 2022).

*Salmonella* is unlike *E. coli* 0157:H7 because the current in-plant interventions such as separating hide-on from hide-off areas and sanitization of equipment, personnel, and carcass surfaces are directed towards surface decontamination do not affect *Salmonella* that is within lymph nodes (Arthur et al., 2008; Haneklaus et al., 2012). Infection of *Salmonella* in the lymph nodes is of importance because of the number of lymph nodes within the body. This then contributes to the amount of lymph nodes that are consequently included in beef trimmings that are used in further processed products such as ground beef (Gragg et al., 2013). The lymphatic system is responsible for the movement of many substance. *Salmonella* has been reported in mesenteric, subiliac, and mandibular lymph nodes. Mesenteric are not of great concern due to being removed during evisceration portion of harvest (Hanlon et al., 2016). The objectives of this study were 1) to evaluate various management practices of feedlots with historically high *Salmonella* occurrence in comparison of feedlots with lower *Salmonella* levels, and 2) management practice implications to epithelial integrity of the hindgut with prevalence in SLN and liver severity.

## Materials and Methods

### *Feedlot survey*

Feedlots ($n$ = 6), from the Texas Panhandle, identified based upon historic sampling data for high and low prevalence of *Salmonella* were surveyed for this trial. Interviews were conducted on-site between personnel from West Texas A&M University and the feedlot manager at each of six feedlots (A, B, C, D, E, F). Personnel of West Texas A&M University transcribed interview responses from feedlot managers onto a survey form (Appendix A). The survey questionnaire contained 31 questions that were categorized into the following six topics: receiving cattle management and type of cattle received (4 questions); general cattle management (11 questions); ration ingredients and adjustments (5 questions); capacity and pen management (8 questions); pest management (3 questions). The questions on the survey form were designed to identify differences in cattle source type, care, and management among feedlots. Surveys were reassessed every quarter to account for seasonal changes or adjustments made due to the commodity markets. Observational tours of each feedlot were conducted to visually assess cattle condition, pen condition, yard maintenance, pest management, environmental challenges, and were denoted by West Texas A&M personnel. Additionally, weather data including temperatures, relative humidity, and precipitation were recorded for each feedlot using the Texas Tech Mesonet. Weather data was recorded for a day of harvest and six days prior.

*Sample collection*

Samples were collected from commercial beef processing plants located in the High Plains region of the United States. Collections were made quarterly beginning January 2021 and ending February 2022 for each of the six feedlots. The aim of each quarterly collection was to obtain paired intact liver tissue samples ($n$ =8; 4 edible and 4 severely abscessed) and their subsequent subiliac lymph node samples (LN), as well as, paired colonic mucosal tissue ($n$ = 8; 4 #1 graded colons and 4 #2 graded colons) and their subsequent subiliac lymph node samples from each feedlot enrolled in the trial. Categorization of colon tissue score was based on the gross anatomy and integrity of the epithelial cell layer, #1 colon layers resembled a ridged surface and #2 colon surface was smooth. Overall, 192 liver, 176 colon, and 368 subiliac lymph node samples were collected. Liver tissue, colonic tissue and subiliac LN were collected and placed in individual sterile sample collection bags. Following collection, samples were refrigerated and shipped on ice overnight to the USDA-ARS laboratory in College Station, TX for bacterial culture. Individual carcass ID were collected and cross reference to a unique carcass ID which were transferred to any samples collected. Individual animal ear tag information was recorded for each sampled carcass. Ear tag number was used to obtain feedlot and lot information such as, sex, head per pen, origin, days on feed, average initial body weight, average final body weight, pulls and dead per pen, and cattle type.

## *Salmonella* Prevalence and Enumeration

### *Sample processing*

Edible liver tissue or intact liver abscess capsules were excised from the liver. Sub-samples from abscessed livers always contained an abscess with viable purulent material. Edible liver tissue or abscess was surface-sterilized by submersion into boiling water for approximately 5 seconds. The surface-sterilized sample was then placed in a filtered Whirl-Pak bag, weighed, and pulverized with a rubber mallet.

Colonic mucosal tissue was collected by excising an approximately 6 inch section of the distal colon. Luminal contents of the distal colon were collected by removing a small section of colon and zip-tying the end of each segment of the colon. The sectioned off colon sample was placed in a Ziploc bag. Colon segments were surface-sterilized by submersion into boiling water for approximately 5 seconds. Next, a sterile small incision was made into the wall of the colon, and the colon fecal contents emptied into a Whirl-Pak bag.

LN were processed by trimming excess fat tissue. For quantification of Salmonella, SLN were sterilized in boiling water submersion for 3 to 5 s. Then SLN samples were placed into a filtered stomacher bag (Nasco), weights were recorded, and each sample was pulverized utilizing a rubber mallet. Quantification of SLN samples were described previously.

*Salmonella culture*

Aerobic quantification (Brichta-Harhay et la., 2012; Wottlin et al., 2022) of liver and SLN samples are as follows: each sample bag had tryptic soy broth (TSB) (80 mL) added and for 30 s, using a laboratory blender (BagMixer 400VW, Interscience Laboratories, Inc., Weymouth, MA), samples were homogenized. Duplicates of 1 mL of the homogenized sample were added to Petrifilm EB (3M Health Care, St. Paul, MN) and incubated overnight at 37 ºC, for enumeration. Bacterial growth that was observed and films were moved to XLD plates with 15 µg/mL novobiocin and 10 µg/mL cefsoludin and incubated at 37 ºC for 24 h. The number of black colonies were recorded and converted to $\log_{10}$CFU/g SLN. The original SLN-TSB mixture was incubated for 2 h at room temperature and then again for 12 h at 42 ºC, for prevalence analysis. Then, anti-*Salmonella* immunomagnetic separation added to 1 mL of each enrichment culture with 20 µL of anti-*Salmonella* beads (Invitrogen, Waltham, MA) and incubated at room temperature for 15 min with shaking at 800 rpm. The beads were removed from the enriched samples and washed twice in PBS-Tween 20, then transferred to Rapport-Vassiliadis R10 (RV) broth (3 mL) and incubated at 42 ºC for 24 h. Then, 100 µL of RV broth was plated each to brilliant green agar (BGA) with 80 µg/mL and BAG with 30 µg/mL tetracycline for incubation at 37 ºC overnight. Lysine iron and triple sugar iron agars were used to biochemically confirm suspect colonies. A sample of 1 mL was taken of the SLN-TSB mixture and tetrathionate broth overnight enrichments on the morning of d 2, and the RV enrichment on the morning of d 3. This sample was centrifuged, supernatant was disposed of, and then the pellet saved for whole-genome sequencing. Pellets from *Salmonella*-negative samples were discarded of. Liver samples were also

anaerobically tested by plating the RV enrichment onto MacConkey(S) plates and incubating anaerobically for 72 h. After incubation, plates that had colorless colonies was then sub-cultured to BGA and incubated aerobically for 24 h.

Colon samples were processed by enriching 10 g luminal contents in 90 mL of tetrathionate broth with 1.8 mL of iodine solution at 37 ºC for 24 h. A portion of the pre-incubation mixture (50 µL) was plated onto XLD using a spiral plater and incubated at 37 ºC for 24h, and then for an additional 24 h at room temperature, for enumeration of *Salmonella.* After incubation of the fecal-tetrathionate mixture, a 100 µL portion was moved to 5 mL RV broth and incubated at 42 ºC for 24 h. After enrichment, samples were dual-plated onto BGA with 25 µg/mL of novobiocin and 30 µg/mL of BGA with tetracycline and incubated for 24 h at 37 ºC. After incubation, 3 suspect colonies, these colonies were pink with round distinct borders, per plate were confirmed biochemically using lysine iron and triple sugar iron agars.

## Results

### *Receiving cattle management and type of cattle received*

Cattle arriving to the feedlot traveled on average 334 miles to over 596 miles (Table 1). Feedlot B, on average, received cattle from the greatest distance when compared to other feedlots which resulted in cattle traveling roughly 9 hrs. On average, the shortest distance cattle traveled to the feedlot was to feedlot E (334 miles) which was roughly 5 hrs. Half of the feedlots averaged over 400 miles of travel for newly received cattle while the other half averaged 300 to 400 miles. Stocker type cattle (Table 2) were the most predominant type fed ranging from 65% of the total cattle fed to as little as 20%.

Feedlots A, B, C, D, and E all fed primarily stocker cattle. Auction market cattle were another type of cattle that feedlot managers reported. Cattle that typically come from an auction market may or may not have had any vaccinations or been introduced to a bunk feeding system. Auction market cattle represented approximately 20% of the feedlot's capacity Feedlots C, D, and F fed less than 20% auction market cattle whereas, A and B feedlots fed 10% or less, and feedlot E fed only 3%. Mexican type cattle are cattle that originated from Mexico; these cattle would come into the feedlot usually at lower body weights and would have recently crossed the Mexican border. Feedlot F is the only feedlot without Mexican cattle on feed (Table 2). Feedlots B, C, D, and E fed 21% or less Mexican type cattle, and Feedlot A fed the most with over 70% of cattle fed at their feedlot being cattle of Mexican origin. Dairy and dairy cross cattle were the least common type of cattle fed with the exception of Feedlot F which fed 44% dairy and dairy cross cattle,

All feedlots fed a mixture of heifers and steers but they were typically fed separately, except for feedlot A which would feed mixed sex pens. Mixed pen feeding at feedlot A was because of the high percentage of Mexican cattle fed at the feedlot and because Mexican heifers are all supposed to be spayed when crossing the border from Mexico over into the US.

Generally, arrival management practices were observed to be similar across the six feedlots. Vaccinations and implants were used in all feedlot processing. The range for average initial weight of cattle was 273.83 kg (feedlot C) to 371.27 kg (feedlot F). Average final BW ranged from 560.55 kg (Feedlot C) to 616.28 kg (Feedlot F). Feedlot C

also had the lowest average final weights of cattle sampled in this study. Mean days on feed had a range of 173.7 d (feedlot F) to 256.1 d (feedlot A).

*Capacity and pen management*

Capacity of each feedlot varied from only 24,000 to 125,000 head per feedlot (Table 1). Feedlot B reported to give cattle within the feedlot 12 inches of bunk space and feedlot A reported to give cattle as little as 6 inches of bunk space per head

*Ration ingredients and adjustments*

Feedlots A, B, C, and D all utilized the same nutritionist. For feedlots E and F, a different nutritionist was used for each of those feedlots. Feedlots A, B, C, D all initially reported that they do not make seasonal adjustments to their rations whereas, feedlots E and F reported that there may be a need for seasonal ration ingredient adjustments. Rations for each feedlot were comprised of different ingredients (Table 1). Feedlot E initially fed a wet distillers grain (WDG), flaked corn, and sorghum silage based diet and during late spring they commonly switch from sorghum silage to corn silage. During this study starting in June of 2021 and until the end of 2021 there was a switch from flaked corn to flaked wheat in their ration. Feedlot F fed flaked corn from January (2021) to June (2021). In June (2021), feedlot F stopped the feeding of flaked corn and began feeding flaked wheat. Though feedlots A, B, C, and D first reported that no seasonal adjustments are made to their rations, when reached out to the feedlots at the end of the study C and D feedlots both reported that a change in the ration was made during this study, due to the large winter and ice storm in February (2021). The feedlot of feedlot D reported at the end of this study that there 8 ration changes over the course of the study.

Both C and D feedlots fed a dry rolled corn inclusive diet and in April (2021) dry rolled corn was replaced with wheat until November (2021). Wheat has been reported to have higher starch intake than corn (Huntington et al., 1997). Feed additives such as, beta adrenergic agonists, ionophores, and feed-grade antibiotics were commonly fed in almost all of the feedlots enrolled in the study such as, Rumensin, Tylosin phosphate (Tylan), and Optaflexx (Table 1). Feedlot B did not feed the beta adrenergic agonist, Optaflexx. Feedlot A did not feed Tylosin phosphate. Melengestrol Acetate was used for suppressant of estrus in heifers in all feedlots except A. Pathogen interventions were also used within the ration ingredients. Bovamine Defend (Nutrition Physiology Company, LLC, Overland Park, KS) was fed to cattle in feedlots: C, D, E, and F.

*Pest management*

Flies were controlled with the release of parasitic wasps (Kunafin, Quemado, TX) in all feedlots, except feedlot B. However, feedlot B had the most extensive in-house management for fly control. Feed and drive alleys were sprayed twice daily (AM and PM) and fly bait was applied along bunks and around all permanent structures, as needed. Feedlots C and D use the fly control program from April to September whereas Feedlots E and F implement the fly control program from May to September. Bird issues and control programs varied from each feedlot. Feedlots A, E, and F had no bird control program. Feedlot B, annually, paints all roosting areas to repel and minimize bird issues. Feedlots C and D both have an issue with the amount of birds present at the feedlot but both have not implemented a bird control program. For other pests, such as rodents, all feedlots used bait or traps for rodent control.

*Weather*

Weather data for each feedlot was evaluated for changes in temperature, relative humidity, and precipitation (Figure 6, Figure 7, Figure 8). The averages used were from the 6 d prior to and the day of harvest. The biggest affect that weather had was seen across quarters. The average highest temperatures (Figure 3) and precipitation (Figure 4) was recorded during quarter 3 where prevalence of *Salmonella* was also the highest.

*Feedlot prevalence*

Feedlots in the Texas Panhandle were surveyed for differences in management practices on the prevalence in *Salmonella* in liver, colons, and SLN samples during harvest. A total of 128 samples were taken from feedlots A, D, E, and F whereas 92 and 126 total samples were taken from feedlots B and C, respectively. Feedlots A, C, and F had the lowest total prevalence rates for *Salmonella* across all quarters and sample types of 17.19%, 20.63%, and 19.53%, respectively (Table 3). Feedlots B, D, and E had the highest prevalence rates for *Salmonella* at 29.35% 24.22%, and 24.22%, respectively. In Quarter 1 (sampled January –February 2021), only two feedlots had samples that were positive for *Salmonella*, feedlots B (8.33%) and F (6.25%) feedlots (Table 3). Quarter 2 (sampled March 2021) had 0.0% of feedlots with positive *Salmonella* samples. In quarter 3(July- October 2021), Feedlot D had the highest prevalence of *Salmonella* (90.63%) followed closely by feedlot C with 81.25% (Table 3). There was an overall prevalence of 20.45% in quarter 4 (sampled December 2021- February 2022). Feedlot E had the highest with 90.63% positive *Salmonella* samples in quarter 4. Prevalence of *Salmonella* of each samples type was recorded by sample date (Figure 2). There was one sampling days that

100% of the liver samples that were collected were positive for *Salmonella* (August 2, 2021) and two sampling days that 100% of the lymph node samples collected were positive for *Salmonella* (August 2, 2021, December 16, 2021). Samples began testing positive approximately 188 days into the year.

*Sample prevalence*

Prevalence and concentration of individual sample types are reported in Figure 1. *Salmonella* prevalence for all samples analyzed was 22.33% ($n$ = 163/730). In total, 365 LN were collected. Total *Salmonella* prevalence of LN samples was 25.25% ($n$ = 92/365). Liver samples ($n$ = 190) were collected and divided up between edible ($n$ =96) and abscessed ($n$ = 94) livers. The total prevalence of livers was 19.27% ($n$ = 37/190). Edible liver samples prevalence rate of *Salmonella* was 18.75% ($n$ = 18) whereas abscessed liver samples were 19.15% ($n$ = 18/94) (Figure 5). Corresponding LN samples of liver samples had a 24.21% ($n$ = 46) prevalence. Colon samples ($n$ =175) were also collected and divided between #1 ($n$ = 88) and #2 ($n$ = 87) colons throughout the entirety of the study. In total, 19.43% ($n$ = 34/175) colon samples were positive for *Salmonella.* The #1 colons were 19.32% ($n$ =17) positive and #2 colons had the samples prevalent with *Salmonella* of 19.54% ($n$ =17) (Figure 5). The samples of SLNs corresponding to colons had the highest prevalence (26.29%; $n$ = 46) (Figure 5).The relative risk of a SLN being positive for *Salmonella* when associated with a #1 colon or #2 colon was 0.77 ($P$ = 0.37) and 0.71 ($P$ = 0.22), respectively. The relative risk of a SLN being positive for *Salmonella* when associated with an edible or abscessed liver was 0.75 ($P$ = 0.30) and 0.83 ($P$ = 0.49), respectively. This highlights the fact that there is little risk of having both samples being *Salmonella* positive.

*Concentration*

Concentration of *Salmonella* varied across sample types (Figure 5). The concentrations of *Salmonella* ranged from 0.10 LogCFU/g (liver tissue) to 2.16 LogCFU/g (#2 colons). Both edible and abscess liver tissue samples were observed for the lowest concentrations of *Salmonella* with a LogCFU/g of 0.10 and their associated SLNs had LogCFU/g of 0.65 and 0.55, respectively. Concentrations of *Salmonella* were the highest in colon samples with concentrations reaching 1.75 and 2.16 LogCFU/g in #1 and #2 colons, respectively. *Salmonella* concentrations in SLN from carcasses with #1 and #2 colons were 0.24 and 0.35 LogCFU/g, respectively.

**Discussion**

Managing receiving cattle play an important role in performance of cattle. Newly received cattle have weaning, transportation, commingling, and the introduction of new environmental stresses placed on them in a short amount of time. When comparing the distance traveled for received cattle between the feedlots with the highest and lowest *Salmonella* positive samples, feedlots B and A, respectively, feedlot B received cattle from the second shortest distance averaging 370 miles and feedlot A received cattle from the greatest distance with cattle traveling an average of 596 miles. Transportation stress would induce the shedding of *Salmonella* (Corrier et al., 1990). Feedlots with the highest capacity also associated to the yard with the highest overall prevalence of *Salmonella* (Figure 1). However, the feedlot with the lowest prevalence did not have the lowest capacity of cattle. Feedlot C had the lowest capacity (24,000 cattle) but an overall prevalence of 20.63% whereas, feedlot B had the highest capacity of cattle (125,000

cattle) and also the highest prevalence of *Salmonella* (29.35%). Wells et al. (2000) observed dairies with higher volume of cattle also had higher prevalence of *Salmonella.* T Receiving cattle are newly received, younger cattle, which may or may not have come from a backgrounding facility that first introduced them to a bunk where they were trained on how to eat from one before entering a feedlot and younger cattle usually prefer to eat feed at the same time. For these reasons, receiving phase cattle would need to have more bunk space of 18 inches per head when finishing cattle would need around 9 to 12 inches per head (Harner et al., 2021). In contrast of the low prevalence of *Salmonella* within samples from feedlot A, this feedlot allotted the lowest amount of bunk space per head (6 to 9 inches). Whereas, feedlot B had the highest prevalence of *Salmonella* with 29.35% and the most bunk space. The variation of bunk space amount allotted to cattle throughout the different feedlots could have been dependent on moisture conditions within pens and more bunk space per head could be used to improve those conditions. Bunk space could also be dependent on whether cattle were in more of a receiving or finishing phase. This feedlot also allowed for the most bunk space for its cattle with 12 inches allotted per head. Feedlot B also was the feedlot with the highest capacity of cattle by feeding 125,000 head. Fedorka-Cray et al. (1998) along with Wells et al. (2000) have reported higher *Salmonella* prevalence within larger operations.

Feedlot A had the lowest prevalence for *Salmonella* in all samples and quarters at 17.19%. This feedlot fed the lowest percentage of stocker cattle (20%) and the highest of Mexican cattle (70%). Literature has shown Numerous studies have described the difference in *Salmonella* prevalence of feedlot, dairy cattle, and cull cattle of however,

differences of *Salmonella* in different cattle within the feedlots has been studied little (Brown et al., 2015).

Splitting the prevalence of *Salmonella* by quarters, there were different feedlots each quarter that had the highest prevalence of *Salmonella* in their samples. Feedlots B, D, and E were the feedlots that had the highest number of samples have a positive result of for *Salmonella.* Though feedlot B, in total, had the highest amount of *Salmonella* positive samples. It should also be noted that there were less colon samples collected than liver samples because from one feedlot there were no colon samples collected during the quarters of 1 and 4. For these specific feedlots, the quarter before the high incidence of *Salmonella*, quarter 2 for feedlots C and D and quarter 3 for quarter E, there was a ration change from corn diet to a wheat diet. This increase of *Salmonella* positive could have been due to this ration change from lower starch to a higher starch inclusive diet. Huntington (1997) observed an increased starch intake with cattle being fed dry-rolled and steam-flaked wheat in comparison to dry-rolled and steam-flaked corn. Increased starch can increase the risk for acidosis to occur. Slyter (1976) reported that wheat is more inclined to cause acidosis than other grain sources. Chronic acidosis results in a decrease of feed intake (Owens et al., 1998). Higher starch diets are more likely to cause acidosis (Slyter, 1976; Huntington, 1997) which can allow for a decrease in rumen health (Owens et al., 1998) and a pathway for *Salmonella* to escape the gastrointestinal tract (Boyle et al., 2006). The environment and nutritional stressors that feedlot B cattle underwent could have caused certain gastrointestinal irritation that allowed for *Salmonella* to escape. This has been reported by Sousa et al., (2005) when tight junctions within the intestinal tract breakdown.

Prevalence and concentration variability observed in sample types are due to where *Salmonella* is colonized and how it travels and escapes throughout the body (Figure 5). It has been observed that *Salmonella* infection most often occurs via fecal-oral route (Gal-More et al., 2014) which allows for the bacteria to colonize within the epithelium of the intestines. Encoded virulence factors by *Salmonella* pathogenicity islands allow for penetration of the epithelium tissue and distribution of the bacteria into the bloodstream and other organs (Ly and Casanova, 2007). Because *Salmonella* colonizes in the intestinal epithelium and would explain the higher concentration seen in the colon samples compared to SLN and liver samples. Vazquez-Torrs and Fang (2000) observed that in the immune system; *Salmonella* is able to survive in phagocytes and dendritic cells that capture the bacteria after invading M cells of the Peyer's patches which leads to the infection of lymph nodes. Prevalence of *Salmonella* was the highest among all samples types during all quarters within the SLN samples. The high prevalence within SLN samples may be contributed to the survivability of *Salmonella* within the lymphatic system. Vipham et al. (2015) fed cattle diets that included *Lactobacillus acidophus* and *Propionibacterium freudenreichii* which are the active ingredients within Bovamine Defend and a control diet. Of cattle fed the active ingredients within Bovamine Defend, only 4.7% of the SLNs collected tested positive for *Salmonella* whereas, the control diet fed cattle had 25.9% of the SLNs collected test positive for *Salmonella.* Feedlot B had the highest prevalence for *Salmonella* (29.35%) and did not report feeding Bovamine Defend or a product similar. However, Feedlot A did not report feeding any pathogen intervention products either, but had the lowest *Salmonella* prevalence (17.19%). Furthermore, feedlot A had 23.44% *Salmonella* positive SLN whereas feedlot

B had 34.78% SLNs positive for *Salmonella*. Though *Salmonella* within the lymph nodes of cattle is of great concern because they contribute to the prevalence within ground beef, it should also be noted that *Salmonella* is also a concern in edible organ meat such as liver tissue that is being marketed to consumers. This leads into the discussion of what other marketable products are tainted with *Salmonella.*

Variation of *Salmonella* prevalence across quarters is an outcome that was expected and interpreted within the literature. Wells et al. (2001) reported the same seasonal outcomes of higher *Salmonella* prevalence in cattle that were sampled during the months of May – July that correspond with our quarter 3 months and lower prevalence in cattle sampled February – April which correspond with this studies quarter 1 and 2. The increased averaged temperature observed in months that fall in quarter 3 are also the same months that *Salmonella* is repeatedly seen to increase as well. This peaks the speculation that heat stress weaken cattle's immune system and allow for *Salmonella* numbers to increase within the body and consequently escape the gastrointestinal tract to other bodily organs (Edrington et al., 2008). This seasonal change would indicate cattle feeders and beef processors need to be prepared to implement strategies to minimize *Salmonella* in beef cattle and products. It is also important to note that though precipitation and relative humidity increased in quarter 3 as did *Salmonella* prevalence it is sporadic when each weather data point and prevalence is compared.

## Conclusion

Many different factors can play a part in the uptake of *Salmonella* by feedlot cattle. Transportation, capacity, and diet are some of the hypothetical conclusions that

were drawn that could potentially increase the prevalence of *Salmonella* in cattle. A more detailed study is needed to draw confirmed conclusions that they could be a reliable solution to *Salmonella* increase in feedlot cattle. This study also looked into cattle type, days on feed, specific ration ingredients, and other management practices as a plausible explanation of *Salmonella* in cattle though further investigation is need to determine if these factors influence *Salmonella* in a positive or negative action. Furthermore, when looking at the three different sample types, lymph nodes, colon and liver, lymph nodes are of much concern because of their ability to go into beef trim that is used for further manufactured products. It also needs to be noted that edible offal products (Arthur et al., 2008) such as liver should also be a concern to consumers because of the amount of *Salmonella* positive samples that were found in livers that were deemed edible. Further investigation is need to see what other organs harbor *Salmonella* and the way in which *Salmonella* eventually escapes the gastrointestinal tract and infects those organs.

## Literature Cited


Amachawadi, R. G., Thomas, M., Nagaraja, T. G., & Scaria, J. (2016). Genome Sequences of *Salmonella* enterica subsp. enterica Serovar Lubbock Strains Isolated from Liver Abscesses of Feedlot Cattle. *Genome Announcements (Washington, DC)*, *4*(3). https://doi.org/10.1128/genomeA.00319-16

Arthur, T. M., Brichta-Harhay, D. M., Bosilevac, J. M., Guerini, M. N., Kalchayanand, N., Wells, J. E., Shackelford, S. D., Wheeler, T. L., & Koohmaraie, M. (2008). Prevalence and Characterization of *Salmonella* in Bovine Lymph Nodes Potentially Destined for Use in Ground Beef. *Journal of Food Protection*, *71*(8), 1685–1688. https://doi.org/10.4315/0362-028X-71.8.1685

Boyle, E. C., Brown, N. F., and Finlay, B. B. (2006). *Salmonella* enterica serovar Typhimurium effectors SopB, SopE, SopE2 and SipA disrupt tight junction structure and function. *Cellular Microbiology*, *8*(12), 1946–1957. https://doi.org/10.1111/j.1462-5822.2006.00762.x

Brichta-Harhay, D. M., Arthur, T. M., Bosilevac, J. M., Kalchayandand, N., Schmidt, J. W., Wang, R., Shackelford, S. D., Loneragan, G. H., and Wheeler, T. L. (2012). Microbiological Analysis of Bovine Lymph Nodes for the Detection of *Salmonella* enterica. *Journal of Food Protection, 75*(5), 854-858. https://doi.org/10.4315/0362-028X.JFP-11-434

Brown, T. R., Edrington, T. S., Loneragan, G. H., Hanson, D. L., Malin, K., Ison, J. J., & Nisbet, D. J. (2015). Investigation into Possible Differences in *Salmonella* Prevalence in the Peripheral Lymph Nodes of Cattle Derived from Distinct Production Systems

and of Different Breed Types. *Journal of Food Protection*, *78*(11), 2081–2084. https://doi.org/10.4315/0362-028X.JFP-15-198

Chinivasagam, H. N., Tran, T., Maddock, L., Gale, A., & Blackall, P. J. (2009). Mechanically Ventilated Broiler Sheds: a Possible Source of Aerosolized *Salmonella*, Campylobacter, and Escherichia coli. *Applied and Environmental Microbiology*, *75*(23), 7417–7425. https://doi.org/10.1128/AEM.01380-09

Chomel, B. B., Belotto, A., and Meslin, F.-X. (2007). Wildlife, exotic pets, and emerging zoonoses. *Emerging Infectious Diseases*, *13*(1), 6–11. https://doi.org/10.3201/eid1301.060480

Corrier, D. E., Purdy, C. W., and DeLoach, J. R. (1990). Effects of marketing stress on fecal excretion of *Salmonella* spp in feeder calves. *American Journal of Veterinary Research*, *51*(6), 866–869.

Edrington, T. S., Ross, T. T., Callaway, T. R., Martinez, C. H., Hume, M. E., Genovese, K. J., Poole, T. L., Anderson, R. C., and Nisbet, D. J. (2008). Investigation into the seasonal salmonellosis in lactating dairy cattle. *Epidemiology and Infection*, *136*(3), 381-390. https://doi.org/10.1017/S0950268807008680

Fedorka-Cray, P. J., Dargatz, D. A., Thomas, L. A., and Gray, J. T. (1998). Survey of *Salmonella* serotypes in feedlot cattle. *Journal of Food Protection*, *61*(5), 525–530. https://doi.org/10.4315/0362-028X-61.5.525

Gal-Mor, O., Boyle, E. C., and Grassl, G. A. (2014). Same species, different diseases: how and why typhoidal and non-typhoidal *Salmonella* enterica serovars differ. *Frontiers in Microbiology*, *5*, 391–391. https://doi.org/10.3389/fmicb.2014.00391

Gragg, S. E., Loneragan, G. H., Brashears, M. M., Nightingale, K. K., Brichta-Harhay, D. M., Ruiz, H., Elder, J. R., Garcia, L. G., Miller, M. F., Echeverry, A., and Ramirez Porras, R. G. (2013). Substantial Within-Animal Diversity of *Salmonella* Isolates

Gutema, F. D., Agga, G. E., Abdi, R. D., De Zutter, L., Duchateau, L., and Gabriël, S. (2019). Corrigendum: Prevalence and Serotype Diversity of *Salmonella* in Apparently Healthy Cattle: Systematic Review and Meta-Analysis of Published Studies, 2000-2017. *Frontiers in Veterinary Science*, *6*, 184–184. https://doi.org/10.3389/fvets.2019.00184

Haneklaus, A. N., Harris, K. B., Griffin, D. B., Edrington, T. S., Lucia, L. M., and Savell, J. W. (2012). *Salmonella* Prevalence in Bovine Lymph Nodes Differs among Feedyards. *Journal of Food Protection*, *75*(6), 1131–1133. https://doi.org/10.4315/0362-028X.JFP-11-530

Hanlon, K. E , Miller, M. F., Guillen, L. M., and Brashears, M. M. (2016). *Salmonella* Presence in Mandibular, Mesenteric, and Subiliac Lymph Nodes Collected from Sheep and Goats in the United States. *Journal of Food Protection*, *79*(11), 1977–1981. https://doi.org/10.4315/0362-028X.JFP-16-193

Harner, J., Murphy, J. P., Boyer, W., Davidson, J., George, H., Graber, R., Minson, S., and Harvey, M. (2021). Planning and Designing Cattle Feedlots.

Herrick, R., Rogers, C., McEvers, T., Amachawadi, R., Nagaraja, T., Maxwell, C., and Lawrence, T. (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*(2), 170–182. https://doi.org/10.15232/aas.2021-02228

Huntington, G. B. (1997). Starch Utilization by Ruminants: From Basics to the Bunk. *Journal of Animal Science, 75*(1), 852-867. https://doi.org/10.2527/1997.753852x

Im, M. C., Seo, K. W., Bae, D. H., and Lee, Y. J. (2016). Bacterial Quality and Prevalence of Foodborne Pathogens in Edible Offal from Slaughterhouses in Korea. *Journal of Food Protection*, *79*(1), 163–168. https://doi.org/10.4315/0362-028X.JFP-15-251

Kallapura, G., Morgan, M. J., Pumford, N. R., Bielke, L. R., Woldfenden, A. D., Faulkner, O. B., Latorre, J. D., Menconi, A., Hernandez-Velasco, X., Kuttappan, V. A., Hargis, B. M., and Tellez, G. (2014). Evaluation of the respiratory route as a viable portal of entry for *Salmonella* in poultry via intratracheal challenge of *Salmonella* Enteritidis and *Salmonella* Typhimurium. *Poultry Science*, *93*(2), 340–346. https://doi.org/10.3382/ps.2013-03602

Ly, K. T., and Casanova, J. E. (2007). Mechanisms of *Salmonella* entry into host cells. *Cellular Microbiology*, *9*(9), 2103–2111. https://doi.org/10.1111/j.1462-5822.2007.00992.x

Mian, L. S., Maag, H., Tacal, J. V. (2002). Isolation of *Salmonella* from muscoid flies at commercial animal establishments in San Bernardino County, California. J. Vector Ecol. 27:82–85

Nagaraja, T. G., and Chengappa, M. M. (1998). Liver abscesses in feedlot cattle: a review. *Journal of Animal Science*, *76*(1), 287–298. https://doi.org/10.2527/1998.761287x

Nickelson, K. J., Taylor, T. M., Griffen, D. B., Savell, J. W., Gehring, K. B., and Arnold, A. N. (2019). Assessment of *Salmonella* Prevalence in Lymph Nodes of U.S. and

Mexican Cattle Presented for Slaughter in Texas. *Journal of Food Protection*, *82*(2), 310–315. https://doi.org/10.4315/0362-028X.JFP-18-288

Owens, F. N., Secrist, D. S., Hill, W. J., and Gill, D. R. (1998). Acidosis in Cattle: A Review. *Journal of Animal Science, 76*(1), 275.

Rescigno, M., Urbano, M., Valzasina, B., Francolini, M., Rotta, G., Bonasio, R., Granucci, F., Kraehenbuhl, J., and Ricciardi-Castagnoli, P. (2001). Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. *Nature Immunology, 2*(4), 361-367. https://doi.org/10.1038/86373

Slyter, L. L (1976). Influence of Acidosis on Rumen function. *Journal of Animal Science, 43*(4), 910-929. https://doi.org/10.2527/jas1976.434910x

Sofos, J. N., Kochevar, S. L., Reagan, J. O., and Smith, A. C. (1999). Incidence of *Salmonella* on Beef Carcasses Relating to the U.S. Meat and Poultry Inspection Regulations. *Journal of Food Protection*, *62*(5), 467–473. https://doi.org/10.4315/0362-028X-62.5.467

Sousa, S., Lecuit, M., and Cossart, P. (2005). Microbial strategies to target, cross or disrupt epithelia. *Current Opinion in Cell Biology*, *17*(5), 489–498. https://doi.org/10.1016/j.ceb.2005.08.013

Vazquez-Torres, A., and Fang, F. C. (2000). Cellular routes of invasion by enteropathogens. *Current Opinion in Microbiology*, *3*(1), 54–59. https://doi.org/10.1016/S1369-5274(99)00051-X

Vieira-Pinto, M., Vieira, M. J., Oliveria, M., Fontes, M. C., Themudo, P., and Semedo-Lemsaddek, T. (2011). *Salmonella* sp. in edible offal (liver and tongue) from pigs slaughtered for consumption. Iowa State University Digital Repository.

Vipham, J. L., Loneragen, G. H., Guillen, L. M., Brooks, J. C., Johnson, B. J., Pond A., Pond, N., and Brashears, M. M. (2015). Reduced Burden of *Salmonella* enterica in Bovine Subiliac Lymph Nodes Associated with Administration of a Direct-fed Microbial. *Zoonoses and Public Health, 62*(8), 599-608. https://doi.org/10.1111/zph.12187

Wang, M., Qazi, I. H., Wang, L., Zhou, G., and Han, H. (2020). *Salmonella* Virulence and Immune Escape. *Microorganisms (Basel), 8*(3), 407. https://doi.org/10.3390/microorganisms8030407

Wells, S. J., Fedorka-Cray, P. J., Dargatz, D. A., Ferris, K., and Green, A. (2001). Fecal Shedding of *Salmonella* spp. by Dairy Cows on Farm and at Cull Cow Markets. *Journal of Food Protection, 64*(1), 3–11. https://doi.org/10.4315/0362-028X-64.1.3

Wottlin, L. R., Edrington, T. S., and Anderson, R. C. (2022). *Salmonella* Carriage in Peripheral Lymph Nodes and Feces of Cattle at Slaughter Is Affected by Cattle Type, Region, and Season. *Frontiers in Animal Science*, *3*. https://doi.org/10.3389/fanim.2022.859800

Wray, C., and Davies, R. H. (2000). *Salmonella* Infections in Cattle. *CAB International*

Xu, Y., Tao, S., Hinkle, N., Harrison, M., and Chen, J. (2018). *Salmonella*, including antibiotic-resistant *Salmonella*, from flies captured from cattle farms in Georgia,

U.S.A. *The Science of the Total Environment, 616-617*, 90–96.

https://doi.org/10.1016/j.scitotenv.2017.10.32

**Table 3.1**. Different observations and outcomes among different feedlots

| | Feedlots | | | | | |
|---|---|---|---|---|---|---|
| Items | A | B | C | D | E | F |
| Initial BW,[1] kg | 296 | 301 | 274 | 279 | 333 | 371 |
| Final BW,[2] kg | 593 | 579 | 561 | 563 | 617 | 616 |
| Distance travelled to feedlot,[3] miles | 370 | 596 | 410 | 380 | 334 | 570 |
| Sorting methods[4] | on arrival | on arrival with min. terminal | on arrival | on arrival | terminal | terminal |
| Capacity,[5] head | 70,000 | 125,000 | 24,000 | 36,000 | 61,000 | 100,155 |
| Feed additives[6] | Rumensin, Optaflexx | Rumensin, Tylan MGA | Rumensin, Tylan, Optalfexx, MGA | Rumensin, Tylan, Optalfexx, MGA | Rumensin, Tylan, Optalfexx, Heifermax/MGA | Rumensin, Tylan, Optalfexx, MGA |
| Bunk space,[7] inches/animal | 6" - 9" | 12" | 9.2" - 12" | 9.2" - 12" | 8" - 12" | 7.75" |
| Ration ingredients[8] | Steam flaked corn, Corn gluten, Alfalfa hay, Corn silage, Whey, Fat, Supplements | Corn, Molasses, Fat, Ensilage, Alfalfa pellets, Cotton burrs, Corn gluten, Supplements | N/A | N/A | Wet distillers, Flaked corn, Sorghum silage, Alfalfa hay, Whey, Tallow, Supplements | Corn silage, Flaked corn, Sweet bran-plus, Tallow, Supplements |

[1]Average BW (body weight) that cattle started at in the feedlot.

[2]Average BW that cattle were shipped out at the feedlot and to the beef processing plant.

[3]Miles that newly received cattle had to travel to arrive at the particular feedlot.

[4]At what time cattle are sorted.

[5]Total capacity of each feedlot by head of cattle.

[6]Feed additives that are found in the ration fed to cattle.

[7]Bunk space allotted per head of cattle in a pen

[8]Common finisher ration ingredients.

**Table 3.2.** Type of cattle fed by each feedlot

| Item | Feedlot | | | | | |
|---|---|---|---|---|---|---|
| Type of cattle,[1] % | A | B | C | D | E | F |
| Stocker | 20 | 65 | 60 | 60 | 66.5 | 40 |
| Auction market | 10 | 9 | 20 | 20 | 3.5 | 16 |
| Mexican | 70 | 21 | 15 | 15 | 9 | 0 |
| Dairy/dairy cross | 0 | 4 | 0 | 0 | 21 | 44 |
| Other[2] | 0 | 1 | 5 | 5 | 0 | 0 |

[1]Percentage of particular cattle type this fed at each feedlot.

[2]Other cattle type would those that did not fit in the categories above such as cull cattle

**Table 3.3.** Prevalence of *Salmonella* by feedlot and quarter with the inclusion of all sample types

| | Prevalence | | | | |
|---|---|---|---|---|---|
| Feedlot | Quarter 1 | Quarter 2 | Quarter 3 | Quarter4 | Total |
| A | 0.00% (0/32) | 0.00% (0/32) | 65.63% (21/32) | 3.13% (1/32) | 17.19% (22/128) |
| B | 8.33% (1/12) | 0.00% (0/32) | 78.13% (25/32) | 6.25% (1/16) | 29.35% (27/92) |
| C | 0.00% (0/32) | 0.00% (0/30) | 81.25% (26/32) | 0.00% (0/32) | 20.63% (26/126) |
| D | 0.00% (0/32) | 0.00% (0/32) | 90.63% (29/32) | 6.25% (2/32) | 24.22% (31/128) |
| E | 0.00% (0/32) | 0.00% (0/32) | 6.25% (2/32) | 90.63% (29/32) | 24.22% (31/128) |
| F | 6.25% (2/32) | 0.00%) (0/32) | 62.50% (20/32) | 9.38% (3/32) | 19.53% (25/128) |
| Total | 1.74% (3/172) | 0.00% (0/190) | 63.40% (123/192) | 20.45% (36/176) | 22.33% (163/730) |

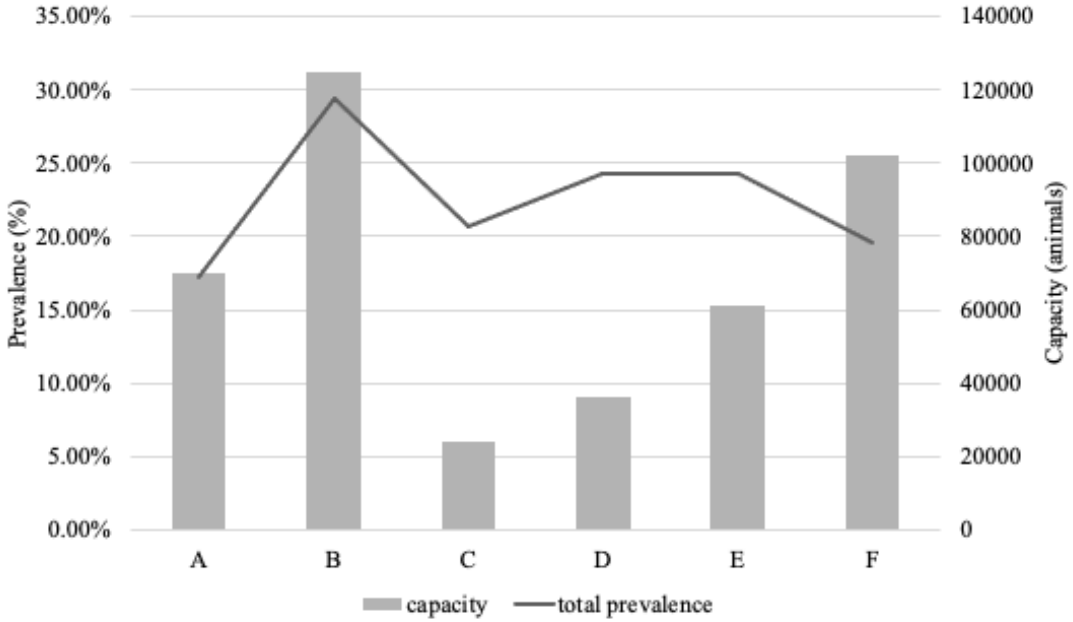


**Figure 3.1**. Capacity of each feedlot by head of cattle in comparison to the total prevalence of *Salmonella* of each feedlot.

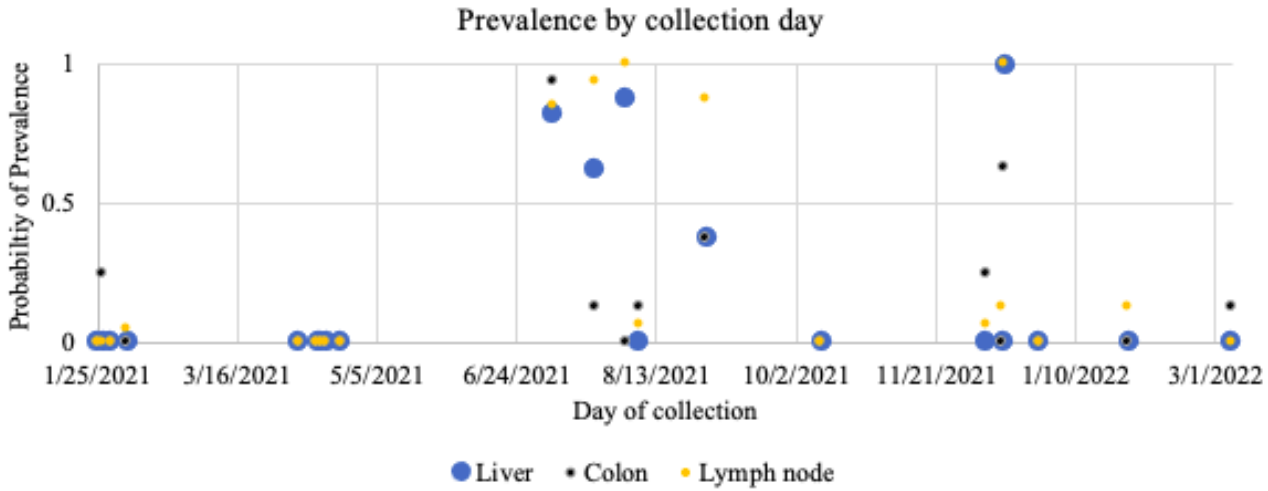


**Figure 3.2.** Prevalence of different sample types by day of collection.

/

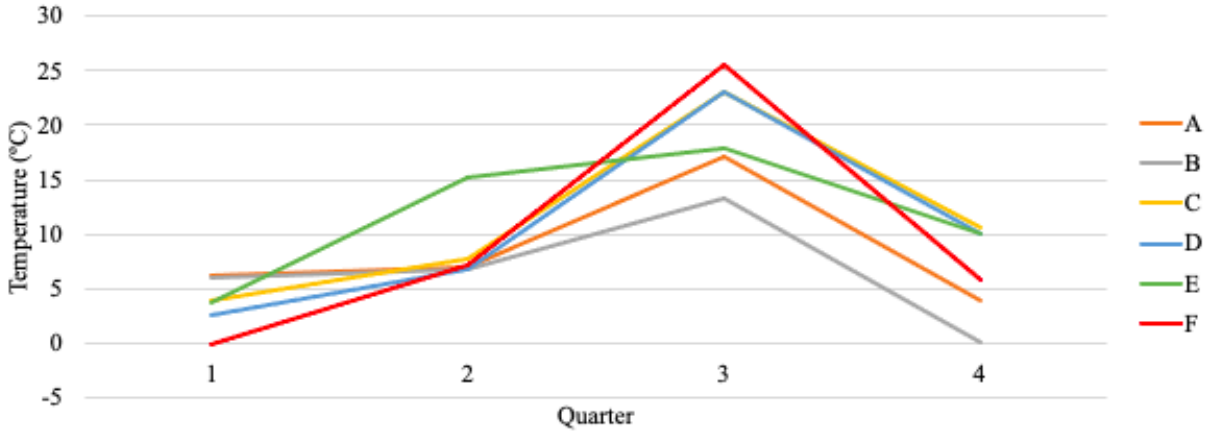


**Figure 3.3.** Average temperature taken six days prior to harvest and day of harvest for each feedlot by quarter.

*I*

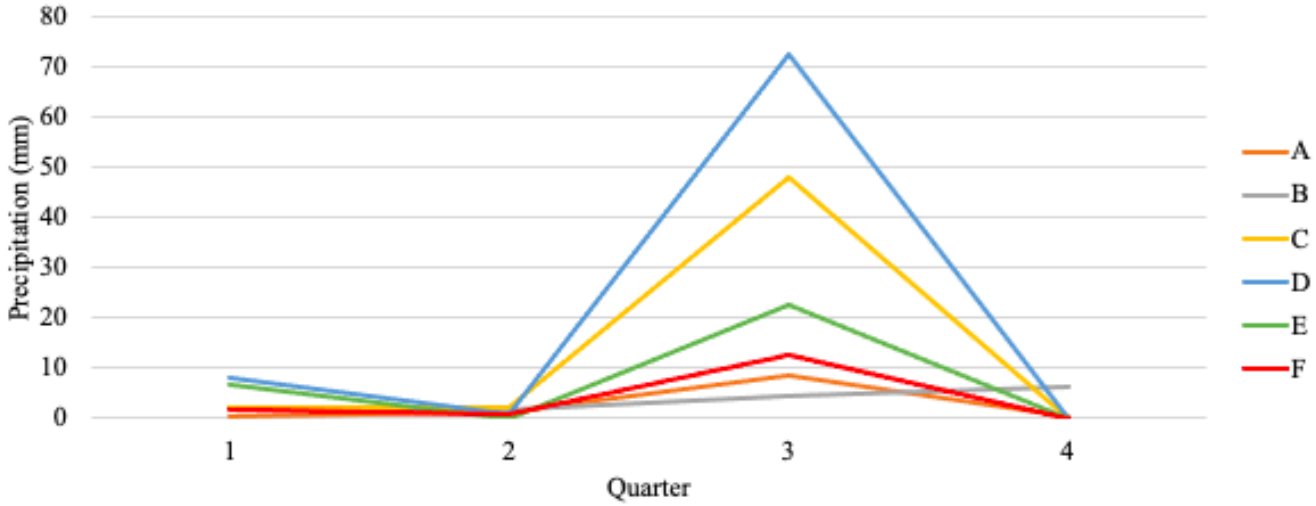


**Figure 3.4.** Total precipitation taken six days prior to harvest and day of harvest for each feedlot by quarter.

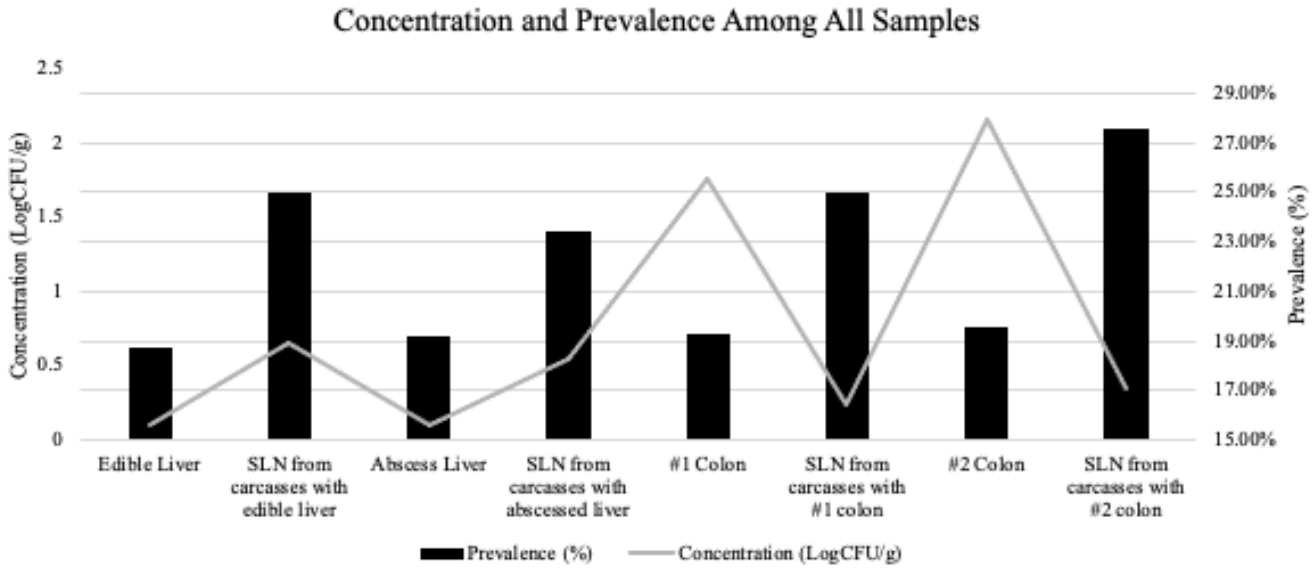


**Figure 3.5.** Concentration and prevalence of *Salmonella* across different samples type for inclusion of the entire study.

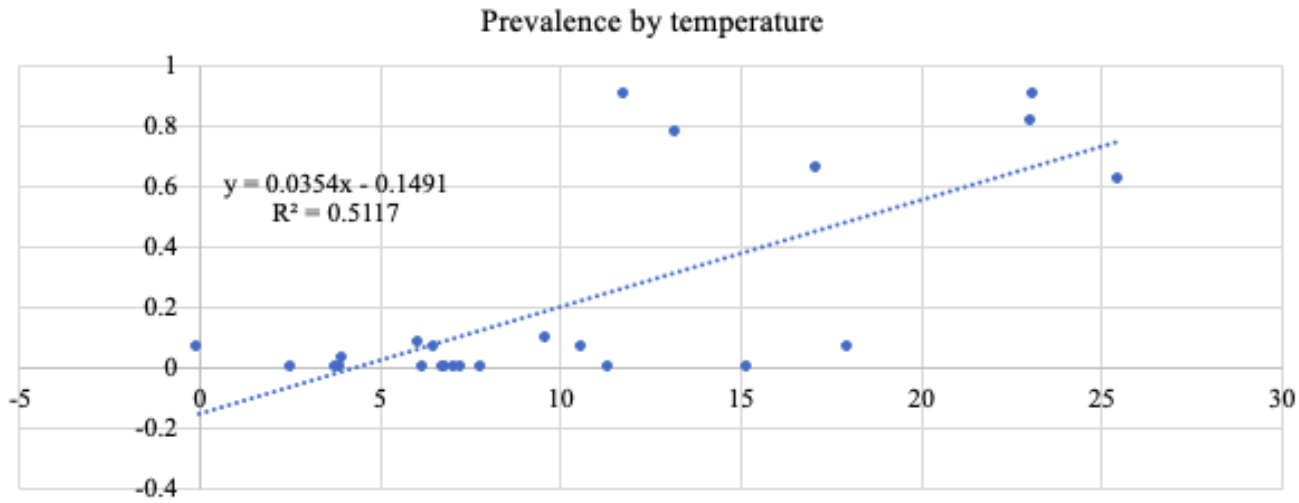


**Figure 3.6.** Sample type prevalence variability based on average temperature taken six days prior to sampling and day of sampling.

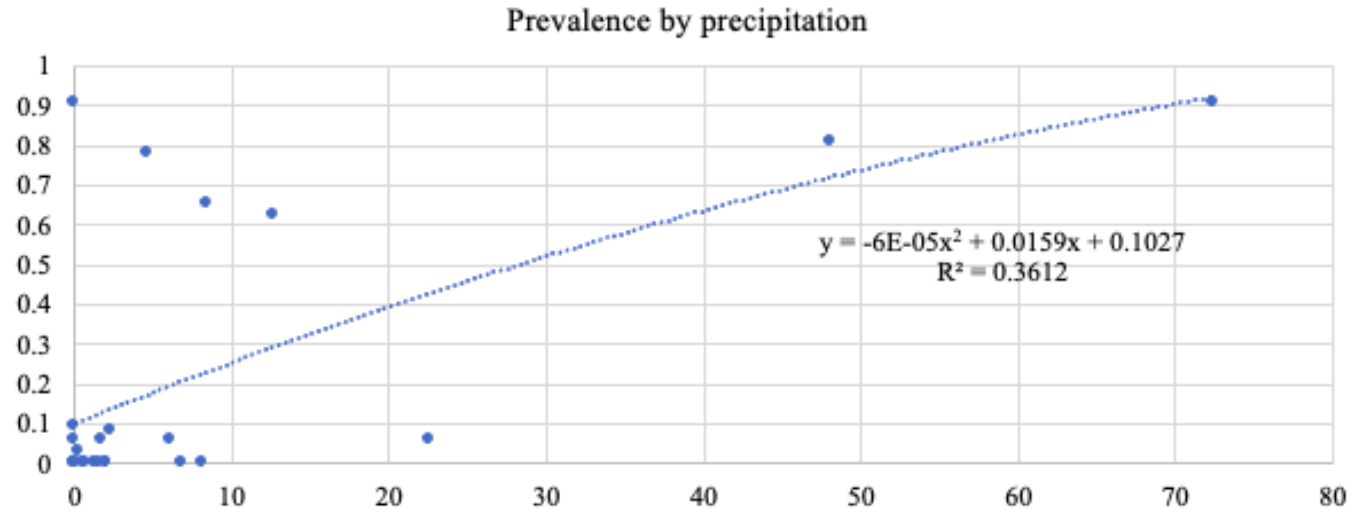


**Figure 3.7.** Sample type prevalence variability based on average precipitation taken six days prior to sampling and day of sampling.

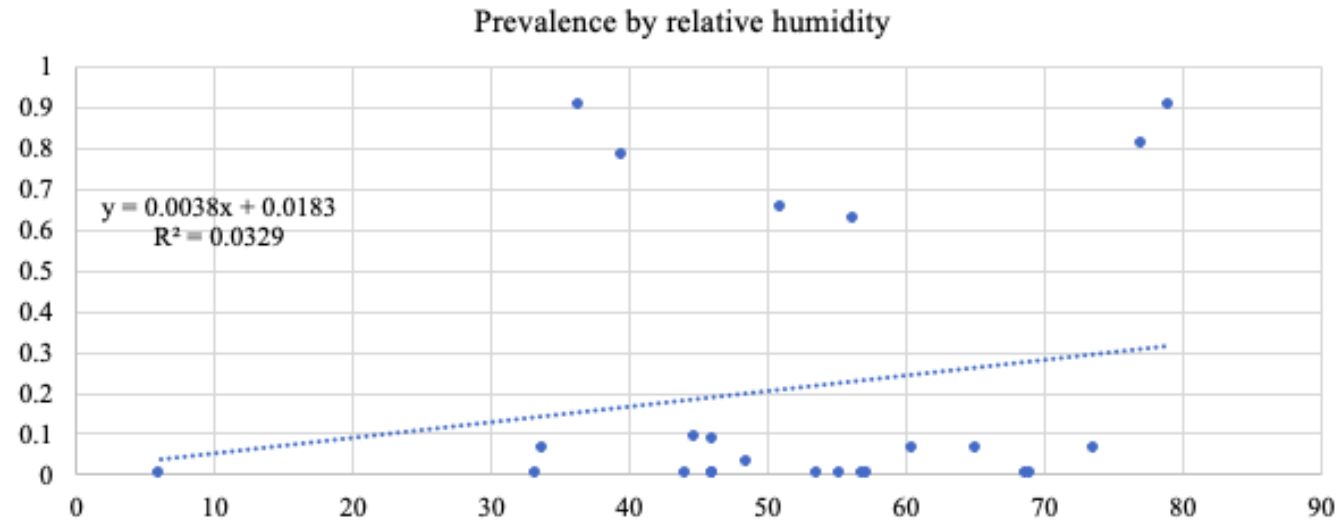


**Figure 3.8.** Sample type prevalence variability based on average relative humidity taken six days prior to sampling and day of sampling.