SALMONELLA SEROVAR DYNAMICS IN CATTLE

by

BRYAN LARSEN

(Under the Direction of Nikki Shariat)

ABSTRACT

Salmonella is a food safety issue in beef products. This work investigated *Salmonella* prevalence and serovar populations in four cattle feedlots using deep serotyping by CRISPR-SeroSeq. The first study investigated *Salmonella* diversity and serovar persistence in feedlot soil layers, cattle pens, and the feedlot environment. In the second study, we assessed *Salmonella* transmission, between fecal and dried manure across two feedlots, for two placements. We identified 37 serovars and showed that 80% of samples contained two or more serovars. On average, samples contained 2.9 serovars (range 1 – 10). In study one, shipping/receiving pens had the highest prevalence (87.5%;14/16), and most complex samples (avg 3.9 serovars/sample). In the second study, we observed that manure samples showed higher serovar complexity than the fecal samples. Our data highlights, the enormous *Salmonella* complexity in feedlot environments.

INDEX WORDS: *Salmonella*, serovars, beef cattle, CRISPR-SeroSeq, food safety

SALMONELLA SEROVAR DYNAMICS IN CATTLE

by

BRYAN LARSEN

BS, University of Georgia, 2020

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment

of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2023

© 2023

Bryan Larsen

All Rights Reserved

SALMONELLA SEROVAR DYNAMICS IN CATTLE

by

BRYAN LARSEN

Major Professor: Nikki Shariat
Committee: Todd R. Callaway
Govindaraj Dev Kumar

Electronic Version Approved:

Ron Walcott
Vice Provost for Graduate Education and Dean of the Graduate School
The University of Georgia
May 2023

DEDICATION

I want to dedicate this to all the friends I've made during my Master's. You all have been incredibly supportive, and this was only possible due to all of you. This is also dedicated to my family, for being there for me during times of stress. I also wanted to thank Nikki for giving me this opportunity, I will always be grateful for you giving me this opportunity.

ACKNOWLEDGEMENTS

I want to thank Dr. Nikki Shariat[1] for being my mentor and main advisor during my Master's. For allowing me to join your lab as an undergraduate and sparking my research interest.

I want to thank Diego Casas[2], Tayler M. Hays[2], Dr. Dale R. Woerner[2], and Dr. Kendra Nightingale[2] at Texas Tech University for their collaboration during this project, namely sample collection and *Salmonella* culturing and detection.

Finally, thanks to Dr. Pejman Rohani[3] and Dr. Christian E. Gunning[3] from the University of Georgia for help with the statistical analysis and figure design.

[1]Department of Population Health, College of Veterinary Medicine, University of Georgia, Athens, Georgia, USA

[2] Department of Animal and Food Sciences, Davis College of Agricultural Science and Natural Resources, Texas Tech University, Lubbock, Texas, USA

[3]Odum School of Ecology, University of Georgia, Athens, Georgia, USA

TABLE OF CONTENTS

Page

ACKNOWLEDGEMENTS v

LIST OF TABLES viii

LIST OF FIGURES ix

CHAPTER

1 INTRODUCTION 1

REFERENCES 4

2 LITERATURE REVIEW 10

FOODBORNE PATHOGEN 10

SALMONELLA SEROVARS 12

MOLECULAR SEROTYPING TECHNIQUES 14

SALMONELLA IN THE CATTLE INDUSTRY 19

SUMMARY 25

REFERENCES 27

3 *SALMONELLA* SEROVAR DYNAMICS IN CATTLE 51

INTRODUCTION 51

MATERIALS AND METHODS 55

RESULTS 61

DISCUSSION 88

CONCLUSION 94

REFERENCES 96

4 SUMMARY 108

REFERENCES 110

LIST OF TABLES

Page

Table 1: Relative frequency of each serovar..72

Table 2: Relative frequency of each serovar by feedlot 77

Table 3: Relative frequency of each serovar by season..78

LIST OF FIGURES

Page

Figure 1: Vectors for Salmonella within feedlots 9

Figure 2: Estimated mean *Salmonella* prevalence by experimental unit 69

Figure 3: Population complexity per feedlot 70

Figure 4: Population complexity per season in feedlot 4 71

Figure 5: Relative frequency of serovars recovered from Feedlot 1 73

Figure 6: Relative frequency of serovars recovered from Feedlot 2 74

Figure 7: Relative frequency of serovars recovered from Feedlot 3 75

Figure 8: Relative frequency of serovars recovered from Feedlot 4 76

Figure 9: *Salmonella* prevalence in fresh feces versus manure pad samples 79

Figure 10: *Salmonella* serovar complexity 80

Figure 11: Sampling schematic for collection of fecal and manure samples 81

Figure 12: Serovar profiles in manure and fecal samples per day in feedlot 2 pen 1 in the summer placement 82

Figure 13: Serovar profiles in manure and fecal samples per day in feedlot 2 Pen 2 summer placement 83

Figure 14: Serovar profiles in manure and fecal samples per day in feedlot 4 Pen 1 summer placement 84

Figure 15: Serovar profiles in manure and fecal samples per day in feedlot 4 pen 2 summer placement 85

Figure 16: Serovar profiles in manure and fecal samples per day in feedlot 4 pen 1 winter placement 86

Figure 17: Serovar profiles in manure and fecal samples per day in feedlot 4 pen 2 winter placement 87

CHAPTER 1

INTRODUCTION

Salmonellosis is the second most commonly diagnosed bacterial foodborne illness in the United States, causing approximately 1.3 million human illnesses yearly (Tack et al., 2020). *Salmonella* enterica is a diverse bacterial species comprised of more than 2,500 serovars; serovars are defined by O and H antigens (Grimont & Weill, 2007). According to an interagency report, 6.0% of *Salmonella* cases are estimated to result from eating contaminated beef (IFSAC,2022). *Salmonella* enterica is associated with many different animals, including many food animal species (IFSAC, 2022)

Salmonella is commonly isolated from cattle feces and hide; while most *Salmonella* infections in cattle are asymptomatic, there are some notable exceptions, such as infections caused by serovars Dublin and Typhimurium (Clinton et al., 1981; Nielsen et al., 2004). *Salmonella* has been isolated from cattle feces, lymph nodes, hide, liver, and colon (Amachawadi & Nagaraja, 2015; Gragg et al., 2013; Porwollik et al., 2018). Contaminated ground beef is thought to be caused by the inclusion of infected lymph nodes, during slaughter, adipose tissue is used to get the desired degree of fat content within the product; lymph nodes contained within the adipose tissue can contaminate the end product (Gutiérrez et al., 2020; Samuel et al., 1979; Webb et al., 2017). Numerous outbreaks have been linked to contaminated ground beef, such as an outbreak of serovar Dublin in 2019, serovar Newport in 2018, and serovar Typhimurium in 2013 (Laufer et al., 2015; Roels" et al., 1997).

Most *Salmonella* mitigation methods have been aimed toward reducing pathogen load prior to slaughter, while some studies have looked at reducing *Salmonella* from other incoming vectors (Hamilton et al., 2021; LeJeune et al., 2001; Parker et al., 2022; Smith et al., 2022).

Studies have investigated potential sources of *Salmonella* in feedlots, for example, wild birds, flies, contaminated feed, and contaminated water troughs (figure 1)(Elser et al., 2019; LeJeune et al., 2001; Olafson et al., 2016; Parker et al., 2022; You et al., 2006). Studies have also investigated the efficacy of the mitigation methods at slaughter and incoming *Salmonella* dynamics due to the co-mingling of cattle of different origins; to better understand the effects this has on *Salmonella* in the cattle pens (Levent et al., 2021). Currently, little has been done to investigate the transmission of *Salmonella* in the feedlot environment and *Salmonella* colonizing cattle.

Serovars are defined by their lipopolysaccharide (O) and flagellar (H) antigens (Grimont & Weill, 2007). Serovars have different phenotypes; these phenotypes make *Salmonella* ubiquitous in the environment; some serovars have increased antibiotic resistance, are better biofilm producers, or have increased thermal tolerance (Ng et al., 1969; Obe et al., 2022; Vosik et al., 2018). These phenotypic differences can yield survival advantages in different environments, including environments with low water activity or high pH (Santillana Farakos et al., 2013; Wilmes-Riesenberg et al., 1996).

The main objective of this work was to examine *Salmonella* dynamics between the soil and cattle in feedlot environments and to track these dynamics over time. Four commercial feedlots were visited during this work, and all *Salmonella*-positive samples were analyzed with CRISPR-SeroSeq (CSS). This is a genomic deep serotyping tool that differentiates between *Salmonella* serovars based on their native CRISPR sequences and reports the relative frequency of each serovar within a sample (Thompson et al., 2018). This work showed, for the first time, that *Salmonella* populations in cattle feedlot environments are complex, consisting of an average of

2.9 serovars per sample. Across the two studies presented, four out of five samples contained two or more serovars.

REFERENCES

Amachawadi, R. G., & Nagaraja, T. G. (2015). First Report Of Anaerobic Isolation Of *Salmonella* Enterica From Liver Abscesses Of Feedlot Cattle. In *Journal Of Clinical Microbiology* (Vol. 53, Issue 9, Pp. 3100–3101). American Society For Microbiology. https://doi.org/10.1128/jcm.01111-15

Clinton, N. A., Weaver, R. W., & Hidalgo, R. J. (1981). Transmission Of *Salmonella* Typhimurium Among Feedlot Cattle After Oral Inoculation. *Journal Of Applied Bacteriology*, *50*(1), 149–155. Https://Doi.Org/10.1111/J.1365-2672.1981.Tb00879.X

Elser, J. L., Adams Progar, A. L., Steensma, K. M. M., Caskin, T. P., Kerr, S. R., & Shwiff, S. A. (2019). Economic And Livestock Health Impacts Of Birds On Dairies: Evidence From A Survey Of Washington Dairy Operators. In *Plos ONE* (Vol. 14, Issue 9). Public Library Of Science. https://doi.org/10.1371/journal.pone.0222398

Gragg, S. E., Loneragan, G. H., Nightingale, K. K., Brichta-Harhay, D. M., Ruiz, H., Elder, J. R., Garcia, L. G., Miller, M. F., Echeverry, A., Ramírez Porras, R. G., & Brashears, M. M. (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

Grimont, P., & Weill, F. (2007). Antigenic Formulae Of The *Salmonella* Serovars. *WHO Collaborating Centre For Reference And Research On Salmonella*, *9th Editio*.

Gutiérrez, T. P., Lozano, M. S. R., Suárez, E. J. D., Guzmán, N. R., Ramos, O. S., Pérez, C. F. H., & Medina, R. D. M. (2020). Lymph Nodes And Ground Beef As Public Health Importance Reservoirs Of *Salmonella* Spp. *Revista Mexicana De Ciencias Pecuarias*, *11*(3), 795–810. https://doi.org/10.22319/rmcp.v11i3.5516

Hamilton, A. M., Paulsen, D. J., Trout Fryxell, R. T., Orta, V. E., Gorman, S. J., Smith, D. M., Buchanan, J. R., Wszelaki, A. L., & Critzer, F. J. (2021). Prevalence Of *Salmonella* Enterica In Flies On A Diversified Cattle And Fresh Produce Farm Across Two Growing Seasons. *Journal Of Food Protection*, *84*(6), 1009–1015. https://doi.org/10.4315/jfp-20-339

Ifsac. (2021). *Foodborne Illness Source Attribution Estimates For 2019 For Salmonella, Escherichia Coli O157, Listeria Monocytogenes, And Campylobacter Using Multi-Year Outbreak Surveillance Data, United States*. Https://Www.Cdc.Gov/Foodsafety/Ifsac/Projects/Index.Html.

Ifsac. (2022). *Foodborne Illness Source Attribution Estimates For 2020 For Salmonella, Escherichia Coli O157, And Listeria Monocytogenes Using Multi-Year Outbreak Surveillance Data, United States*. https://www.cdc.gov/foodsafety/ifsac/projects/index.html.

Laufer, A. S., Grass, J., Holt, K., Whichard, J. M., Griffin, P. M., & Gould, L. H. (2015). Outbreaks Of *Salmonella* Infections Attributed To Beef --United States, 1973-2011. *Epidemiology And Infection*, *143*(9), 2003–2013. https://doi.org/10.1017/s0950268814003112

Lejeune, J. T., Besser, T. E., Merrill, N. L., Rice, D. H., & Hancock, D. D. (2001). Livestock Drinking Water Microbiology And The Factors Influencing The Quality Of Drinking Water Offered To Cattle. *Journal Of Dairy Science*, *84*(8), 1856–1862. https://doi.org/10.3168/jds.s0022-0302(01)74626-7

Levent, G., Schlochtermeier, A., Ives, S. E., Norman, K. N., Lawhon, S. D., Loneragan, G. H., Anderson, R. C., Vinasco, J., Den Bakker, H. C., & Scott, H. M. (2021). High-Resolution Genomic Comparisons Within *Salmonella* Enterica Serotypes Derived From Beef Feedlot Cattle: Parsing The Roles Of Cattle Source, Pen, Animal, Sample Type, And Production Period. *Applied And Environmental Microbiology*, *87*(12), 1–20. https://doi.org/10.1128/aem.00485-21

Ng, H., Bayne, H. G., & Garibaldi, J. A. (1969). Heat Resistance Of *Salmonella*: The Uniqueness Of *Salmonella* Senftenberg 775W. In *APPLIED MICROBIOLOGY*.

Nielsen, L. R., Schukken, Y. H., Gröhn, Y. T., & Ersbøll, A. K. (2004). *Salmonella* Dublin Infection In Dairy Cattle: Risk Factors For Becoming A Carrier. *Preventive Veterinary Medicine*, *65*(1–2), 47–62. Https://Doi.Org/10.1016/J.Prevetmed.2004.06.010

Obe, T., Richards, A. K., & Shariat, N. W. (2022). Differences In Biofilm Formation Of *Salmonella* Serovars On Two Surfaces Under Two Temperature Conditions. *Journal Of Applied Microbiology*, *132*(3), 2410–2420. https://doi.org/10.1111/jam.15381

Olafson, P. U., Brown, T. R., Lohmeyer, K. H., Harvey, R. B., Nisbet, D. J., Loneragan, G. H., & Edrington, T. S. (2016). Assessing Transmission Of *Salmonella* To Bovine Peripheral Lymph Nodes Upon Horn Fly Feeding. *Journal Of Food Protection*, *79*(7), 1135–1142. https://doi.org/10.4315/0362-028x.jfp-15-414

Parker, E. M., Parker, A. J., Short, G., O'Connor, A. M., & Wittum, T. E. (2022). *Salmonella* Detection In Commercially Prepared Livestock Feed And The Raw Ingredients And Equipment Used To Manufacture The Feed: A Systematic Review And Meta-Analysis. *Preventive Veterinary Medicine*, *198*. Https://Doi.Org/10.1016/J.Prevetmed.2021.105546

Porwollik, S., Genovese, K., Chu, W., Loneragan, G. H., Edrington, T., & Mcclelland, M. (2018). Neutral Barcoding Of Genomes Reveals The Dynamics Of *Salmonella* Colonization In Cattle And Their Peripheral Lymph Nodes. *Veterinary Microbiology*, *220*(April), 97–106. https://doi.org/10.1016/j.vetmic.2018.05.007

Roels", T. H., Frazak$, P. A., Kazmierczak#, J. J., Mackenzie, W. R., Proctor#, M. E., Kurzynski% □□□, T. A., & Davis#, J. P. (1997). Incomplete Sanitation Of A Meat Grinder And

Ingestion Of Raw Ground Beef : Contributing Factors To A Large Outbreak Of *Salmonella* Typhimurium Infection. In *Epidemiol. Infect* (Vol. 119). Cambridge University Press.

Samuel, J. L., O'Boyle, D. A., Mathers, W. J., & Frost, A. J. (1979). Isolation Of *Salmonella* From Mesenteric Lymph Nodes Of Healthy Cattle At Slaughter. *Research In Veterinary Science*, 238–241.

Santillana Farakos, S. M., Frank, J. F., & Schaffner, D. W. (2013). Modeling The Influence Of Temperature, Water Activity And Water Mobility On The Persistence Of *Salmonella* In Low-Moisture Foods. *International Journal Of Food Microbiology*, *166*(2), 280–293. https://doi.org/10.1016/j.ijfoodmicro.2013.07.007

Smith, K. V., Delong, K. L., Boyer, C. N., Thompson, J. M., Lenhart, S. M., Strickland, W. C., Burgess, E. R., Tian, Y., Talley, J., Machtinger, E. T., & Trout Fryxell, R. T. (2022). A Call For The Development Of A Sustainable Pest Management Program For The Economically Important Pest Flies Of Livestock: A Beef Cattle Perspective. *Journal Of Integrated Pest Management*, *13*(1). https://doi.org/10.1093/jipm/pmac010

Tack, D. M., Ray, L., Griffin, P. M., Cieslak, P. R., Dunn, J., Rissman, T., Jervis, R., Lathrop, S., Muse, A., Duwell, M., Smith, K., Tobin-D'angelo, M., Duc, ;, Vugia, J., Joanna, ;, Kufel, Z., Beverly, ;, Wolpert, J., Tauxe, ; Robert, & Payne, D. C. (2020). *Preliminary Incidence And Trends Of Infections With Pathogens Transmitted Commonly Through Food — Foodborne Diseases Active Surveillance Network, 10 U.S. Sites, 2016–2019*. https://www.cdc.gov/mmwr/mmwr_continuingeducation.html

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 CRISPR-Seroseq FOOD MICROBIOLOGY Crossm

Downloaded From. *Aem.Asm.Org 1 Applied And Environmental Microbiology*, *84*, 1859–1877. Https://Doi.Org/10.1128/AEM.01859-18

Vosik, D., Tewari, D., Dettinger, L., M'ikanatha, N. M., & Shariat, N. W. (2018). CRISPR Typing And Antibiotic Resistance Correlates With Polyphyletic Distribution In Human Isolates Of *Salmonella* Kentucky . *Foodborne Pathogens And Disease*, *15*(2), 101–108. https://doi.org/10.1089/fpd.2017.2298

Webb, H. E., Brichta-Harhay, D. M., Brashears, M. M., Nightingale, K. K., Arthur, T. M., Bosilevac, J. M., Kalchayanand, N., Schmidt, J. W., Wang, R., Granier, S. A., Brown, T. R., Edrington, T. S., Shackelford, S. D., Wheeler, T. L., & Loneragan, G. H. (2017). *Salmonella* In Peripheral Lymph Nodes Of Healthy Cattle At Slaughter. *Frontiers In Microbiology*, *8*(NOV), 1–10. https://doi.org/10.3389/fmicb.2017.02214

Wilmes-Riesenberg, M. R., Bearson, B., Foster, J. W., & Iii, R. C. (1996). Role Of The Acid Tolerance Response In Virulence Of *Salmonella* Typhimurium. In *INFECTION AND IMMUNITY* (Vol. 64, Issue 4).

You, Y., Rankin, S. C., Aceto, H. W., Benson, C. E., Toth, J. D., & Dou, Z. (2006). Survival Of *Salmonella* Enterica Serovar Newport In Manure And Manure-Amended Soils. *Applied And Environmental Microbiology*, *72*(9), 5777–5783. https://doi.org/10.1128/aem.00791-06

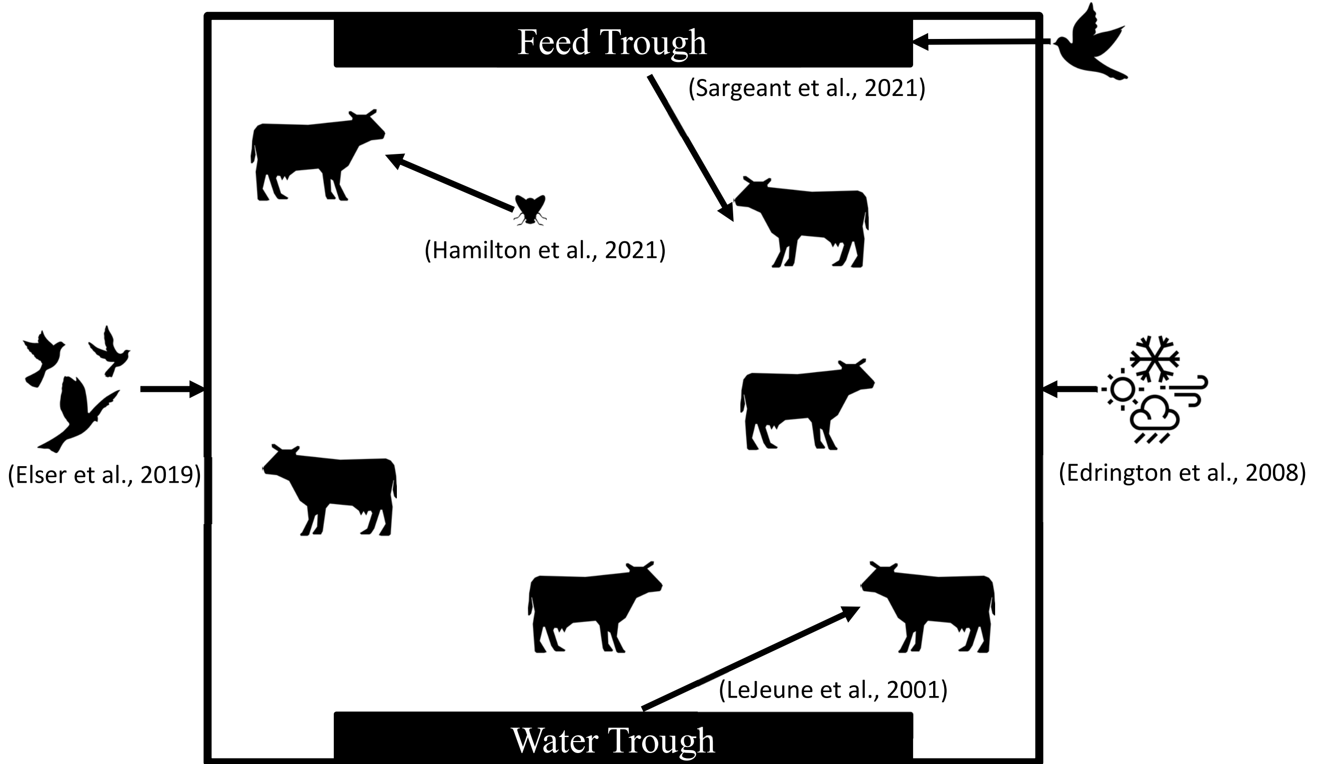


Figure 1. Vectors for *Salmonella* within the feedlot.

This figure shows, some of the vectors for *Salmonella* to enter the feedlot and infect the cattle. Those vectors being wild birds, animal feed, horn flies, season stress induced shedding, and *Salmonella* in the water trough.

CHAPTER 2

LITERATURE REVIEW

Foodborne Pathogens

In the United States, in 2018 there were 49 million estimated cases of foodborne illness acquired from *Campylobacter*, *Cyclospora*, *Listeria*, *Salmonella*, shiga-toxin producing *Escherichia coli*, *Shigella*, *Vibrio*, and *Yersinia* (Tack et al., 2020). The five most common foodborne pathogens are Norovirus, *Campylobacter*, *Clostridium Perfringens*, non-typhoidal *Salmonella*, and *Staphylococcus aureus* (CDC 2020). Globally, there are an estimated 600 million cases of foodborne illness and 420,000 deaths (World Health Organization. Foodborne Disease Burden Epidemiology Reference Group, 2015). Among these foodborne pathogens, *Campylobacter* and *Salmonella* are the top bacterial cause of foodborne illness in the U.S. (CDC 2020). *Campylobacter* causes 1.7 million illnesses annually in the United States, whereas *Salmonella* causes 1.3 million (Collins et al., 2022).

<u>Salmonella</u>

Salmonella is a gram-negative rod-shaped bacterium that can be divided into two species, *S. bongori* and *S. enterica* (Christensen et al., 1998; McQuiston et al., 2008). *Salmonella* enterica is further divided into six subspecies; *enterica, indica, salamae, houtenae, diarizonae,* and *arizonae* (Desai et al., 2013). Of the six subspecies, subspecies *enterica* is responsible for ~99% of illnesses associated with *Salmonella* (Desai et al., 2013; McQuiston et al., 2008; Porwollik et al., 2004). *Salmonella* enterica subspecies *enterica* contains more than 1,500 serovars (Grimont & Weill, 2007). The 1.3 million annual cases of Salmonellosis result in an economic loss of more than $3.5 million (Hoffmann et al., 2015; Majowicz et al., 2010; Scallan et al., 2011; Scharff, 2012; Tack et al., 2020). *Salmonella* is ubiquitous; and has been linked to numerous

outbreaks in products such as peanut butter, cereal, chocolate, and beef (Centers for Disease Control and Prevention, 2018; Laufer et al., 2015; Sheth et al., 2011; Werber et al., 2005). Environmentally, *Salmonella* has been found in water and soil (Hekman et al., 1994; Liu et al., 2018); this is relevant to outbreaks due to contamination of produce (Jacobsen & Bech, 2012). This demonstrates that *Salmonella* can survive under various conditions, such as low pH, low water activity, and high temperature (Dev Kumar et al., 2018; Holley et al., 2006; Jechalke et al., 2019; H. Liu et al., 2018; Ng et al., 1969; Samadpour et al., 2006) and that it is adapted to grow in different animal hosts.

Salmonellosis in Humans

Salmonellosis is an infection caused by the bacterium *Salmonella*, typically caused by invasive serovars (Cheng et al., 2019). In humans, Salmonellosis is often denoted by symptoms of diarrhea, vomiting, and nausea; caused by invasion of the gastrointestinal tract (Arthur, Bosilevac, et al., 2008; Ijabadeniyi et al., 2011; Sánchez-Vargas et al., 2011; Sheth et al., 2011; Strauss et al., 2001). After the initial infection, *Salmonella* invades the epithelial cells via a type three secretion system (T3SS) (Rosselin et al., 2011). *Salmonella* infection causes 23,000 hospitalizations and 450 deaths annually (Tack et al., 2020). In many instances, *Salmonella* can colonize animals but does not result in symptoms, and this poses a challenge when monitoring food animals for potential contamination. *Salmonella* infections are often the result of consuming food products from animals colonized with *Salmonella*; some examples are poultry, cattle, and swine. (Andrés-Barranco et al., 2014; Cason et al., 2011; Gutema et al., 2019; Holschbach & Peek, 2018; Rostagno et al., 2005; Shah et al., 2017; Webb et al., 2017).

Salmonella Serovars

Differences in serovar phenotypes

Serovars are genetically distinct, leading to phenotypic differences between serovars (Worley et al., 2018). Such differences include host restriction, virulence, thermal tolerance, and antimicrobial resistance. Host restricted serovars (i.e., serovars Gallinarum and Typhi), can only be found in one host, as opposed to serovar Typhimurium which is a host generalist (Cohen et al., 2021). Serovar Gallinarum is host restricted to poultry, whereas serovar Typhi is host restricted to humans, causing fowl typhoid and typhoid fever, respectively (Kidgell et al., 2002; Shivaprasad, 2000). An example of a host adapted serovar is serovar Choleraesuis, which is adapted to infect swine (Uzzau et al., 2000). Serovars can differ in their propensity to cause illness due to differences in virulence factors (Andino & Hanning, 2015). One example is serovar Javiana, which has a gene encoding for *Salmonella*-cytolethal distending toxin (S-CDT) (Miller & Wiedmann, 2016). The genetic variances in serovars can yield different advantages for survival; one example is serovar Kentucky, which is known for having increased antimicrobial resistance (Sharma et al., 2020; Vosik et al., 2018). Another example is serovar Senftenberg, which is known for having increased thermal tolerance (Ng et al., 1969). The ability to form biofilms can vary between serovars; an example is Schwarzengrund, which despite changes in temp and attachment surface, forms robust biofilms as opposed to serovar Newport, which was consistently a weak biofilm former (Obe et al., 2022). Serovars can survive in different environments with varying pH (Berrang et al., 2015; Cox et al., 2013; Kataria et al., 2020). Serovar Tennessee can survive in low water activity environments that do not support microbial growth, making it a concern for contamination in other food products like pasta and peanut butter

(CDC, 1993; Morasi et al., 2022; Sheth et al., 2011). While most serovars cause enteric distress, serovars typhi and paratyphi cause typhoid and paratyphoid fever, respectively (Santos et al., 2001). In cattle, serovar Dublin causes systemic infection and respiratory distress in calves (Paudyal et al., 2019; Srednik et al., 2021). The severity of infections varies by serovar; T. F Jones et al. (2008) found that hospitalization rates vary by serovar, and the rate of invasive disease also varies by serovar.

Polyphyly in *Salmonella*

Additionally, ten percent of *Salmonella* serovars are polyphyletic, meaning while genetically distinct, the serovars share O and H antigens; this makes it difficult to distinguish between the two via serotyping (Barsoum & Awad, 1972; Worley et al., 2018). Some examples of polyphyletic serovars are Montevideo, Kentucky, and Newport (Yin et al., 2020). Yin et al. (2020) showed the genetic differences and clustering of polyphyletic serovars Bareilly, Saintpaul, Muenchen, and Montevideo using a maximum likelihood tree based on seven MLST genes. Showing that one group of Saintpaul clusters closely to Typhimurium while the other clusters closely to Paratyphi B (Yin et al., 2020). Another example of how polyphyletic serovars differ from each other are the CRISPR arrays. Examination of the CRISPR arrays of polyphyletic serovar Kentucky revealed completely different arrays; further examination into the differences between the two revealed different antimicrobial resistance profiles; serovar Kentucky I was resistant to amoxicillin, cefoxitin, and ceftriaxone whereas, serovar Kentucky II was resistant to nalidixic acid, sulfisoxazole, gentamicin, and ciprofloxacin (Vosik et al., 2018).

Molecular Serotype Identification

Aside from traditional serum agglutination, there are many methods for serovar identification, such as SeqSero, *Salmonella* in silico typing resource (SISTR), Intergenic Sequence Ribotyping (ISR), *Salmonella* Multiplex Assay for Rapid Typing (SMART) assay, Clustered Regulatory Interspersed Short Palindromic Repeats (CRISPR) typing, and qPCR (Bugarel et al., 2018; Fabre et al., 2012; Guard et al., 2012, 2012b; Richards et al., 2020; Yoshida et al., 2016; Zhang et al., 2019). Seqsero and SISTR use whole genome sequence (WGS) information to determine the serovar identity. SeqSero is a platform that predicts serovar identification based on the genes that encode the somatic and flagellar antigens (Zhang et al., 2019). In contrast, SISTR uses genoserotyping paired with seven multilocus sequence typing genes (MLST) and core genome multilocus sequence typing (cgMLST) to determine the serovar identity based on the WGS (Yoshida et al., 2016). Other molecular methods not utilizing WGS for identification include ISR, a PCR-based method that amplifies the region between the 23S and 5S ribosomal genes (Guard et al., 2012). The SMART assay uses 16 gene targets in a high throughput multiplex PCR (Leader et al., 2009). When paired with traditional serotyping through pulse-field gel electrophoresis (PFGE), it correctly identifies serovars 89.6% of the time. Fabre et al. (2012) showed that the two CRISPR arrays in *Salmonella* contain unique spacer sequences, which can be used to differentiate serovars. The fact that serovars contain unique spacer sequences allows for a real-time PCR-based approach. This approach uses probes to attach to specific spacer sequences (Bugarel et al., 2018; Richards et al., 2020). Another molecular typing tool, multi-locus sequence typing (MLST), uses seven housekeeping gene loci to characterize

Salmonella serovars (Achtman et al., 2012). These gene loci can characterize *Salmonella* into sequence types, which can differentiate within a serovar (Achtman et al., 2012).

Salmonella isolation

The Food and Drug Administration Bacterial Analytic Manual (FDA BAM) procedure for *Salmonella* isolation is a multi-step process that takes several days (Andrews et al., 2022). Samples are first pre-enriched in buffered peptone water (BPW) and then subcultured into selective enrichment media, which prevent the growth of bacteria other than *Salmonella*. Rappaport Vassiliadis (RV) and tetrathionate (TT) broths are selective for *Salmonella* isolation. Finally, a selective indicator media such as xylose lysine tergitol 4 (XLT-4) or brilliant green sulfa (BGS) is used to identify presumptive *Salmonella* colonies. BPW is used for the pre-enrichment of *Salmonella and* is a non-selective media that allows the resuscitation of injured bacteria. RV broth is a selective enrichment broth for *Salmonella* composed of two selective agents, magnesium chloride and malachite green. These ingredients work by suppressing the growth of gram-positive bacteria and preventing the growth of other coliforms (Rappaport et al., 1956; Vassiliadis et al., 1981). TT enriches *Salmonella* by providing an electron acceptor that can be utilized in place of oxygen as a terminal electron acceptor (Knox, 1943; Palumbo & Alford, 1970). Serovars that can survive at a low pH may be better able to survive in RV broth than serovars that are less acid tolerant. The biases introduced by culturing media skew the *Salmonella* serovar population recovered. RV and TT are commonly used in parallel for *Salmonella* isolation due to their different selection methods; using them in parallel results in a higher chance of isolating *Salmonella*.

XLT-4 media differentiates *Salmonella* based on the production of H_2S, resulting in black colonies, on BGS *Salmonella* colonies appear as pink-white colonies surrounded by a red

halo (Miller et al., 1991; Poisson, 1992; Richardson et al., 2019). H_2S-negative *Salmonella* colonies cannot be detected via standard isolation on XLT-4, and multiple media are suggested to be able to capture these. Some other media used for *Salmonella* selection and differentiation include (BGS), Hektoen Enteric agar (HE), and Chromogenic agar for *Salmonella* Esterase (CASE). These other selective differential agar media can be used as additional methods for *Salmonella* isolation and detection. Due to different methods for indicating *Salmonella*, CASE and HE agar colonies are identified as blue-green colonies (Hektoen et al., 1968; Moats & Kinner, 1974). Further confirmation can be done with biochemical tests such as Triple Sugar Iron Agar (TSI), or Lysin Iron Agar (LIA) can be used as a confirmation of *Salmonella* via the color of the slant and butt of the media, pH shifts in the media, and production of H_2S (Andrews et al., 2022).

Limitations to *Salmonella* culture isolation

Salmonella isolation is not without faults; both TT and RV broth need to be deployed in parallel to ensure isolation; there is a selection bias during enrichment (Cox et al., 2019; Gorski, 2012; Larsen et al., 2021; Obe et al., 2021; Singer et al., 2009). It has been observed that TT and RV can preferentially enrich different serovar(s); for example, TT broth enriches serovar Enteritidis, whereas RV broth was observed to enrich serovar Schwarzengrund preferentially (Cox et al., 2019; Gorski, 2012; Singer et al., 2009). Plating media can add another layer of bias, exacerbating existing biases (Larsen et al., 2021). Larsen et al. (2021) found that competing serovars Montevideo and Typhimurium, in set ratios of 1:1, 10:1, and 100:1 and their inverse in BPW, RV, and TT, resulted in a consistent pattern; Typhimurium consistently outcompeted Montevideo when starting as the minority serovar in TT media. Singer et al. (2009) competed one strain of serovar Newport, two strains of serovar Typhimurium, and one strain of serovar

Enteritidis against each other in tetrathionate broth. Singer et al. (2009) found that serovar Newport routinely outcompeted the other serovars, despite the starting concentration. Gorski et al. (2012) competed serovars from different serogroups and observed serogroups C1, C2, and E were most often the dominant serogroup within RV culture.

The microbiology laboratory guidebook (MLG) recommends picking at least three colonies (USDA FSIS, 2023). Serovars that can survive at a low pH may be better able to survive in RV broth than serovars that are less acid tolerant. The biases introduced by culturing media skew the *Salmonella* serovar population recovered.

Salmonella Populations

After identifying *Salmonella* via selective differential media, 1-3 colonies are picked. This introduces a significant limitation: where multiple serovars are present in the same sample, picking a small number of colonies will typically only identify the most abundant serovar in a sample. Therefore, low-abundance serovars remain undetected. Siceloff et al. (2022) showed from 28 serovars found in the broiler breeder environment, 16 appeared more often in the minority of a sample. Serovars hidden due to low abundance in a population can be overlooked but could be highly associated with human illness or antimicrobial resistance. Failure to identify serovars commonly associated with human illness because it's not highly abundant in a sample can result in a false sense of security. The incredible diversity within serovars' means that the inability to characterize whole populations could spell disaster, potentially the majority serovar could be serovar Kentucky, which is not commonly associated with human illness, while one background serovar could be Typhimurium (Haley et al., 2016; Rauch et al., 2018; Vosik et al., 2018).

One method to fully characterize *Salmonella* populations is CSS, a deep serotyping method that resolves the relative frequency of multiple serovars in a single sample (Thompson et al., 2018). CRISPR-SeroSeq exploits the serovar-specific spacer sequences within the *Salmonella* CRISPR arrays (Bugarel et al., 2018; Fabre et al., 2012; Thompson et al., 2018). CRISPR-SeroSeq has been used to characterize media biases in *Salmonella* isolation (Larsen et al., 2021), freshwater (Deaven et al., 2021), animal feed and feed ingredients (N. W. Shariat et al., 2022) and in broiler production and processing (Rasamsetti et al., 2021, 2022; Rasamsetti & Shariat, 2023; Siceloff et al., 2022; Thompson et al., 2018). In the feed study, up to 11 serovars were detected in a single sample from blood meal (Shariat et al., 2022), as well as from the broiler breeder environment (Siceloff et al., 2022). In the broiler breeder poultry study, 32% of *Salmonella*-positive samples contained two or more serovars (Siceloff et al., 2022), and in water samples, 80% contained more than one serovar (Deaven et al., 2021). This new research avenue has highlighted that *Salmonella* often occurs in complex populations that consist of multiple serovars. Characterizing the entire population rather than the serovar in the majority can greatly help food safety by showing serovars commonly associated with human illness hidden in the minority of a sample.

<u>CRISPR-SeroSeq</u>

Salmonella has two CRISPR arrays composed of invariable direct repeats and variable spacers (Bugarel et al., 2018; N. Shariat et al., 2015). The combination of the spacers in the CRISPR arrays creates a profile that differentiates between serovars; this includes the differentiation of polyphyletic serovars (Bugarel et al., 2018; N. Shariat et al., 2013; N. Shariat & Dudley, 2014; Thompson et al., 2018). The overall relative frequencies of serovars can also be determined based on the amount of sequence reads attributed to each serovar. Reads are

attributed to serovars by running the sequences through a database and comparing them to sequences submitted to the NCBI BLAST database. The CRISPR-SeroSeq database comprises the CRISPR profiles of over 140 serovars. CSS has been used to identify as many as 11 serovars in one sample and serovars in as little as 0.5% of the population (Shariat et al., 2022; Thompson et al., 2018).

The entire *Salmonella* population will have their CRISPR amplified via PCR. CSS does this by utilizing broth cultures instead of colonies from a plate (Wallace, H Andrews, and Thomas, 2009). Using RV and TT broths for CSS prevents the need to pick multiple colonies from a plate. Using the CRISPR array to create CRISPR profiles or CRISPR types identifies differences between strains of the same serovar via duplication of deletion within the CRISPR array (Vosik et al., 2018).

<u>Salmonellosis in Cattle</u>

Symptoms of salmonellosis in cattle are characterized by fever, dehydration, anorexia, and diarrhea (Costa et al., 2012). The feces of sick animals can be watery and contain mucus and blood clots (Costa et al., 2012). Clinical cases are commonly associated with infections by serovars Typhimurium and Dublin (Clinton et al., 1981; Paudyal et al., 2019). Serovar Dublin has increased morbidity and mortality in calves and has been associated with systemic infections and respiratory distress in calves; serovar Dublin infections can also cause spontaneous abortions in heifers. (Nielsen, 2013; Nielsen et al., 2004). *Salmonella* infections caused by serovar Dublin are associated with enterocolitis and septicemia (Nielsen, 2013). During infection, serovar Dublin is often shed in the feces. Serovar Dublin is also found to exist in a latent carrier stage and colonize internal organs (Nielsen, 2013). The carrier state is thought to be how serovar Dublin infections sporadically spread throughout herds (Nielsen et al., 2004). In many instances,

Salmonella colonization, with serovars other than Dublin, cause asymptomatic infections in cattle; during the asymptomatic infection, *Salmonella* is often shed in the feces (Alam et al., 2009; Cummings et al., 2009; Rodriguez-Rivera et al., 2014; Sorensen et al., 2002).

Cattle are prone to abscesses in the liver, and while not commonly associated with *Salmonella*, a quarter of abscesses have recoverable *Salmonella* (Amachawadi & Nagaraja, 2015; Asakura et al., 2022; Pinnell et al., 2022). The incidence of liver abscesses within feedlots ranges from 12-32% (Nagaraja & Chengappa, 1998). Another common site of infection in cattle is the colon; *Salmonella* infection can cause enterocolitis resulting in inflammation within the colon and small intestine (Eckmann, 2006). Chronic *Salmonella* infections cause the thickening of the colonic wall and a buildup of yellow/gray necrotic tissue (Zha et al., 2019). During a challenge assay, *Salmonella* isolated from the colon was found to be highly similar to the initial culture orally ingested by the cattle (Porwollik et al., 2018), suggesting that orally ingested *Salmonella* disseminates throughout the cattle.

Salmonella is a food safety concern in beef cattle

Salmonella associated with beef products accounts for an estimated 6.0% of *Salmonella* infections (IFSAC, 2022). Beef is the 6th largest single contributor to *Salmonella* infections (IFSAC, 2022). Between 1973 to 2011, *Salmonella* was associated with 28,599 outbreaks, with 96 outbreaks attributed to beef products (Laufer et al., 2015). *Salmonella* has been found in cattle offal (entrails and edible internal organs); one study from the republic of Korea showed that it was found in 7.1% of offal samples (Im et al., 2016). *Salmonella* colonization of the offal is important due to the inclusion of these parts in ground beef or processed meats. *Salmonella* has also been found in the lymph nodes; the lymph nodes are often included in ground beef and

thought to be one route of contamination (Arthur, Brichta-Harhay, et al., 2008; Gutiérrez et al., 2020)

One of the major outbreaks that changed how the beef industry mitigates foodborne pathogens was the *E. coli* O157:H7 outbreak in 1993. This was caused by contaminated ground beef which made more than 700 people sick and caused four deaths (Department of Health and Human Services, 1993). This outbreak resulted in *E. coli* O157:H7 being considered an adulterant in ground beef. Most mitigations during slaughter are aimed at reducing *E. coli* but are effective against *Salmonella* as well (Buege & Ingham, 2003; Buncic & Sofos, 2012; Fegan et al., 2004; Samuel et al., 1979; Young et al., 2016). In 2018 there was an outbreak of serovar Newport in ground beef. This outbreak affected people in multiple states and caused more than 400 cases of salmonellosis and 117 hospitalizations (CDC, 2019).

Post-harvest *Salmonella* control

Most intervention strategies for *Salmonella* in cattle production are in slaughter or post-processing. Upon entry to the slaughter facility, cattle are stunned or immobile. Stunning is typically performed using gas, percussion, or electrical methods. After stunning, the cattle will be hung and bled ("Animal Slaughter," 2010; Buncic & Sofos, 2012; Leary & American Veterinary Medical Association., 2016). The carcass is washed with antimicrobials, most commonly lactic acid but also peracetic acid (PAA) or ozonated water (Antic et al., 2021; Buege & Ingham, 2003; Koohmaraie et al., 2005). Hide washing wets the hide to prevent hair from dispersing and contaminating processes downstream. The hide is then vacuumed to remove excess water (Koohmaraie et al., 2005). Hide removal is an essential step in controlling bacterial pathogens during slaughter, as past studies have shown a wide range of *Salmonella* incidence on cattle hides, ranging from 0-100% (Arthur et al., 2007; Bosilevac et al., 2005; Gragg et al., 2013;

Stephens et al., 2007). Post-hide removal, the prevalence of *Salmonella* decreases by 50% (Brichta-Harhay et al., 2008). After removal of the hide, the carcass is spot cleaned via knife trimming, steam vacuuming, or ambient water washes (Koohmaraie et al., 2005; Wheeler et al., 2014). Subsequent evisceration is another critical step to avoid product contamination by removing the GI tract.

Pre-harvest sources of *Salmonella* introduction to beef cattle

The cattle industry is not vertically integrated, and animals in a single pen at a feedlot can originate from multiple cow-calf operations or backgrounding operations across the country. Further, dairy beef cattle can also be co-mingled with beef cattle at feedlots. During finishing, cattle are often re-organized by weight, gender, breed, and moved into different pens accordingly. Thus, any *Salmonella* present on a single animal or subset of animals has the potential to spread to other animals within the same pen and feedlot.

Environmental sources of *Salmonella* in feedlots include the pen floors; *Salmonella* was found in the water troughs on dairy farms and feedlots (Carlson, Engeman, et al., 2011; LeJeune et al., 2001). From 473 samples taken from various water troughs, only 0.8% were found to be positive (LeJeune et al., 2001), suggesting this may not be a significant source of *Salmonella*. Contaminated feed is also a source of *Salmonella* to beef cattle (Hsieh et al., 2014). Studies have shown that animal feed can be contaminated with *Salmonella*, albeit at a low prevalence (Jones & Richardson, 2004; Shariat et al., 2022). *Salmonella* has been found in feedlots, feed storage areas, and feed manufacturing equipment (Sargeant et al., 2021). Porwollik et al. (2018) orally challenged cattle with *Salmonella* and showed *Salmonella* disseminated throughout the cattle's lymph nodes, GI tract, and other organs. Other animals colonized with *Salmonella* can shed it into the environment can be a source of *Salmonella*, including rodents, wild birds, and insects

(Carlson et al., 2020; Carlson, Franklin, et al., 2011; Olafson et al., 2016). Wild birds have long been a concern for *Salmonella* transmission in agricultural environments (Elser et al., 2019; Faddoul et al., 1966). The dissemination of *Salmonella* via migratory birds can spread *Salmonella* across large stretches of land (Callaway et al., 2014). The increased presence of starlings in a feedlot have been shown to have a positive relation to the level of *Salmonella* found in both the feed and water troughs (Carlson, Franklin, et al., 2011). The United States Department of Agriculture found that only 34% of feedlots with more than 1,000 cattle have some intervention to reduce the number of wild birds (U.S. Department of Agriculture National Animal Health Monitoring System, 2013). One biosecurity method within feedlots is, having separate equipment for removing sick animals or spreading manure than for moving dirt. This prevents cross-contamination with healthy animals or spreading bacteria in the manure to other pens. Insects have been shown to harbor *Salmonella*, and the horse fly, which bites the cattle and commonly defecates upon landing, can be a vector for introducing *Salmonella* into the blood of the cattle (Hamilton et al., 2021; Machtinger et al., 2021; Olafson et al., 2016).

Bacterial survival and transmission within the soil column have been observed to be dependent on the pore size of the soil (Hekman et al., 1994; Mosaddeghi et al., 2009). Smaller pore sizes better retain water, which in turn allows for survival within the soil (Hekman et al., 1994). It was found that bacterial recovery was decreased based on soil depth, but the transport was linked to the water activity within the soil (Hekman et al., 1994)

It should be noted that these are not always direct *Salmonella* transmission, and *Salmonella* that colonizes cattle can come from multiple sources (figure 1). For example, in a study on the role of wild birds on *Salmonella* prevalence in feed and water troughs (50/120), trough samples were positive (Carlson, Engeman, et al., 2011). The presence of *Salmonella* in

water and feed troughs can be influenced by the wild bird populations (Carlson, Engeman, et al., 2011). After introducing controls for wild birds, the *Salmonella* prevalence decreased from 28% to 5% after the controls were introduced (Carlson, Engeman, et al., 2011). When comparing those results to *Salmonella* contamination in feed troughs, 8% were contaminated, then after controls were added, 0% were found to be contaminated with *Salmonella* (Carlson, Engeman, et al., 2011). Many other factors other than wild birds could be contributing to the *Salmonella* prevalence in the water troughs, such as the placement of the trough, season, how often the trough is cleaned, the type of trough, etc. (Carlson, Engeman, et al., 2011; Carlson, Franklin, et al., 2011; Kirk et al., 2002; LeJeune et al., 2001). To add to this complexity, Levent et al. (2019) showed that *Salmonella* infections within cattle are not universal, one serovar does not infect the entire animal, and different serovars can be found on the hide, in the lymph nodes, and the feces.

Pre-harvest *Salmonella* control

Feed additives are one method of reducing foodborne pathogens in different types of food animal production. In cattle, using probiotic feed additives can help reduce potential pathogens in the GI tract (Callaway et al., 2012). There have been few studies in cattle investigating the use of pre-and probiotics; Celmanax has shown promise and acts against the adhesion of Enterohemorrhagic *E. coli* and mycotoxin binder (Baines et al., 2011). Another is direct-fed microbial (DFM), DFM such as *lactobacillus*, which can reduce the shedding of *E. coli* up to 50%; further studies have shown that 13% of cattle fed a DFM shed E. coli, whereas normally 46% of cattle will shed E. coli (Callaway et al., 2014). Cattle are vaccinated based on diseases affecting the respiration or reproduction of the animals (USDA APHIS, 2009). Cattle are not commonly vaccinated against *Salmonella*; 0.3% of feedlot cattle are vaccinated against *Salmonella* (USDA APHIS, 2009). Additional approaches have included considering how shade

can affect cattle; having some form of shade can help reduce heat stress on the animals, which can, in turn, reduce *Salmonella* shedding (Cummings et al., 2009; Edrington et al., 2004).

Salmonella has been isolated from various cattle organs, at slaughter, and from cattle feces. *Salmonella* population and serovar complexity in feedlots have not been observed. Complex serovar populations have been found in water, cattle, and animal feed (Deaven et al., 2021; N. W. Shariat et al., 2022; Siceloff et al., 2021). Deep serotyping of cattle feedlots can lead to a better understanding of *Salmonella* populations. Examining serovar populations in feedlots can create a baseline for understanding *Salmonella* transmission in feedlots and survival within the soil environment.

Summary

Salmonella is a diverse pathogen that causes 1.3 million illnesses annually in the U.S. and is commonly acquired from eating or drinking contaminated products (Maciorowski et al., 2006). One source is beef products, which are estimated to be responsible for 6.0% of *Salmonella* cases (IFSAC, 2022). Identification and in-depth characterization of *Salmonella* serovars found within the beef industry are needed to help understand the hurdles in getting rid of *Salmonella*. The large diversity between serovars, and the complexity of multiserovar populations, dictate the need for deep serotyping methods.

Salmonella is a commensal organism in cattle, commonly found on the hide, in the feces, and in the lymph nodes (Gragg et al., 2013; Gutiérrez et al., 2020; Haneklaus et al., 2012; Webb et al., 2017). Typically, the recovery of *Salmonella* is a three-step process before identifying *Salmonella* serovars, starting with pre-enrichment, selective enrichment, and culminating in selective differential plating. Further identification via serotyping, SeqSero, ISR, SMART, CSS, and qPCR are needed to determine the serovar causing the infection, but each is not without its

faults. Multiserovar populations have been observed in water, feed, poultry litter, and more; due to the limitation via traditional culturing, serovars hidden in the minority are overlooked. Serovar Kentucky is not commonly associated with human illness, but if serovar Infantis is in the minority, Infantis might not be found via traditional culturing methods.

In cattle, most mitigation methods are aimed at slaughter to reduce *Salmonella* before it reaches the consumer; while research has been dedicated to finding ways to reduce *Salmonella* being introduced to the cattle via vectors such as wild birds, feed, and water, not much has been dedicated to looking into the pen environment before cattle arrival. Is *Salmonella* from the previous herd interacting with the new cohorts entering the pen?

REFERENCES

Achtman, M., Wain, J., Weill, F. X., Nair, S., Zhou, Z., Sangal, V., Krauland, M. G., Hale, J. L., Harbottle, H., Uesbeck, A., Dougan, G., Harrison, L. H., & Brisse, S. (2012). Multilocus Sequence Typing As A Replacement For Serotyping In *Salmonella* Enterica. *Plos Pathogens*, *8*(6). https://doi.org/10.1371/journal.ppat.1002776

Alam, M. J., Renter, D. G., Ives, S. E., Thomson, D. U., Sanderson, M. W., Hollis, L. C., & Nagaraja, T. G. (2009). Potential Associations Between Fecal Shedding Of *Salmonella* In Feedlot Cattle Treated For Apparent Respiratory Disease And Subsequent Adverse Health Outcomes. *Veterinary Research*, *40*(1). https://doi.org/10.1051/vetres:2008040

Amachawadi, R. G., & Nagaraja, T. G. (2015). First Report Of Anaerobic Isolation Of *Salmonella* Enterica From Liver Abscesses Of Feedlot Cattle. In *Journal Of Clinical Microbiology* (Vol. 53, Issue 9, Pp. 3100–3101). American Society For Microbiology. https://doi.org/10.1128/jcm.01111-15

Andino, A., & Hanning, I. (2015). *Salmonella* Enterica: Survival, Colonization, And Virulence Differences Among Serovars. In *Scientific World Journal* (Vol. 2015). Hindawi Publishing Corporation. https://doi.org/10.1155/2015/520179

Andrés-Barranco, S., Vico, J. P., Garrido, V., Samper, S., Herrera-León, S., De Frutos, C., & Mainar-Jaime, R. C. (2014). Role Of Wild Bird And Rodents In The Epidemiology Of Subclinical Salmonellosis In Finishing Pigs. *Foodborne Pathogens And Disease*, *11*(9), 689–697. https://doi.org/10.1089/fpd.2014.1755

Andrews, W. H., Wang, H., Jacobson, A., Ge, B., Zhang, G., & Hammack, T. (2022). *Bam Chapter 5_ Salmonella _ Fda*.

Antic, D., Houf, K., Michalopoulou, E., & Blagojevic, B. (2021). Beef Abattoir Interventions In A Risk-Based Meat Safety Assurance System. In *Meat Science* (Vol. 182). Elsevier Ltd. https://doi.org/10.1016/j.meatsci.2021.108622

Arthur, T. M., Bosilevac, J. M., Brichta-Harhay, D. M., Kalchayanand, N., King, D. A., Shackelford, S. D., Wheeler, T. L., & Koohmaraie, M. (2008). Source Tracking Of Escherichia Coli O157:H7 And *Salmonella* Contamination In The Lairage Environment At Commercial U.S. Beef Processing Plants And Identification Of An Effective Intervention †. In *Journal Of Food Protection* (Vol. 71, Issue 9). http://meridian.allenpress.com/jfp/article-pdf/71/9/1752/1681415/0362-028x-71_9_1752.pdf

Arthur, T. M., Bosilevac, J. M., Brichta-Harhay, D. M., Kalchayanand, N., Shackelford, S. D., Wheeler, T. L., & Koohmaraie, M. (2007). Effects Of A Minimal Hide Wash Cabinet On The Levels And Prevalence Of Escherichia Coli O157:H7 And *Salmonella* On The Hides Of Beef Cattle At Slaughter †. In *Journal Of Food Protection* (Vol. 70, Issue 5). http://meridian.allenpress.com/jfp/article-pdf/70/5/1076/1681365/0362-028x-70_5_1076.pdf

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

Asakura, H., Yamamoto, S., Sasaki, Y., Okada, Y., Katabami, S., Fujimori, A., Munakata, K., Shiraki, Y., Nishibu, H., Hisamoto, C., Kawase, J., Ojima, Y., Kiyoshima, A., & Shiroma, K. (2022). Bacterial Distribution And Community Structure In Beef Cattle Liver And Bile At Slaughter. *Journal Of Food Protection*, *85*(3), 424–434. https://doi.org/10.4315/jfp-21-288

Baines, D., Erb, S., Lowe, R., Turkington, K., Sabau, E., Kuldau, G., Juba, J., Masson, L., Mazza, A., & Roberts, R. (2011). A Prebiotic, Celmanax, Decreases Escherichia Coli O157:H7 Colonization Of Bovine Cells And Feed-Associated Cytotoxicity In Vitro. *BMC Research Notes*, *4*. https://doi.org/10.1186/1756-0500-4-110

Barsoum, I. S., & Awad, A. Y. (1972). *Microtiter Plate Agglutination Test For Salmonella Antibodies* (Vol. 23, Issue 2).

Berrang, M. E., Cosby, D. E., Cox, N. A., Cason, J. A., & Richardson, K. E. (2015). Optimizing Buffering Chemistry To Maintain Near Neutral Ph Of Broiler Feed During Pre-Enrichment For *Salmonella*. *Poultry Science*, *94*(12), 3048–3051. https://doi.org/10.3382/ps/pev277

Bosilevac, J. M., Nou, X., Osborn, M. S., Allen, D. M., & Koohmaraie, M. (2005). Development And Evaluation Of An On-Line Hide Decontamination Procedure For Use In A Commercial Beef Processing Plant †. In *Journal Of Food Protection* (Vol. 68, Issue 2).

Brichta-Harhay, D. M., Guerini, M. N., Arthur, T. M., Bosilevac, J. M., Kalchayanand, N., Shackelford, S. D., Wheeler, T. L., & Koohmaraie, M. (2008). *Salmonella* And Escherichia Coli O157:H7 Contamination On Hides And Carcasses Of Cull Cattle Presented For Slaughter In The United States: An Evaluation Of Prevalence And Bacterial Loads By Immunomagnetic Separation And Direct Plating Methods. *Applied And Environmental Microbiology*, *74*(20), 6289–6297. https://doi.org/10.1128/aem.00700-08

Buege, D., & Ingham, S. (2003). *Small Plant Intervention Treatments To Reduce Bacteria On Beef Carcasses At Slaughter*.

Bugarel, M., Bakker, H. Den, Grout, J., Vignaud, M. L., Loneragan, G. H., Fach, P., & Brisabois, A. (2018). CRISPR-Based Assay For The Molecular Identification Of Highly

Prevalent *Salmonella* Serotypes. *Food Microbiology*, *71*(March), 8–16.
https://doi.org/10.1016/j.fm.2017.03.016

Buncic, S., & Sofos, J. (2012). Interventions To Control *Salmonella* Contamination During Poultry, Cattle And Pig Slaughter. *Food Research International*, *45*(2), 641–655.
https://doi.org/10.1016/j.foodres.2011.10.018

Callaway, T. R., Edrington, T. S., Harvey, R. B., Anderson, R. C., & Nisbet, D. J. (2012). Prebiotics In Food Animals, A Potential To Reduce Foodborne Pathogens And Disease. *Romanian Biotechnological Letters*.

Callaway, T. R., Edrington, T. S., & Nisbet, D. J. (2014a). Isolation Of Escherichia Coli O157:H7 And *Salmonella* From Migratory Brown-Headed Cowbirds (Molothrus Ater), Common Grackles (Quiscalus Quiscula), And Cattle Egrets (Bubulcus Ibis). *Foodborne Pathogens And Disease*, *11*(10), 791–794. https://doi.org/10.1089/fpd.2014.1800

Callaway, T. R., Edrington, T. S., & Nisbet, J. (2014b). Meat Science And Muscle Biology Symposium: Ecological And Dietary Impactors Of Foodborne Pathogens And Methods To Reduce Fecal Shedding In Cattle 1,2. *J. Anim. Sci*, *92*, 1356–1365.
https://doi.org/10.2527/jas2013-7308

Carlson, J. C., Chandler, J. C., Bisha, B., Lejeune, J. T., & Wittum, T. E. (2020). Bird-Livestock Interactions Associated With Increased Cattle Fecal Shedding Of Ciprofloxacin-Resistant Escherichia Coli Within Feedlots In The United States. *Scientific Reports*, *10*(1).
https://doi.org/10.1038/s41598-020-66782-4

Carlson, J. C., Engeman, R. M., Hyatt, D. R., Gilliland, R. L., Deliberto, T. J., Clark, L., Bodenchuk, M. J., & Linz, G. M. (2011). Efficacy Of European Starling Control To Reduce

Salmonella Enterica Contamination In A Concentrated Animal Feeding Operation In The Texas Panhandle. *BMC Veterinary Research*, *7*. https://doi.org/10.1186/1746-6148-7-9

Carlson, J. C., Franklin, A. B., Hyatt, D. R., Pettit, S. E., & Linz, G. M. (2011). The Role Of Starlings In The Spread Of *Salmonella* Within Concentrated Animal Feeding Operations. *Journal Of Applied Ecology*, *48*(2), 479–486. https://doi.org/10.1111/j.1365-2664.2010.01935.x

Cason, J., Cox, N., Buhr, R., Bourassa, D., & Richardson, L. (2011). Probability Of Identitying Different *Salmonella* Serotypes In Poultry Samples. *International Poultry Scientific Forum. Southern Poultry Sciencesociety, Mississippi State, MS.*, P75–76; Abstr 262P.

Cdc. (1993). *Salmonella* Serotype Tennessee In Powdered Milk Products And Infant Formula -- Canada And United States, 1993. *Morbidity And Mortality Weekly Report, Cdc*, *42*(26), 516–517.

Centers For Disease Control And Prevention. (2018). *Multistate Outbreak Of Salmonella Mbandaka Infections Linked To Kellogg's Honey Smacks Cereal (Final Update)*.

"Outbreak Of *Salmonella* Infections Linked To Ground Beef." *Centers For Disease Control And Prevention*, Centers For Disease Control And Prevention, 22 Mar. 2019, https://www.cdc.gov/*salmonella*/newport-10-18/index.html.

Cheng, R. A., Eade, C. R., & Wiedmann, M. (2019). Embracing Diversity: Differences In Virulence Mechanisms, Disease Severity, And Host Adaptations Contribute To The Success Of Nontyphoidal *Salmonella* As A Foodborne Pathogen. In *Frontiers In Microbiology* (Vol. 10, P. 1368).

Christensen, H., Nordentoft, S., & Olsen, J. E. (1998). NOTE: Phylogenetic Relationships Of *Salmonella* Based On rRNA Sequences. *International Journal Of Systematic Bacteriology*, *48*(2), 605–610. https://doi.org/10.1099/00207713-48-2-605

Clinton, N. A., Weaver, R. W., & Hidalgo, R. J. (1981). Transmission Of *Salmonella* Typhimurium Among Feedlot Cattle After Oral Inoculation. *Journal Of Applied Bacteriology*, *50*(1), 149–155. Https://Doi.Org/10.1111/J.1365-2672.1981.Tb00879.X

Cohen, E., Azriel, S., Austeri, O., Gal, A., Zitronblat, C., Mikhlin, S., Scharte, F., Hensei, M., Rahav, G., & Mor, O. G. (2021). Pathoadaptation Of The Passerine-Associated *Salmonella* Enterica Serovar Typhimurium Lineage To The Avian Host. *Plos Pathogens*, *17*(3). https://doi.org/10.1371/journal.ppat.1009451

Collins, J. P., Shah, H. J., Lowell Weller, D., Ray, L. C., Smith, K., Mcguire, S., Trevejo, R. T., Jervis, R. H., Vugia, D. J., Rissman, T., Garman, K. N., Lathrop, S., Laclair, B., Boyle, M. M., Harris, S., Zablotsky Kufel, J., Tauxe, R. V, Bruce, B. B., Billig Rose, E., … Payne, D. C. (2022). *Morbidity And Mortality Weekly Report Preliminary Incidence And Trends Of Infections Caused By Pathogens Transmitted Commonly Through Food-Foodborne Diseases Active Surveillance Network, 10 U.S. Sites, 2016-2021*. https://www.cdc.gov/*salmonella*/oranienburg-09-21/details.html

Costa, L. F., Paixão, T. A., Tsolis, R. M., Bäumler, A. J., & 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

Cox, N. A., Berrang, M. E., House, S. L., Medina, D., Cook, K. L., & Shariat, N. W. (2019). Population Analyses Reveal Preenrichment Method And Selective Enrichment Media Affect *Salmonella* Serovars Detected On Broiler Carcasses. *Journal Of Food Protection*, *82*(10), 1688–1696. https://doi.org/10.4315/0362-028x.jfp-19-166

Cox, N. A., Cason, J. A., Buhr, R. J., Richardson, K. E., Richardson, L. J., Rigsby, L. L., & Fedorka-Cray, P. J. (2013). Variations In Preenrichment Ph Of Poultry Feed And Feed

Ingredients After Incubation Periods Up To 48 Hours. *Journal Of Applied Poultry Research*, *22*(2), 190–195. https://doi.org/10.3382/japr.2012-00552

Cummings, K. J., Warnick, L. D., Alexander, K. A., Cripps, C. J., Gröhn, Y. T., James, K. L., Mcdonough, P. L., & Reed, K. E. (2009). The Duration Of Fecal *Salmonella* Shedding Following Clinical Disease Among Dairy Cattle In The Northeastern USA. *Preventive Veterinary Medicine*, *92*(1–2), 134–139. https://doi.org/10.1016/j.prevetmed.2009.07.002

Deaven, A. M., Ferreira, C. M., Reed, E. A., Chen See, J. R., Lee, N. A., Almaraz, E., Rios, P. C., Marogi, J. G., Lamendella, R., Zheng, J., Bell, R. L., & Shariat, N. W. (2021). *Salmonella* Genomics And Population Analyses Reveal High Inter- And Intra- Serovar Diversity In Freshwater. *Applied And Environmental Microbiology*, AEM.02594-20. https://doi.org/10.1128/aem.02594-20

Department Of Health And Human Services. (1993, April 16). *Update: Multistate Outbreak Of Escherichia Coli O157:H7 From Hamburgers--Western United States, 1992-1993*. Morbidity And Mortality Weekly Report. https://www.cdc.gov/mmwr/preview/mmwrhtml/00020219.htm

Desai, P. T., Porwollik, S., Long, F., Cheng, P., Wollam, A., Clifton, S. W., Weinstock, G. M., & Mcclelland, M. (2013). Evolutionary Genomics Of *Salmonella* Enterica Subspecies. *Mbio*, *4*(2). https://doi.org/10.1128/mbio.00579-12

Dev Kumar, G., Williams, R. C., Sriranganathan, N., Boyer, R. R., & Eifert, J. D. (2018). Survival Of Tomato Outbreak Associated *Salmonella* Serotypes In Soil And Water And The Role Of Biofilms In Abiotic Surface Attachment. *Foodborne Pathogens And Disease*, *15*(9), 548–553. https://doi.org/10.1089/fpd.2017.2416

Eckmann, L. (2006). Animal Models Of Inflammatory Bowel Disease: Lessons From Enteric Infections. *Annals Of The New York Academy Of Sciences*, *1072*, 28–38. https://doi.org/10.1196/annals.1326.008

Edrington, T. S., Hurne, M. E., Looper, M. L., Schultz, C. L., Fitzgerald, A. C., Callaway, T. R., Genovese, K. J., Bischoff, K. M., Mcreynolds, J. L., Anderson, R. C., & Nisbet, D. J. (2004). Variation In The Faecal Shedding Of *Salmonella* And E. Coli O157:H7 In Lactating Dairy Cattle And Examination Of *Salmonella* Genotypes Using Pulsed-Field Gel Electrophoresis. *Letters In Applied Microbiology*, *38*(5), 366–372. https://doi.org/10.1111/j.1472-765x.2004.01495.x

Elser, J. L., Adams Progar, A. L., Steensma, K. M. M., Caskin, T. P., Kerr, S. R., & Shwiff, S. A. (2019). Economic And Livestock Health Impacts Of Birds On Dairies: Evidence From A Survey Of Washington Dairy Operators. In *Plos ONE* (Vol. 14, Issue 9). Public Library Of Science. https://doi.org/10.1371/journal.pone.0222398

Fabre, L., Zhang, J., Guigon, G., Le Hello, S., Guibert, V., Accou-Demartin, M., De Romans, S., Lim, C., Roux, C., Passet, V., Diancourt, L., Guibourdenche, M., Issenhuth-Jeanjean, S., Achtman, M., Brisse, S., Sola, C., & Weill, F.-X. (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

Faddoul, G. P., Fellows, G. W., Baird, J., & Ba, J. (1966). *A Survey On The Incidence Of Salmonellae In Wild Birds* (Vol. 10, Issue 1).

Fegan, N., Vanderlinde, P., Higgs, G., & 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

Gorski, L. (2012). Selective Enrichment Media Bias The Types Of *Salmonella* Enterica Strains Isolated From Mixed Strain Cultures And Complex Enrichment Broths. *Plos ONE*, *7*(4). https://doi.org/10.1371/journal.pone.0034722

Gragg, S. E., Loneragan, G. H., Nightingale, K. K., Brichta-Harhay, D. M., Ruiz, H., Elder, J. R., Garcia, L. G., Miller, M. F., Echeverry, A., Ramírez Porras, R. G., & Brashears, M. M. (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

Grimont, P., & Weill, F. (2007). Antigenic Formulae Of The *Salmonella* Serovars. *WHO Collaborating Centre For Reference And Research On Salmonella*, *9th Editio*.

Guard, J., Sanchez-Ingunza, R., Morales, C., Stewart, T., Liljebjelke, K., Van Kessel, J., Ingram, K., Jones, D., Jackson, C., Fedorka-Cray, P., Frye, J., Gast, R., & Hinton, A. (2012a). Comparison Of Dkgb-Linked Intergenic Sequence Ribotyping To DNA Microarray Hybridization For Assigning Serotype To *Salmonella* Enterica. *FEMS Microbiology Letters*, *337*(1), 61–72. https://doi.org/10.1111/1574-6968.12010

Guard, J., Sanchez-Ingunza, R., Morales, C., Stewart, T., Liljebjelke, K., Van Kessel, J., Ingram, K., Jones, D., Jackson, C., Fedorka-Cray, P., Frye, J., Gast, R., & Hinton, A. (2012b). Comparison Of Dkgb-Linked Intergenic Sequence Ribotyping To DNA Microarray Hybridization For Assigning Serotype To *Salmonella* Enterica. *FEMS Microbiology Letters*, *337*(1), 61–72. https://doi.org/10.1111/1574-6968.12010

Gutema, F. D., Agga, G. E., Abdi, R. D., De Zutter, L., Duchateau, L., & Gabriël, S. (2019). 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*(Apr), 1–11. https://doi.org/10.3389/fvets.2019.00102

Gutiérrez, T. P., Lozano, M. S. R., Suárez, E. J. D., Guzmán, N. R., Ramos, O. S., Pérez, C. F. H., & Medina, R. D. M. (2020). Lymph Nodes And Ground Beef As Public Health Importance Reservoirs Of *Salmonella* Spp. *Revista Mexicana De Ciencias Pecuarias*, *11*(3), 795–810. https://doi.org/10.22319/rmcp.v11i3.5516

Haley, B. J., Kim, S. W., Pettengill, J., Luo, Y., Karns, J. S., & Van Kessel, J. A. S. (2016). Genomic And Evolutionary Analysis Of Two *Salmonella* Enterica Serovar Kentucky Sequence Types Isolated From Bovine And Poultry Sources In North America. *Plos ONE*, *11*(10). https://doi.org/10.1371/journal.pone.0161225

Hamilton, A. M., Paulsen, D. J., Trout Fryxell, R. T., Orta, V. E., Gorman, S. J., Smith, D. M., Buchanan, J. R., Wszelaki, A. L., & Critzer, F. J. (2021). Prevalence Of *Salmonella* Enterica In Flies On A Diversified Cattle And Fresh Produce Farm Across Two Growing Seasons. *Journal Of Food Protection*, *84*(6), 1009–1015. https://doi.org/10.4315/jfp-20-339

Haneklaus, A. N., Harris, K. B., Griffin, D. B., Edrington, T. S., Lucia, L. M., & 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

Hekman, W. E., Heijnen, C. E., Burgers, S. L. G. E., Van Veen, J. A., & Van Elsas, J. D. (1994). Transport Of Bacterial Inoculants Through Intact Cores Of Two Different Soils As Affected By Water Percolation And The Presence Of Wheat Plants. *Microbial Ecology*, *16*, 143–158.

Hektoen, I., Agar, E., King, S., & Metzger, W. I. (1968). A New Plating Medium For The Isolation Of Enteric Pathogens. In *APPLIED MICROBIOLOGY* (Vol. 16, Issue 4).

Hoffmann, S., Maculloch, B., & Batz, M. (2015). *United States Department Of Agriculture Economic Burden Of Major Foodborne Illnesses Acquired In The United States*. www.ers.usda.gov

Holley, R. A., Arrus, K. M., Ominski, K. H., Tenuta, M., & Blank, G. (2006). *Salmonella* Survival In Manure-Treated Soils During Simulated Seasonal Temperature Exposure. *Journal Of Environmental Quality*, *35*(4), 1170–1180. https://doi.org/10.2134/jeq2005.0449

Holschbach, C. L., & Peek, S. F. (2018). *Salmonella* In Dairy Cattle. *Veterinary Clinics Of North America - Food Animal Practice*, *34*(1), 133–154. https://doi.org/10.1016/j.cvfa.2017.10.005

Hsieh, Y., Lee, K., Poole, T., Runyon, M., Jones, B., Herrman, T. J., Plains, S., States, U., & Station, C. (2014). *Detection And Isolation Of Salmonella Spp . In Animal Feeds From 2007-2011*. *2*(1), 14–27.

Ifsac. (2022). *Foodborne Illness Source Attribution Estimates For 2020 For Salmonella, Escherichia Coli O157, And Listeria Monocytogenes Using Multi-Year Outbreak Surveillance Data, United States*. https://www.cdc.gov/foodsafety/ifsac/projects/index.html.

Ijabadeniyi, O. A., Debusho, L. K., Vanderlinde, M., & Buys, E. M. (2011). Irrigation Water As A Potential Preharvest Source Of Bacterial Contamination Of Vegetables. *Journal Of Food Safety*, *31*(4), 452–461. https://doi.org/10.1111/j.1745-4565.2011.00321.x

Im, M. C., Seo, K. W., Bae, D. H., & 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

Jacobsen, C. S., & Bech, T. B. (2012). Soil Survival Of *Salmonella* And Transfer To Freshwater And Fresh Produce. In *Food Research International* (Vol. 45, Issue 2, Pp. 557–566). https://doi.org/10.1016/j.foodres.2011.07.026

Jechalke, S., Schierstaedt, J., Becker, M., Flemer, B., Grosch, R., Smalla, K., & Schikora, A. (2019). *Salmonella* Establishment In Agricultural Soil And Colonization Of Crop Plants Depend On Soil Type And Plant Species. *Frontiers In Microbiology*, *10*(MAY), 1–17. https://doi.org/10.3389/fmicb.2019.00967

Jones, F. T., & Richardson, K. E. (2004). *Salmonella* In Commercially Manufactured Feeds. *Poultry Science*, *83*(3), 384–391. https://doi.org/https://doi.org/10.1093/ps/83.3.384

Kataria, J., Vaddu, S., Rama, E. N., Sidhu, G., Thippareddi, H., & Singh, M. (2020). Evaluating The Efficacy Of Peracetic Acid On *Salmonella* And Campylobacter On Chicken Wings At Various Ph Levels. *Poultry Science*, *99*(10), 5137–5142. https://doi.org/10.1016/j.psj.2020.06.070

Kidgell, C., Reichard, U., Wain, J., Linz, B., Torpdahl, M., Dougan, G., & Achtman, M. (2002). *Salmonella* Typhi, The Causative Agent Of Typhoid Fever, Is Approximately 50,000 Years Old. *Infection, Genetics And Evolution*, *2*(1), 39–45. https://doi.org/10.1016/S1567-1348(02)00089-8

Kirk, J., Atwill, E., Holmberg, C., Arana, M., Ghirardelli, D., Higginbotham, G., Markagaard, G., Mullinax, D., Wubishet, A., & Kirk, J. (2002). *Prevalence Of And Risk Factors For Salmonella In Water Offered To Weaned Dairy Calves In California, USA*.

Knox, R. (1943). The Effect Of Tetrathionate On Bacterial Growth. *Science*, *98*(2540), 224. https://doi.org/10.1126/science.98.2540.224

Koltuniewicz, A. (2010). Animal Slaughter. In *Encyclopedia Of Meat Sciences*.

Koohmaraie, M., Arthur, T. M., Bosilevac, J. M., Guerini, M., Shackelford, S. D., & Wheeler, T. L. (2005). Post-Harvest Interventions To Reduce/Eliminate Pathogens In Beef. *Meat Science*, *71*(1), 79–91. https://doi.org/10.1016/j.meatsci.2005.03.012

Larsen, B. R., Richardson, K. E., Obe, Tomi., Schaeffer, Cheryl., & Shariat, N. W. (2021). Mixed *Salmonella* Cultures Reveal Competitive Advantages Between Strains During Pre-Enrichment And Selective Enrichment. *Journal Of Food Safety*, *41*(6). https://doi.org/10.1111/jfs.12934

Laufer, A. S., Grass, J., Holt, K., Whichard, J. M., Griffin, P. M., & Gould, L. H. (2015). Outbreaks Of *Salmonella* Infections Attributed To Beef --United States, 1973-2011. *Epidemiology And Infection*, *143*(9), 2003–2013. https://doi.org/10.1017/S0950268814003112

Leader, B. T., Frye, J. G., Hu, J., Fedorka-Cray, P. J., & Boyle, D. S. (2009). High-Throughput Molecular Determination Of *Salmonella* Enterica Serovars By Use Of Multiplex PCR And Capillary Electrophoresis Analysis. *Journal Of Clinical Microbiology*, *47*(5), 1290–1299. https://doi.org/10.1128/JCM.02095-08

Leary, S. L., & American Veterinary Medical Association. (N.D.). *AVMA Guidelines For The Humane Slaughter : 2016 Edition*.

Lejeune, J. T., Besser, T. E., Merrill, N. L., Rice, D. H., & Hancock, D. D. (2001). Livestock Drinking Water Microbiology And The Factors Influencing The Quality Of Drinking Water Offered To Cattle. *Journal Of Dairy Science*, *84*(8), 1856–1862. https://doi.org/10.3168/jds.S0022-0302(01)74626-7

Liu, H., Whitehouse, C. A., & Li, B. (2018). Presence And Persistence Of *Salmonella* In Water: The Impact On Microbial Quality Of Water And Food Safety. In *Frontiers In Public Health* (Vol. 6). Frontiers Media S.A. https://doi.org/10.3389/fpubh.2018.00159

Machtinger, E. T., Gerry, A. C., Murillo, A. C., & Talley, J. L. (2021). Filth Fly Impacts To Animal Production In The United States And Associated Research And Extension Needs. *Journal Of Integrated Pest Management*, *12*(1). https://doi.org/10.1093/jipm/pmab026

Maciorowski, K. G., Herrera, P., Kundinger, M. M., & Ricke, S. C. (2006). Animal Feed Production And Contamination By Foodborne *Salmonella*. *Journal Fur Verbraucherschutz Und Lebensmittelsicherheit*, *1*(3), 197–209. https://doi.org/10.1007/s00003-006-0036-z

Majowicz, S. E., Musto, J., Scallan, E., Angulo, F. J., Kirk, M., O'Brien, S. J., Jones, T. F., Fazil, A., Hoekstra, R. M., & Studies, For The I. C. On E. D. "Burden Of I. (2010). The Global Burden Of Nontyphoidal *Salmonella* Gastroenteritis. *Clinical Infectious Diseases*, *50*(6), 882–889. https://doi.org/10.1086/650733

Mcquiston, J. R., Herrera-Leon, S., Wertheim, B. C., Doyle, J., Fields, P. I., Tauxe, R. V., & Logsdon, J. M. (2008). Molecular Phylogeny Of The *Salmonella*e: Relationships Among *Salmonella* Species And Subspecies Determined From Four Housekeeping Genes And Evidence Of Lateral Gene Transfer Events. *Journal Of Bacteriology*, *190*(21), 7060–7067. https://doi.org/10.1128/JB.01552-07

Miller, R. G., Tate, C. R., Mallinson, E. T., & Scherrer, J. A. (1991). Xylose-Lysine-Tergitol 4: An Improved Selective Agar Medium For The Isolation Of *Salmonella*. *Poultry Science*, *70*(12), 2429–2432. https://doi.org/10.3382/ps.0702429

Moats, W. A., & Kinner, J. A. (1974). Factors Affecting Selectivity Of Brilliant Green-Phenol Red Agar For *Salmonella*e. In *APPLIED MICROBIOLOGY*.

Morasi, R. M., Rall, V. L. M., Dantas, S. T. A., Alonso, V. P. P., & Silva, N. C. C. (2022). *Salmonella* Spp. In Low Water Activity Food: Occurrence, Survival Mechanisms, And Thermoresistance. *Journal Of Food Science*, *87*(6), 2310–2323. https://doi.org/10.1111/1750-3841.16152

Mosaddeghi, M. R., Mahboubi, A. A., Zandsalimi, S., & Unc, A. (2009). Influence Of Organic Waste Type And Soil Structure On The Bacterial Filtration Rates In Unsaturated Intact Soil

Columns. *Journal Of Environmental Management*, *90*(2), 730–739. https://doi.org/10.1016/j.jenvman.2008.01.009

Nagaraja, T. G., & Chengappa, M. M. (1998). Liver Abscesses In Feedlot Cattle: A Review. In *Journal Of Animal Science* (Vol. 76, Issue 1, Pp. 287–298). American Society Of Animal Science. https://doi.org/10.2527/1998.761287x

Ng, H., Bayne, H. G., & Garibaldi, J. A. (1969). Heat Resistance Of *Salmonella*: The Uniqueness Of *Salmonella* Senftenberg 775W. In *APPLIED MICROBIOLOGY*.

Nielsen, L. R. (2013). Review Of Pathogenesis And Diagnostic Methods Of Immediate Relevance For Epidemiology And Control Of *Salmonella* Dublin In Cattle. In *Veterinary Microbiology* (Vol. 162, Issue 1, Pp. 1–9). https://doi.org/10.1016/j.vetmic.2012.08.003

Nielsen, L. R., Schukken, Y. H., Gröhn, Y. T., & Ersbøll, A. K. (2004). *Salmonella* Dublin Infection In Dairy Cattle: Risk Factors For Becoming A Carrier. *Preventive Veterinary Medicine*, *65*(1–2), 47–62. https://doi.org/10.1016/j.prevetmed.2004.06.010

Obe, T., Berrang, M. E., Cox, N. A., House, S. L., & Shariat, N. W. (2021). Comparison Of Selective Enrichment And Plating Media For *Salmonella* Isolation From Broiler Carcasses. *Journal Of Food Safety*, *41*(6). https://doi.org/10.1111/jfs.12928

Obe, T., Richards, A. K., & Shariat, N. W. (2022). Differences In Biofilm Formation Of *Salmonella* Serovars On Two Surfaces Under Two Temperature Conditions. *Journal Of Applied Microbiology*, *132*(3), 2410–2420. https://doi.org/10.1111/jam.15381

Olafson, P. U., Brown, T. R., Lohmeyer, K. H., Harvey, R. B., Nisbet, D. J., Loneragan, G. H., & Edrington, T. S. (2016). Assessing Transmission Of *Salmonella* To Bovine Peripheral Lymph Nodes Upon Horn Fly Feeding. *Journal Of Food Protection*, *79*(7), 1135–1142. https://doi.org/10.4315/0362-028X.JFP-15-414

Palumbo, S. A., & Alford, J. A. (1970). Inhibitory Action Of Tetrathionate Enrichment Broth. *Applied Microbiology*, *20*(6), 970–976. https://doi.org/10.1128/aem.20.6.970-976.1970

Paudyal, N., Pan, H., Elbediwi, M., Zhou, X., Peng, X., Li, X., Fang, W., & Yue, M. (2019). Characterization Of *Salmonella* Dublin Isolated From Bovine And Human Hosts. *BMC Microbiology*, *19*(1). https://doi.org/10.1186/s12866-019-1598-0

Phan-Thien, K., Metaferia, M. H., Bell, T. L., Bradbury, M. I., Sassi, H. P., Van Ogtrop, F. F., Suslow, T. V., & Mcconchie, R. (2020). Effect Of Soil Type And Temperature On Survival Of *Salmonella* Enterica In Poultry Manure-Amended Soils. *Letters In Applied Microbiology*, *71*(2), 210–217. https://doi.org/10.1111/lam.13302

Pinnell, L. J., Whitlow, C. W., Huebner, K. L., Bryant, T. C., Martin, J., Belk, K. E., & Morley, P. S. (2022). Not All Liver Abscesses Are Created Equal: The Impact Of Tylosin And Antibiotic Alternatives On Bovine Liver Abscess Microbial Communities And A First Look At Bacteroidetes-Dominated Communities. *Frontiers In Microbiology*, *13*. https://doi.org/10.3389/fmicb.2022.882419

Poisson, D. M. (1992). Novobiocin, Brilliant Green, Glycerol, Lactose Agar: A New Medium For The Isolation Of *Salmonella* Strains. *Research In Microbiology*, *143*(2), 211–216. https://doi.org/10.1016/0923-2508(92)90010-L

Porwollik, S., Boyd, E. F., Choy, C., Cheng, P., Florea, L., Proctor, E., & Mcclelland, M. (2004). Characterization Of *Salmonella* Enterica Subspecies I Genovars By Use Of Microarrays. *Journal Of Bacteriology*, *186*(17), 5883–5898. https://doi.org/10.1128/JB.186.17.5883-5898.2004

Rasamsetti, S., Berrang, M., Cox, N. A., & Shariat, N. W. (2021). Selective Pre-Enrichment Method To Lessen Time Needed To Recover *Salmonella* From Commercial Poultry Processing Samples. *Food Microbiology*, *99*. https://doi.org/10.1016/j.fm.2021.103818

Rasamsetti, S., Berrang, M. E., Cox, N. A., & Shariat, N. W. (2022). Assessing *Salmonella* Prevalence And Complexity Through Processing Using Different Culture Methods. *Poultry Science*, *101*(7). https://doi.org/10.1016/j.psj.2022.101949

Rasamsetti, S., & Shariat, N. W. (2023). Biomapping *Salmonella* Serovar Complexity In Broiler Carcasses And Parts During Processing. *Food Microbiology*, *110*. https://doi.org/10.1016/j.fm.2022.104149

Rauch, H. E., Vosik, D., Kariyawasam, S., M'ikanatha, N., & Shariat, N. W. (2018). Prevalence Of Group I *Salmonella* Kentucky In Domestic Food Animals From Pennsylvania And Overlap With Human Clinical CRISPR Sequence Types. *Zoonoses And Public Health*, *65*(7), 831–837. https://doi.org/10.1111/zph.12506

Richards, A. K., Hopkins, B. A., & Shariat, N. W. (2020). Conserved CRISPR Arrays In *Salmonella* Enterica Serovar Infantis Can Serve As Qpcr Targets To Detect Infantis In Mixed Serovar Populations. *Letters In Applied Microbiology*, *71*(2), 138–145. https://doi.org/10.1111/lam.13296

Richardson, K. E., Cox, N. A., Cosby, D. E., Berrang, M. E., Holcombe, N. L., & Weller, C. E. (2019). Impact Of Enrichment Media On H2 S-Negative *Salmonella* Isolated From Xylose-Lysine-Tergitol 4 Agar. *Journal Of Applied Poultry Research*, *28*(4), 1255–1261. https://doi.org/10.3382/japr/pfz093

Rodriguez-Rivera, L. D., Wright, E. M., Siler, J. D., Elton, M., Cummings, K. J., Warnick, L. D., & Wiedmann, M. (2014). Subtype Analysis Of *Salmonella* Isolated From Subclinically Infected Dairy Cattle And Dairy Farm Environments Reveals The Presence Of Both Human- And Bovine-Associated Subtypes. *Veterinary Microbiology*, *170*(3–4), 307–316. https://doi.org/10.1016/j.vetmic.2014.02.013

Rosselin, M., Abed, N., Virlogeux-Payant, I., Bottreau, E., Sizaret, P. Y., Velge, P., & Wiedemann, A. (2011). Heterogeneity Of Type III Secretion System (T3SS)-1-Independent Entry Mechanisms Used By *Salmonella* Enteritidis To Invade Different Cell Types. *Microbiology*, *157*(3), 839–847. https://doi.org/10.1099/mic.0.044941-0

Rostagno, M. H., Gailey, J. K., Hurd, H. S., Mckean, J. D., & Leite, R. C. (2005). *Culture Methods Differ On The Isolation Of Salmonella Enterica Serotypes From Naturally Contaminated Swine Fecal Samples*. 80–83.

Samadpour, M., Barbour, M. W., Nguyen, T., Cao, T.-M., Buck, F., Depavia, G. A., Mazengia, E., Yang, P., Alfi, D., Lopes, M., & Stopforth, J. D. (2006). Incidence Of Enterohemorrhagic Escherichia Coli, Escherichia Coli O157, *Salmonella*, And Listeria Monocytogenes In Retail Fresh Ground Beef, Sprouts, And Mushrooms. In *Journal Of Food Protection* (Vol. 69, Issue 2). http://meridian.allenpress.com/jfp/article-pdf/69/2/441/1678007/0362-028x-69_2_441.pdf

Samuel, J. L., O'Boyle, D. A., Mathers, W. J., & Frost, A. J. (1979). Isolation Of *Salmonella* From Mesenteric Lymph Nodes Of Healthy Cattle At Slaughter. *Research In Veterinary Science*, 238–241.

Sánchez-Vargas, F. M., Abu-El-Haija, M. A., & Gómez-Duarte, O. G. (2011). *Salmonella* Infections: An Update On Epidemiology, Management, And Prevention. In *Travel Medicine And Infectious Disease* (Vol. 9, Issue 6, Pp. 263–277). https://doi.org/10.1016/j.tmaid.2011.11.001

Santos, R. L., Zhang, S., Tsolis, R. M., Kingsley, R. A., Adams, L. G., & Bäumler, A. J. (2001). *Animal Models Of Salmonella Infections: Enteritis Versus Typhoid Fever*.

Sargeant, J. M., Totton, S. C., Plishka, M., & Vriezen, E. R. (2021). *Salmonella* In Animal Feeds: A Scoping Review. In *Frontiers In Veterinary Science* (Vol. 8). Frontiers Media S.A. https://doi.org/10.3389/fvets.2021.727495

Scallan, E., Hoekstra, R. M., Angulo, F. J., Tauxe, R. V, Widdowson, M.-A., Roy, S. L., Jones, J. L., & Griffin, P. M. (2011). Foodborne Illness Acquired In The United States--Major Pathogens. *Emerging Infectious Diseases*, *17*(1), 7–15. Https://Doi.Org/10.3201/Eid1701.P11101

Scharff, R. L. (2012). Economic Burden From Health Losses Due To Foodborne Illness In The United States. *Journal Of Food Protection*, *75*(1), 123–131. https://doi.org/10.4315/0362-028X.JFP-11-058

Shah, D. H., Paul, N. C., Sischo, W. C., Crespo, R., & Guard, J. (2017). Microbiology And Food Safety: Population Dynamics And Antimicrobial Resistance Of The Most Prevalent Poultry-Associated *Salmonella* Serotypes. *Poultry Science*, *96*(3), 687–702. https://doi.org/10.3382/ps/pew342

Shariat, N., & Dudley, E. G. (2014). Crisprs: Molecular Signatures Used For Pathogen Subtyping. *Applied And Environmental Microbiology*, *80*(2), 430–439. https://doi.org/10.1128/AEM.02790-13

Shariat, N., Kirchner, M. K., Sandt, C. H., Trees, E., Barrangou, R., & Dudley, E. G. (2013). Subtyping Of *Salmonella* Enterica Serovar Newport Outbreak Isolates By CRISPR-MVLST And Determination Of The Relationship Between CRISPR-MVLST And PFGE Results. *Journal Of Clinical Microbiology*, *51*(7), 2328–2336. https://doi.org/10.1128/JCM.00608-13

Shariat, N., Timme, R. E., Pettengill, J. B., Barrangou, R., & Dudley, E. G. (2015). Characterization And Evolution Of *Salmonella* CRISPR-Cas Systems. *Microbiology (United Kingdom)*, *161*(2), 374–386. https://doi.org/10.1099/mic.0.000005

Shariat, N. W., Larsen, B. R., Schaeffer, C., & Richardson, K. E. (2022). Animal Feed Contains Diverse Populations Of *Salmonella*. *Journal Of Applied Microbiology*. https://doi.org/10.1111/jam.15525

Sharma, N. C., Kumar, D., Sarkar, A., Chowdhury, G., Mukhopadhyay, A. K., & Ramamurthy, T. (2020). Prevalence Of Multidrug Resistant *Salmonella*e With Increasing Frequency Of *Salmonella* Enterica Serovars Kentucky And Virchow Among Hospitalized Diarrheal Cases In And Around Delhi, India. *Japanese Journal Of Infectious Diseases*, *73*(2), 119–123. https://doi.org/10.7883/yoken.JJID.2019.063

Sheth, A. N., Hoekstra, M., Patel, N., Ewald, G., Lord, C., Clarke, C., Villamil, E., Niksich, K., Bopp, C., Nguyen, T.-A., Zink, D., & Lynch, M. (2011). A National Outbreak Of *Salmonella* Serotype Tennessee Infections From Contaminated Peanut Butter: A New Food Vehicle For Salmonellosis In The United States. *Clinical Infectious Diseases*, *53*(4), 356–362. https://doi.org/10.1093/cid/cir407

Shivaprasad, H. L. (2000). Fowl Typhoid And Pullorum Disease. *OIE Revue Scientifique Et Technique*, *19*(2), 405–424. https://doi.org/10.20506/rst.19.2.1222

Siceloff, A. T., Ohta, N., Norman, K. N., Loneragan, G. H., Norby, B., Morgan Scott, H., & Shariat, N. W. (2021). Antimicrobial Resistance Hidden Within Multiserovar *Salmonella* Populations. *Antimicrobial Agents And Chemotherapy*, *65*(6). https://doi.org/10.1128/AAC.00048-21

Siceloff, A. T., Waltman, D., & Shariat, N. W. (2022). Regional *Salmonella* Differences In United States Broiler Production From 2016 To 2020 And The Contribution Of Multiserovar Populations To *Salmonella* Surveillance. *Applied And Environmental Microbiology*, *88*(8). https://doi.org/10.1128/aem.00204-22

Singer, R. S., Mayer, A. E., Hanson, T. E., & Isaacson, R. E. (2009). Do Microbial Interactions And Cultivation Media Decrease The Accuracy Of *Salmonella* Surveillance Systems And Outbreak Investigations? *Journal Of Food Protection*, *72*(4), 707–713. https://doi.org/10.4315/0362-028X-72.4.707

Sorensen, O., Van Donkersgoed, J., Mcfall, M., Manninen, K., Gensler, G., & Ollis, G. (2002). *Salmonella* Spp. Shedding By Alberta Beef Cattle And The Detection Of *Salmonella* Spp. In Ground Beef. In *Journal Of Food Protection* (Vol. 65, Issue 3). http://meridian.allenpress.com/jfp/article-pdf/65/3/484/1674726/0362-028x-65_3_484.pdf

Srednik, M. E., Lantz, K., Hicks, J. A., Morningstar-Shaw, B. R., Mackie, T. A., & Schlater, L. K. (2021). Antimicrobial Resistance And Genomic Characterization Of *Salmonella* Dublin Isolates In Cattle From The United States. *Plos ONE*, *16*(9 September). https://doi.org/10.1371/journal.pone.0249617

Stephens, T. P., Loneragan, G. H., Karunasena, E., & Brashears, A. 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. In *Journal Of Food Protection* (Vol. 70, Issue 10). http://meridian.allenpress.com/jfp/article-pdf/70/10/2386/1677267/0362-028x-70_10_2386.pdf

Strauss, B., King, W., Ley, A., & Hoey, J. R. (2001). A Prospective Study Of Rural Drinking Water Quality And Acute Gastrointestinal Illness. *BMC Public Health*, *1*, 1–6. https://doi.org/10.1186/1471-2458-1-8

Tack, D. M., Ray, L., Griffin, P. M., Cieslak, P. R., Dunn, J., Rissman, T., Jervis, R., Lathrop, S., Muse, A., Duwell, M., Smith, K., Tobin-D'angelo, M., Duc, ;, Vugia, J., Joanna, ;, Kufel, Z., Beverly, ;, Wolpert, J., Tauxe, ; Robert, & Payne, D. C. (2020). *Preliminary Incidence And Trends Of Infections With Pathogens Transmitted Commonly Through Food — Foodborne*

Diseases Active Surveillance Network, 10 U.S. Sites, 2016–2019.
https://www.cdc.gov/mmwr/mmwr_continuingEducation.html

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 CRISPR-Seroseq FOOD MICROBIOLOGY Crossm Downloaded From. *Aem.Asm.Org 1 Applied And Environmental Microbiology*, *84*, 1859–1877. https://doi.org/10.1128/AEM.01859-18

U.S. Department Of Agriculture National Animal Health Monitoring System. (2013). *Part I: Management Practices On U.S. Feedlots With A Capacity Of 1,000 Or More Head*. http://www.aphis.usda.gov/nahms.

Usda Aphis. (2009). *Vaccination Of Cattle And Calves On U.S. Beef Cow-Calf Operations General Vaccination Practices*. http://www.aphis.usda.gov/nahms

Usda Fsis. (2023). *Mlg 4.13 Isolation And Identification Of Salmonella From Meat, Poultry, Pasteurized Egg, Carcass, And Environmental Sponges*.

Uzzau, S., Brown, D. J., Wallis, T., Rubino, S., Leori, G., Bernard, S., Casadesus, J., Platt, D. J., & Olsen, J. E. (2000). REVIEW Host Adapted Serotypes Of *Salmonella* Enterica. In *Epidemiol. Infect* (Vol. 125).

Vosik, D., Tewari, D., Dettinger, L., M'ikanatha, N. M., & Shariat, N. W. (2018). CRISPR Typing And Antibiotic Resistance Correlates With Polyphyletic Distribution In Human Isolates Of *Salmonella* Kentucky . *Foodborne Pathogens And Disease*, *15*(2), 101–108. https://doi.org/10.1089/fpd.2017.2298

Wallace, H Andrews And Thomas, H. (2009). BAM: *Salmonella*. *Bacteriological Analytical Manual*, *December 2007*, 21.

Webb, H. E., Brichta-Harhay, D. M., Brashears, M. M., Nightingale, K. K., Arthur, T. M., Bosilevac, J. M., Kalchayanand, N., Schmidt, J. W., Wang, R., Granier, S. A., Brown, T. R., Edrington, T. S., Shackelford, S. D., Wheeler, T. L., & Loneragan, G. H. (2017). *Salmonella* In Peripheral Lymph Nodes Of Healthy Cattle At Slaughter. *Frontiers In Microbiology*, *8*(NOV), 1–10. https://doi.org/10.3389/fmicb.2017.02214

Werber, D., Dreesman, J., Feil, F., Treeck, U., Fell, G., Ethelberg, S., Hauri, A., Roggentin, P., Prager, R., Fisher, I., Behnke, S., Bartelt, E., Weise, E., Ellis, A., Siitonen, A., Andersson, Y., Tschäpe, H., Kramer, M., & Ammon, A. (2005). International Outbreak Of *Salmonella* Oranienburg Due To German Chocolate. *BMC Infectious Diseases*, *5*, 7. https://doi.org/10.1186/1471-2334-5-7

Wheeler, T. L., Kalchayanand, N., & Bosilevac, J. M. (2014). Pre- And Post-Harvest Interventions To Reduce Pathogen Contamination In The U.S. Beef Industry. *Meat Science*, *98*(3), 372–382. https://doi.org/10.1016/j.meatsci.2014.06.026

World Health Organization. Foodborne Disease Burden Epidemiology Reference Group. (2015). *WHO Estimates Of The Global Burden Of Foodborne Diseases*.

Worley, J., Meng, J., Allard, M. W., Brown, E. W., & Timme, R. E. (2018). *Salmonella Enterica Phylogeny Based On Whole-Genome Sequencing Reveals Two New Clades And Novel Patterns Of Horizontally Acquired Genetic Elements*. https://doi.org/10

Yin, Z., Liu, J., Du, B., Ruan, H. H., Huo, Y. X., Du, Y., & Qiao, J. (2020). Whole-Genome-Based Survey For Polyphyletic Serovars Of *Salmonella* Enterica Subsp. Enterica Provides New Insights Into Public Health Surveillance. *International Journal Of Molecular Sciences*, *21*(15), 1–19. https://doi.org/10.3390/ijms21155226

Yoshida, C. E., Kruczkiewicz, P., Laing, C. R., Lingohr, E. J., Gannon, V. P. J., Nash, J. H. E., & Taboada, E. N. (2016). The *Salmonella* In Silico Typing Resource (SISTR): An Open Web-Accessible Tool For Rapidly Typing And Subtyping Draft *Salmonella* Genome Assemblies. *Plos ONE*, *11*(1). https://doi.org/10.1371/journal.pone.0147101

Young, I., Wilhelm, B. J., Cahill, S., Nakagawa, R., Desmarchelier, P., & Rajić, A. (2016). A Rapid Systematic Review And Meta-Analysis Of The Efficacy Of Slaughter And Processing Interventions To Control Nontyphoidal *Salmonella* In Beef And Pork. In *Journal Of Food Protection* (Vol. 79, Issue 12, Pp. 2196–2210). International Association For Food Protection. https://doi.org/10.4315/0362-028X.JFP-16-203

Zha, L., Garrett, S., & Sun, J. (2019). *Salmonella* Infection In Chronic Inflammation And Gastrointestinal Cancer. *Diseases*, *7*(1), 28. https://doi.org/10.3390/diseases7010028

Zhang, S., Den Bakker, H. C., Li, S., Chen, J., Dinsmore, B. A., Lane, C., Lauer, A. C., Fields, P. I., & Deng, X. (2019). *Seqsero2: Rapid And Improved Salmonella Serotype Determination Using Whole-Genome Sequencing Data*. https://journals.asm.org/journal/aem

CHAPTER 3

SALMONELLA SEROVAR DYNAMICS IN CATTLE

Introduction

Salmonella is a leading bacterial foodborne pathogen, causing 1.3 million illnesses in the U.S. annually (Tack et al., 2020). *Salmonella* is often associated with eating contaminated foods, such as poultry, beef, and produce (IFSAC, 2022). Beef products are the 6th largest commodity associated with *Salmonella* infection (IFSAC, 2022).

Cattle are common carriers of *Salmonella*; these infections are often asymptomatic (Gopinath et al., 2012). *Salmonella* enterica comprises over 2,500 serovars, each defined by their unique combinations of O and H antigens (Grimont & Weill, 2007) The carriage of *Salmonella* in cattle depends on the serovar causing the infection and, secondly, the stress on the animal (Gopinath et al., 2012; Gronstol et al., 1974; Nielsen et al., 2004). Gronstol et al. (1974) showed that stress is enough to induce shedding in cattle.

The inclusion of lymph nodes has been linked with *Salmonella* contaminated ground beef (Gutiérrez et al., 2020; Haneklaus et al., 2012; Webb et al., 2017). Many studies have investigated *Salmonella* in cattle, from studies about mitigation at slaughter, to the efficacy of washing the hide to prevent cross-contamination, to antimicrobial resistance, to investigating the prevalence within the lymph nodes and other organs (Arthur et al., 2007; Asakura et al., 2022; Gragg et al., 2013; Gutierrez-Bañuelos et al., 2011; Im et al., 2016; Samuel et al., 1979)

Salmonella sources include flies, feed and water troughs, which can serve as vectors to colonize a herd (Carlson et al., 2020; Olafson et al., 2016; Sargeant et al., 2021; Shariat et al., 2022). *Salmonella* prevalence varies by season, decreasing in colder months (Barkocy-Gallagher et al., 2003). Pre-harvest studies have examined the rate of *Salmonella* shedding in relation to the

season and other vectors introducing *Salmonella* in feedlots (Chong et al., 2021; Cummings et al., 2009; Gopinath et al., 2012; Levent et al., 2019; Olafson et al., 2016; Porwollik et al., 2018). Gragg et al. (2013) showed that *Salmonella* prevalence on the hide ranged from 91-100%, and prevalence in the feces ranged from 85-97%. *Salmonella* is often shed in cattle feces, with a decrease in the colder months. Pre-harvest studies have examined rates of shedding, stress, and ways to reduce *Salmonella* coming into the feedlot. Still, they do not characterize changes in *Salmonella* prevalence soils or serovar diversity in feedlots.

Genetic differences among *Salmonella* serovars give rise to different phenotypes, such as thermal tolerance, host restriction, and antimicrobial resistance (Ng et al., 1969; Uzzau et al., 2000; Vosik et al., 2018). Phenotypic differences could mean better survival or even increased virulence. Examining *Salmonella* populations, composed of multiple serovars, is needed to understand the potential food safety risks. Some serovar are polyphyletic, where the same O and H antigens are shared by two genetically distinct *Salmonella*e (Yin et al., 2020). One example is serovar Kentucky, which can be identified as Kentucky I and II; these serovars have distinct CRISPR arrays and antimicrobial resistance patterns (Vosik et al., 2018). Polyphyletic serovars are not often differentiated; but can have different hosts, Kentucky I is commonly associated with poultry and Kentucky II is associated with cattle (Haley et al., 2016).

Salmonella isolation involves picking 1-3 colonies from selective indicator media (Andrews et al., 2022). This typically only detects the most abundant serovar(s) present in a sample. In more complex serovar populations, picking three colonies is not enough to characterize the full population. Siceloff et al, (2022) found five serovars that were found in the CDC top ten serovars of concern in 2016, three of which appeared more often in the minority of samples. These serovars could have been overlooked in traditional culturing methods.

Deep serotyping identifies all serovars contained within a sample. CRISPR-SeroSeq (CSS) is a deep serotyping method that utilizes the unique spacers with the CRISPR array to identify serovars. The two CRISPR arrays in *Salmonella* are composed of unique spacer sequences and highly conserved direct repeat sequences (Touchon & Rocha, 2010). The array initially evolved via the stepwise addition of spacers, and while the array is no longer evolving, it remains a fingerprinting region (Fabre et al., 2012; N. Shariat & Dudley, 2014; Touchon & Rocha, 2010). A database containing the spacer sequences of ~140 serovars linked to humans, turkey, poultry, and cattle can tease out the serovars present in a sample. CSS works by PCR amplification of the CRISPR spacers, using dual index Illumina primers. This PCR amplifies the spacers of *Salmonella* with a sample; this can then be sequenced. The sequence reads can then be used to identify the relative frequency of each serovar in a sample using the number of sequence reads for each spacer. *Salmonella* populations are complex microbial communities; several studies have shown multiserovar populations existing in numerous environments, such as creek systems, animal feed, broilers, and cattle (Deaven et al., 2021; Shariat et al., 2022; Siceloff et al., 2021, 2022). As many as 11 serovars have been found within one sample using CRISPR-SeroSeq (Shariat et al., 2022; Siceloff et al., 2021).

The complexity of *Salmonella* populations within feedlots can vary, and individual pens can have unique serovar profiles; the serovars recovered can differ based on which pen is sampled (Levent et al., 2019). The goal of the studies presented here was to explore *Salmonella* serovar dynamics in feedlot environments by sampling various pens, soil depths, feed, water, and common areas in the feedlot. Establishing a baseline for *Salmonella* dynamics within feedlots and the seasonal change in *Salmonella* prevalence will create a starting point for further research. An

additional goal of this work was to investigate how serovar dynamics change over time and to temporally compare freshly shed *Salmonella* in feces to *Salmonella* in dried feces.

In these studies, we examined two feedlots with historically high *Salmonella* prevalence. This led us to test the hypothesis that *Salmonella* was surviving and persisting in the feedlot soil, which has not been deeply investigated. Although not directly relevant to feedlot environments, studies have shown that *Salmonella* can survive in manure-amended soils for 40 days (Franz et al., 2005). *Salmonella* internalization into soil depends on pore size, water activity, and temperature (Hekman W.E et al., 1994; Holley et al., 2006; You et al., 2006). Hekman et al. (1995) also found that bacterial density decreased as soil depth increased. Soil pore size affects the rate of bacterial flow; soil with larger pores allows bacteria to travel only when there is an influx of water that is saturating the soil (Hekman W.E et al., 1995.). Soil with smaller pores can retain water and allow bacteria to move due to water in the micropores (Hekman W.E et al., 1995.; Stevik et al., 2004).

This study sampled four feedlots, sampling various pens and locations in the feedlots to better understand *Salmonella* serovar dynamics within feedlots. Over the course of this study, *Salmonella* decreased in prevalence in the colder months. This study also defined the *Salmonella* populations present in cattle feces and the dried manure in the environment.

MATERIALS AND METHODS

Bio-mapping *Salmonella* Populations Across Four Texas Feedlots

Four feedlots were selected for the one-year sampling period. Two feedlots were selected based on a historic high *Salmonella* prevalence and two based on a historic low *Salmonella* prevalence in trim and lymph nodes from their steers at the harvesting plant. Each feedlot was visited four times a year, once per season, over one year to collect samples. On each visit, eight random pens were chosen within the feedlots. A total of seven locations were sampled at each pen. A bootsock sample was taken by walking around the pen in a Z pattern. At the same time, the manure surface composite sample was collected using sterile gloves and picking at 11 different points along the Z pattern using a sterile 55 oz unfiltered Whirlpack™ (Nasco; Fort Atkinson, WI) bag. A hole was dug in a designated area within the pen using a disinfected shovel. Using three separate sterile 55 oz Whirlpack™ bags, three samples were taken from the hole. One sample from the surface layer (top layer), one sample from the interface layer (middle), and one sample from the soil layer (bottom layer). A fresh mixed ration sample was taken from the feed bunk of the pen, and a sample of the trough water was taken using a specimen cup. In addition to the eight pens, a swab was collected from the receiving and shipping pens.

Soil, manure, fecal, and mixed ration samples were diluted with 25g of sample added to 225 ml of modified tryptic soy broth (mTSB; Neogen, Lansing, MI) with 8 mg/L of novobiocin. Samples were homogenized using a stomacher (Model 400 circulator; Seward, West Sussex, UK) at 230 rpm for 2 min. Homogenized samples were incubated at 42ºC for 18-24 h. Trough water samples were processed by measuring 10 ml of water and diluting with 90 ml of mTSB

and incubated at 42ºC for 24h. For feed truck tire samples, 1 ml from the swab sample was added onto 9 ml of mTSB and incubated at 42ºC for 24h. Bootsocks were processed by adding 100 ml of mTSB onto the filtered 24 oz Whirlpack™ bag that they were contained in, homogenized in a stomacher at 230 rpm for 1 min, and incubated at 42ºC for 24h.

Salmonella detection and isolation

From the enriched samples, 1 ml was added to 9 ml of Rappaport Vassiliadis (RV10; Oxoid, Hampshire, UK) broth and 9 ml of tetrathionate broth (TT; Neogen, Lansing, MI) as secondary enrichments. Both RV and TT broths were incubated at 42ºC for 18-24 hours. Following secondary enrichment, a loopful of the sample was streaked onto xylose lysine tergitol 4 (XLT4; Hardy Diagnostics, Santa Maria, CA) and CHROMagar™ *Salmonella* (ChromSal; Springfield, NJ) and incubated at 37ºC for 24 h. Presumptive *Salmonella* positive colonies, black or yellow with a black center on XLT4 agar and mauve on ChromSal agar, were selected and confirmed using the BAX® real-time *Salmonella* assay (Hygiena, Wilmington, DE) following the manufacturer's instructions.

Deep serotyping by CRISPR-SeroSeq

For each of the RV and TT broths from *Salmonella*-positive samples, the overnight enrichments were briefly vortexed and 1 ml was transferred to a microcentrifuge tube. The samples were centrifuged at max speed (~14,000rpm) on a benchtop centrifuge to pellet the bacteria. After removing the supernatant, the pellet was frozen at -20ºC. Total genomic DNA was isolated from pellets using the Promega Genome Wizard kit (Madison, WI), according to the manufacturer's instructions, and was resuspended in 200 µl of molecular grade water and stored at -20°C. Genomic DNA was diluted 10-fold in molecular grade water and 2 µl was used as a template in the first PCR step for CRISPR-SeroSeq with primers targeting the conserved direct

repeat sequences within *Salmonella* CRISPR arrays. PCR products were purified using the Ampure system (Beckman Coulter, Indianapolis, IN), according to the manufacturer's instructions. For the second PCR to add dual index sequences, 5 µl of the cleaned amplicon was used as a template, following the Illumina Nextera protocol (Illumina, San Diego, CA). PCR products were purified using Ampure and pooled in approximate equimolar ratios. Pooled libraries were multiplexed and sequenced on the Illumina NextSeq platform with 150 cycles, single end reads. Each sequencing run contained two negative control samples: a non-template water control from the first PCR and a non-template water control from the second PCR. A positive control containing *Salmonella* serotype Enteritidis genomic DNA with a known CRISPR profile was also included on each run. CRISPR-SeroSeq analyses were performed using a R script that scans sequence reads and uses BLAST to match sequence reads to a database of over 135 serovars, before writing the output directly to Excel. Serovars were called only if they contained multiple CRISPR spacers that were unique to that serotype. Where we had CRISPR-SeroSeq data for both RV and TT enrichments from the same sample, the relative serotype frequencies were normalized and presented as a single data series.

Statistical Analyses

The analysis focused on three primary sample measures: culture-based detection of *Salmonella* presence, the identity (and number) of *Salmonella* serovars via CRISPR-SeroSeq, and the relative frequencies of these serovars within each sample. All analyses were conducted in the R programming environment. Linear models were constructed using the mgcv R package (function gam, method="REML"). The emmeans R package to compute estimated marginal means (EMM) and (adjusted) 95% confidence intervals.

To assess *Salmonella* prevalence across all 928 samples, a logistic regression model was used with presence as the response. Feedlot, season, and sample type were used as fixed effects, and the pen as a random effect. The EMM (+95% confidence intervals) was computed within each feedlot, season, and sample type.

Serotype identity analysis focused on the 263 samples that had CRISPR-SeroSeq profiles. To minimize the impact of rare events, serovars found in fewer than 5 samples were excluded. Summary statistics were computed across all CRISPR-SeroSeq profiles for each serotype, including percent presence (all CRISPR-SeroSeq profiles) and the mean relative frequency (MRF), percent dominant, and percent alone (present CRISPR-SeroSeq samples). The rank presence and rank MRF were also computed. The total number of unique serovars found in each sample (serotype richness) and in each sampled pen (i.e., among sample types), as well as the mean number of serovars per sample within pen (mean richness) were computed.

Serovars were classified based on relative abundance within samples, the serovar making up the largest portion within a sample was classified as the "majority" serovar. The number of times a serovar appeared, and the number of times the serovar was in the "majority" were then used to calculate how often serovars appear in the majority and minority or samples.

Evaluation of *Salmonella* serovar populations in two cattle feedlots, over two placements

Sample collection

Two feedlots were selected for this objective and two pens in each feedlot were sampled following cattle placement in the winter. This was repeated in the same two feedlots but in different pens for cattle placed in the summer. Samples were collected along six lines within each pen: one along each side, and two diagonals. Along each of these lines, a pair of bootsocks was worn to collect manure pad samples (= "manure" in above analyses) and four fresh fecal pats (= "feces") were collected and composited into one sterile 55 oz Whirlpack^{TM} bag. The

bootsock pairs were each placed into different 24 oz Whirlpack™ bags. Thus, for each pen visit there were six fecal samples and six manure pad samples. Samples were collected on day 0 from the manure pad of an empty pen. We returned later that day to collect fresh fecal samples from cattle after they had been placed in the pen. The subsequent visits were on days 15, 30, 45, 60, 90, and right before slaughter (day 120-124).

Fecal samples were diluted by adding 25g of sample to 225 ml of modified tryptic soy broth (mTSB; Neogen, Lansing, MI) with 8 mg/L of novobiocin. Samples were homogenized using a stomacher (Model 400 circulator; Seward, West Sussex, UK) at 230 rpm for 2 min. Homogenized samples were incubated at 42ºC for 18-24 h. Trough water samples were processed by measuring 10 ml of water and diluting with 90 ml of mTSB and incubated at 42ºC for 24h. For feed truck tire samples, 1 ml from the swab sample was added onto 9 ml of mTSB and incubated at 42ºC for 24h. Dried manure samples were collected via bootsocks; the bootsocks samples were processed by adding 100 ml of mTSB onto the filtered 24 oz Whirlpack™ bag that they were contained in, homogenized in a stomacher at 230 rpm for 1 min, and incubated at 42ºC for 24h.

Salmonella detection and isolation

From the enriched samples, 1 ml was added to 9 ml of Rappaport Vassiliadis (RV10; Oxoid, Hampshire, UK) broth and 9 ml of tetrathionate broth (TT; Neogen, Lansing, MI) as secondary enrichments. Both RV and TT broths were incubated at 42ºC for 18-24 hours. Following secondary enrichment, a loopful of the sample was streaked onto xylose lysine tergitol 4 (XLT4; Hardy Diagnostics, Santa Maria, CA) and CHROMagar™ *Salmonella* (ChromSal; Springfield, NJ) and incubated at 37ºC for 24 h. Presumptive *Salmonella* positive colonies, black or yellow with a black center on XLT4 agar and mauve on ChromSal agar, were selected and

confirmed using the BAX® real-time *Salmonella* assay (Hygiena, Wilmington, DE) following the manufacturer's instructions.

Statistical Analyses

Serotype identity analysis focused on the 263 samples that had CRISPR-SeroSeq profiles. To minimize the impact of rare events, serovars found in fewer than 5 samples were excluded. Summary statistics were computed across all CRISPR-SeroSeq profiles for each serotype, including percent presence (all CRISPR-SeroSeq profiles) and the mean relative frequency (MRF), percent dominant, and percent alone (present CRISPR-SeroSeq samples). The rank presence and rank MRF were also computed. The total number of unique serovars found in each sample (serotype richness) and in each sampled pen (i.e., among sample types), as well as the mean number of serovars per sample within pen (mean richness) were computed.

RESULTS

Study 1: Bio-mapping *Salmonella* Populations Across Four Commercial Feedlots

Salmonella prevalence

Of the four feedlots in this study, feedlot 4 had the highest *Salmonella* presence, with a mean prevalence of 76% across the eight sample types, ranging from 3.1% in mixed rations (1/32) to 100% in the shipping/receiving pen (4/4). *Salmonella* prevalence across the eight sample types in feedlot 4 was shipping/receiving pen (100%;4/4), bootsocks (93.75; 30/32), manure sample composite (87.5%; 28/32), the surface layer (81.3%; 26/32), interface layer (53.1%; 17/32), soil layer (37.5%; 12/32), trough water (31.3%; 10/32), and mixed rations (3.1%; 1/32). In contrast, feedlots 1-3 had a decreased mean *Salmonella* prevalence, feedlot 1 (20%), feedlot 2 (30.5%), and feedlot 3 (23.8%). Comparing the *Salmonella* prevalence from all feedlots per season, summer had the most positive samples (46.5%; 91/228), followed by fall (45.7%; 89/228). *Salmonella* prevalence in the winter (30.5; 50/228) sampling collection had a 15% decrease in prevalence compared to the fall sample collection, followed by a further decrease in prevalence in the spring sample collection (17.6%; 31/228). *Salmonella* prevalence was not equal across all sampling locations, the shipping and receiving pens had the most positive samples (87.5;14/16), followed by manure samples (52.2%;67/128) and bootsock samples (50%;64/128) (figure 2). Mixed ration and trough water samples were rarely positive, with (15/128) and (11/128). Compared to the prevalence of bootsock samples, *Salmonella* prevalence was lesser in the surface layer samples (30.5%;39/128), with further decreases in the interface layer (21.9%; 28/128) and soil layer (19.5%; 25/128). Showing that as depth increases, the number of positive samples decreases. *Salmonella* prevalence was found to vary between sample type, feedlot, and season.

Deep serotyping and overall sample complexity

Deep serotyping revealed an average of 2.94 serovars per sample, with a range of 1-10 serovars per sample (figure 3). Samples also showed variance in their serovar complexity; the shipping and receiving pens had the most serovars with an average of 3.92 serovars per sample, and bootsock samples had the second most diversity with an average of 3.44 serovars per sample. Comparing serovar complexity per sample across all feedlots (figure 3), feedlot 4 was observed to have more serovars per sample. The number of serovars identified over the year varied per feedlot; feedlots 1 and 2 had 19 serovars, feedlot 3 had 20 serovars, and feedlot 4 had 30 serovars identified. The serovar complexity and populations varied between samples and feedlots. Figure (4) shows how often multiserovar populations were found per season. The population complexity also varied based on sample type, and between feedlots. In feedlots 1,2, and 3 samples were more often found to have single serovar populations than any single multiserovar population. In contrast, feedlot 4, most samples had three serovars per sample than any other serovar population. Further examination into multiserovar populations throughout the seasons, showed that within feedlot 4 serovar complexity decreases during the colder months, starting with most sample having five serovars in the summer, 3 in both the fall and winter, and in the spring single serovar populations occurred as frequently as two serovars in a sample (figure 4).

Investigating serovar presence and abundance revealed that the three most identified serovars were Anatum, Montevideo I, and Mbandaka, with 181, 128, and 123 appearances (Table 1). Of the 37 serovars identified, 23 appeared more than five times. Looking at the relative frequency within samples allowed for a ratio to be created; this ratio details how often a serovar occurred with the largest relative frequency in a single sample ("majority") or not

(“minority”). Only serovar Altona appeared more often in the majority than minority (7/12 occasions) (Table 1). Serovars Yoruba (6/12) and Schwarzengrund (4/8) appeared equally in the majority and minority (Table 1). The remaining serovars all appeared more often in the minority. Serovar Infantis never appeared in the majority of samples (0/19). The relative frequency of individual serovars varied, with some more often being found in the background of samples.

Serovar populations by feedlot

A detailed heat map showing the relative frequency of serovar populations with feedlot 1 (figure 5) shows that serovars Anatum, Montevideo I, and Muenchen occurred the most frequently. Individual pen profiles can also be observed; pen 5 had three unique serovars compared to the other seven pens sampled in the summer. Serovar Newport III was only found in the summer sampling. Feedlots 1 & 2 show the least diversity, with 19 serovars. Feedlot 2 showed the same three most common serovars (Anatum, Montevideo I, and Muenchen). Feedlot 2 (figure 6) had zero positive samples in the spring sampling, 13 *Salmonella* positive samples in the winter, 26 positive samples in the fall, and 24 positive samples in the summer. In Feedlot 3 (Figure 7) the serovars found the most often were Anatum, Meleagradis, and Montevideo I. Feedlot 3 had a total of 20 serovars found, whereas feedlot 4 had 30 unique serovars. Feedlot 4 (figure 8) had the most positive samples, with Anatum, Mbandaka, and Montevideo I being the serovars found most often. Serovars Anatum, Montevideo I, Muenchen, and Mbadaka were the four most commonly found serovar, with each feedlot have separate serovar populations as well.

Figure (5) also shows that in feedlot 1, the sample with the most diversity was the mixed rations from pen 5 in the summer collection, with six serovars. In contrast, the samples with the most diversity in feedlots 2 and 3 (figures 6-7) were the shipping/receiving pens with seven and six serovars, respectively. In feedlot 4, (figure 8), the sample with the most diversity was a

bootsock sample from pen 2 during the summer collection. Populations complexity was shown to be the highest in the shipping and receiving pens, with complexity varying between samples with the same feedlot, and even pen.

The relative frequency of serovars varied based on the feedlot, with Anatum appearing more often in the majority in two of the four feedlots. In feedlot 1, only serovars Anatum, Montevideo I, Muenchen, and Newport III, appeared in more than five samples across all four seasons. Serovar Anatum was the only serovar among those four to appear more often in the majority. Feedlot 2 had eight serovars that appeared more than five times (table 2). Feedlot 3 had seven serovars that appeared more often than five times, four appearing more often as the major serovar within a sample. Table (2) shows that in feedlot 4, only serovar Mbandaka appears more often as the major serovar. The relative frequency of serovars varied between feedlots, showing distinctions between serovar prevalence in each feedlot.

Table (3) shows that summer had 17 serovars appear more than five times, with none appearing more often as the major serovar within a sample. The Fall season had nine appear five or more times, with three appearing more often as the major serovar. During the winter season, eight serovars appeared more than five times, with serovars Cerro, Meleagradis, and Schwarzengrund appearing more often in the majority of samples. The spring sampling only had serovars Mbandaka, Anatum, and Montevideo I appear in more than five samples, with Mbandaka appearing more often as the major serovar. Relative frequency varied based on the season, with fewer serovars appearing during the colder months. The frequency at which serovars appear in samples varied; serovar Mbandaka only appeared more often in the majority of the spring samples. Spring had the lowest serovar diversity, with less serovars appearing more

than five times. In contrast, with the most diversity during the summer, no serovars appear more frequently in the major.

Study 2: Evaluation of *Salmonella* serovar populations in two cattle feedlots over two placements

Eight pens were used in this study, of which six produced enough *Salmonella* positive samples for in-depth analysis. The two pens were excluded from the analysis; these were pens 1 and 2 from feedlot 2 during the winter placement, which produced only 10/168 positive samples combined. Figure (9) shows that the most positive samples came from feedlot 4 pen 1 in the winter (84.5%;71/84) and pen 1 in the summer sampling (82.1%;68/84). The manure samples (76.2%;192/252) were more often positive than fecal samples (61.9;159/252). Manure samples were collected via bootsocks over a larger area than four composited fecal samples (figure 11). There was a range of 1-8 serovars found in samples, with an average of 2.9 serovars in the fecal and manure samples from both placements in feedlot 4 and the summer placement of feedlot 2. Over 79.3% (283/357) of the samples contained multiserovar populations. Figure (10a) shows the serovar complexity in fecal and manure samples, with manure having a higher complexity. Over three quarters of samples had multi-serovar populations, with more complex populations being found in manure samples, as shown by the red bar indicating the population that occurred most often. Fecal samples more often appeared as single serovar populations, whereas manure samples more often had 2 serovars per sample (figure 10a). Figure (10b) shows the overall population complexity between the winter and summer placement; in the summer placement, more samples had four serovars than any other population. In contrast, the winter placement had more samples containing three serovars than the summer population.

Hide swabs from cattle originating in feedlot 2 pen 1 had the most diversity, with 12 serovars found within a single sample (figure 12). Figures (12-17) show the *Salmonella* populations based on the combined results from all six manure and fecal samples taken on that sampling day from that pen. Serovars Kentucky II was found in manure samples from day 0 – 45 and day 90 but in the feces on days 0, 30, and 60. Serovar Anatum was found in manure samples on days 15-60 and in the feces on days 0, 30, and 90. Serovar Montevideo I was found on every sampling day in the manure but only on days 0, 15, and 60 in fecal samples. Manure samples had two unique serovars (Altona and Newport III) compared to fecal samples. The hide samples had six serovars previously not observed in the feces or manure samples. The samples collected at slaughter had the highest serovar complexity; in the pens serovar detections were inconsistent, with some serovars appearing sporadically throughout the sampling period.

Serovar Mbandaka appears in both the manure and feces on day 45 and is only detected again once samples were taken at slaughter in the hide and lymph nodes. No *Salmonella* positive fecal samples were found on day 124. Kentucky I was found in the manure over the seventh sampling period but was then found in the fecal swabs at slaughter. Serovars Agona, Cerro I, Kentucky I, and Lille were found in the manure but not in fecal samples. Serovars Enteritidis, Infantis, Muenchen, Newport III, Poona, and Typhimurium were unique to the samples collected at slaughter. During the summer placement, Pen 2 in feedlot 2 was the only sample set to have a positive lymph node sample (figure 13). Serovar Typhimurium and Poona were solely found in the samples taken at slaughter, with serovar Poona being found only on the hide and Typhimurium only found in fecal swabs from both pens during the summer placement of feedlot 2. Serovar populations differed between the fresh fecal samples, the dried desiccated manure samples, and samples taken at slaughter.

This shows the within-pen diversity, but new serovars sporadically appear in all the pens. Across all pens, serovars sporadically appeared; in the summer for feedlot 2 pen 1, serovars Altona and Newport III only appeared once in the manure, on days 30 and 90, respectively (figure 12). In pen 2 of feedlot 2, serovars Agona and Cerro I appeared on day 15, Cerro II appeared on day 60, and Lille appeared on day 30, each appearing only in the manure (figure 13). In the same pen, serovar Meleagradis only appeared in the feces on day 90. In Feedlot 4 during the summer sample collection, pen1 had serovar Lille appear in the manure only on day zero. But on day 30, serovars Barranquilla, Cubana, and Kentucky II appeared in the manure, and serovar Senftenberg II only appeared in the manure on day 124. Pen 2 of feedlot 4 in the summer placement had serovar Senftenberg I appear in the manure on day 30, but serovar Uganda appeared in the feces on day 30. During the winter placement of feedlot 4 (figure 16), pen 1 had serovars Infantis, Liverpool, Orion, and Senftenberg I appear only on day 30 in fecal samples. In pen 2, from that same placement, serovars Uganda and Kentucky II appeared only once in the feces on day 30 (figure 17). Samples collected from the same pen during the same sampling period showed differences in serovar populations.

Samples from the summer placement of feedlot 4 (figure 15) showed Cerro II not present in the feces on day 0, but in the manure. Then, on day 15, Cerro II was observed in fecal and manure samples. In the summer placement of feedlot 4 in pen 1, serovar Minnesota only appeared on day 15 in fecal and manure samples. Muenchen I was found in fecal and manure samples but not at slaughter. Manure samples have six more serovars than fecal samples: Barranquilla, Cubana, Kentucky II, Lille, Senftenberg II, and Uganda. Serovar Schwarzengrund was unique to the samples collected at slaughter. Meleagradis was found on day 15 in the manure, then on the hide swab, but not detected at any other sampling point. Comparing the fecal

and manure serovar populations in the summer placement of feedlot 4 in pen 2 (figure 17) showed only one unique serovar; in the feces, Uganda was found only on day 30. Senftenberg I was unique to manure samples on day 30. The samples taken at slaughter showed the most diversity, with serovars Typhimurium and Kentucky II were never found on the feedlot. Manure samples showed more population complexity than fecal samples, and samples collected at slaughter show serovars that had not previously been detected.

Sixteen serovars were found in feedlot 4 pen 1 of the winter placement, and 14 serovars were found in feedlot 4 pen 2 of the winter placement (figures 16-17). Feedlot 4 pen 1 of the winter placement had six serovars found in the feces that were not detected in the manure samples. Serovar Montevideo I was never found in the fecal samples despite being found in 5/7 manure samples. In the winter placement of feedlot 4 pen 2, five serovars only once in the feces but never in the manure. These serovars are not strictly found on day 0 but also days, 30,45,60, and 90. In contrast, the manure had four unique serovars that also appeared sporadically. Despite consistent sampling serovars appear sporadically throughout the sampling periods.

Serovars Poona and Typhimurium were only found from samples at slaughter; Typhimurium was found in the fecal swab and Poona on the hide from both the Feedlot 2 pens in the summer collection. Serovars Enteritidis, Infantis, and Kentucky I were only found on the hide from feedlot 2. In feedlot 4, serovar Uganda appears in all four pens but sporadically in the manure or fecal samples. In contrast, serovar Minnesota was only recovered from the summer placement of feedlot 4. Serovars Uganda were consistently only found within feedlot 4, but serovars Typhimurium, Poona, Infantis, Kentucky I, and Enteritidis were only found from the samples collected at slaughter.

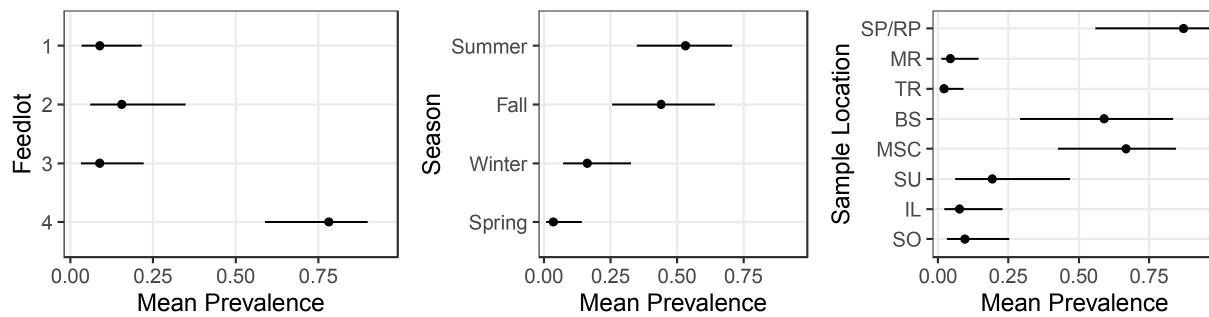


Figure 2. Estimated mean *Salmonella* prevalence by experimental unit.

Presence/absence (prevalence) data was fit using a logistic regression model, and the model was used to compute expected marginal means in each feedlot (left), season (center), or sample location (right). Each panel shows model predictions that average across those experimental units not shown. SP/RP, shipping and receiving pens; MR, mixed rations; TR, trough water; BS, bootsock; MSC, manure surface composite; SU, surface layer; IL, interface layer; SO, soil layer. Figure generated by Christian Gunning.

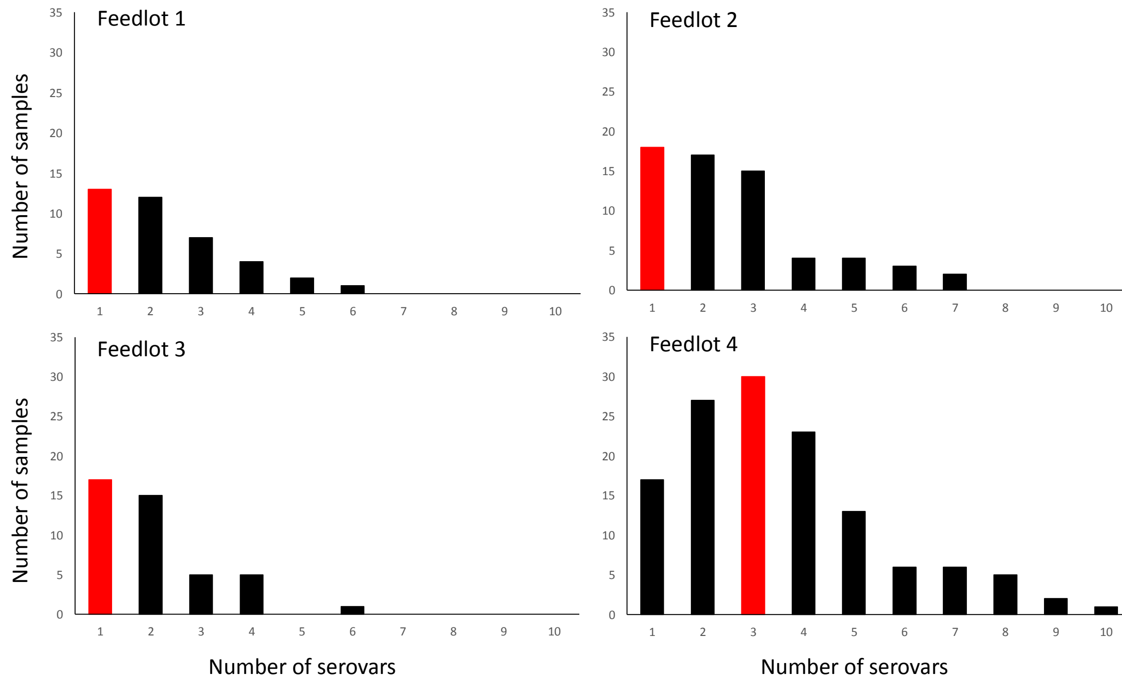


Figure 3. Population complexity per feedlot

The bar graphs above show the number of times serovar populations were found, with the red bar indicating the serovar population that was found most frequently.

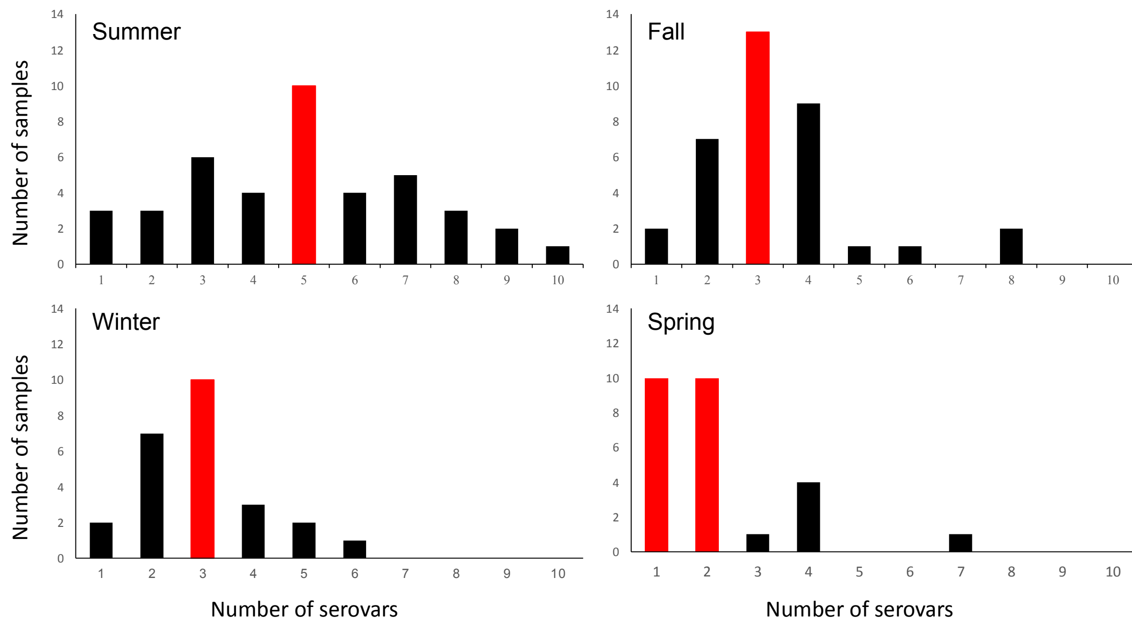


Figure 4. Frequency of Multiserovar Populations in feedlot 4

The bar graphs above show the number of times serovar populations were found, with the red bar indicating the serovar population that was found most frequently. This was strictly done with feedlot 4 due to the other feedlots having few samples in the spring and winter seasons.

Table 1. Relative frequencies of each serovar.

Serotype	Frequency	% Majority	% Minority
Anatum	181	44	56
Montevideo I	128	31	70
Mbandaka	123	45	55
Muenchen	88	35	65
Meleagradis	28	36	64
Kentucky I	25	0	100
Cerro	19	37	63
Infantis	19	0	100
Agona	17	0	100
Altona	12	58	42
Yoruba	12	50	50
Muenster	10	30	70
Newport III	10	20	80
Typhimurium	10	30	70
Schwarzengrund	8	50	50
Kentucky II	7	29	71
Lexington	7	29	71
Liverpool	7	29	71
Minnesota	7	14	86
Montevideo II	7	14	86
Rissen	7	0	100
Reading	6	17	83
Worthington	5	20	80

Footnote: Serovars in the CDC top 10 are shown in bold. Serovars that are more often outnumbered by other serovars when they are present have a higher minority percentage and are shown in red. The darker the shading, the more disparate the majority to minority ratio. Serotype Altona was more often the majority serotype when found and is shown in blue.

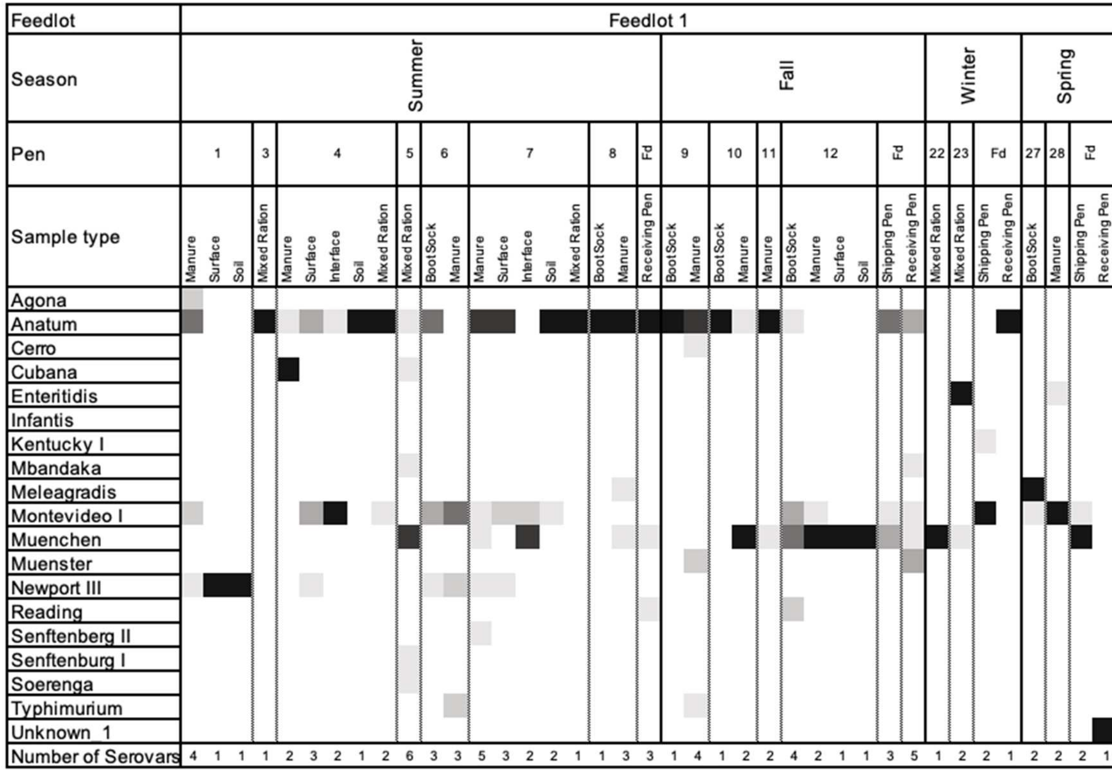
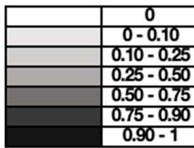


Figure 5: Relative frequency of Serovars found on Feedlot 1

A heatmap indicating the relative frequency of serovars found in positive samples from Feedlot 1. The absence of a serovar is indicated by white, following a gradient of increasingly darker shades to indicate a higher relative frequency of serovars within in a sample.



Feedlot: Feedlot 2 — Season: Summer

Pen	1	2			3		4		5					6					7			8	Fd	
Sample Type	Bootsock	Bootsock	Manure	Surface	Bootsock	Manure	Manure	Trough Water	Bootsock	Manure	Surface	Soil	Mixed Ration	Bootsock	Manure	Surface	Interface	Soil	Bootsock	Manure	Soil	Manure	Shipping Pen	Receiving Pen
Anatum																								
Cerro																								
Enteritidis																								
Infantis																								
Kentucky I																								
Kentucky II																								
Mbandaka																								
Meleagradis																								
Montevideo I																								
Montevideo II/III/IV																								
Muenchen																								
Muenster																								
Newport II																								
Newport III																								
Reading																								
Schwarzengrund																								
Senftenberg I																								
Typhimurium																								
Yoru																								
Number of Serovars	3	3	6	3	2	3	2	1	4	5	5	1	1	5	3	2	1	1	2	3	2	1	6	3

Feedlot: Feedlot 2 — Season: Fall

Pen	9					10				11			12				13		14	15		16			Fd	
Sample Type	Bootsock	Manure	Surface	Interface	Soil	Bootsock	Manure	Interface	Soil	Bootsock	Manure	Interface	Bootsock	Manure	Surface	Soil	Bootsock	Manure	Bootsock	Bootsock	Manure	Bootsock	Manure	Interface	Shipping Pen	Receiving Pen
Anatum																										
Cerro																										
Enteritidis																										
Infantis																										
Kentucky I																										
Kentucky II																										
Mbandaka																										
Meleagradis																										
Montevideo I																										
Montevideo II/III/IV																										
Muenchen																										
Muenster																										
Newport II																										
Newport III																										
Reading																										
Schwarzengrund																										
Senftenberg I																										
Typhimurium																										
Yoru																										
Number of Serovars	4	3	2	1	3	4	3	1	2	3	3	1	3	4	2	2	2	1	2	2	2	1	2	1	7	5

Feedlot: Feedlot 2 — Season: Winter

Pen	17		18			19	20	21		Fd	
Sample Type	Bootsock	Interface	Bootsock	Manure	Interface	Bootsock	Bootsock	Bootsock	Manure	Shipping Pen	Receiving Pen
Anatum											
Cerro											
Enteritidis											
Infantis											
Kentucky I											
Kentucky II											
Mbandaka											
Meleagradis											
Montevideo I											
Montevideo II/III/IV											
Muenchen											
Muenster											
Newport II											
Newport III											
Reading											
Schwarzengrund											
Senftenberg I											
Typhimurium											
Yoru											
Number of Serovars	1	2	2	2	1	3	1	3	1	7	7

Figure 6: Relative frequency of serovars recovered from Feedlot 2

A heatmap indicating the relative frequency of serovars found in positive samples from Feedlot 2. The absence of a serovar is indicated by white, following a gradient of increasingly darker shades to indicate a higher relative frequency of serovars within in a sample.

	0
	0 - 0.10
	0.10 - 0.25
	0.25 - 0.50
	0.50 - 0.75
	0.75 - 0.90
	0.90 - 1

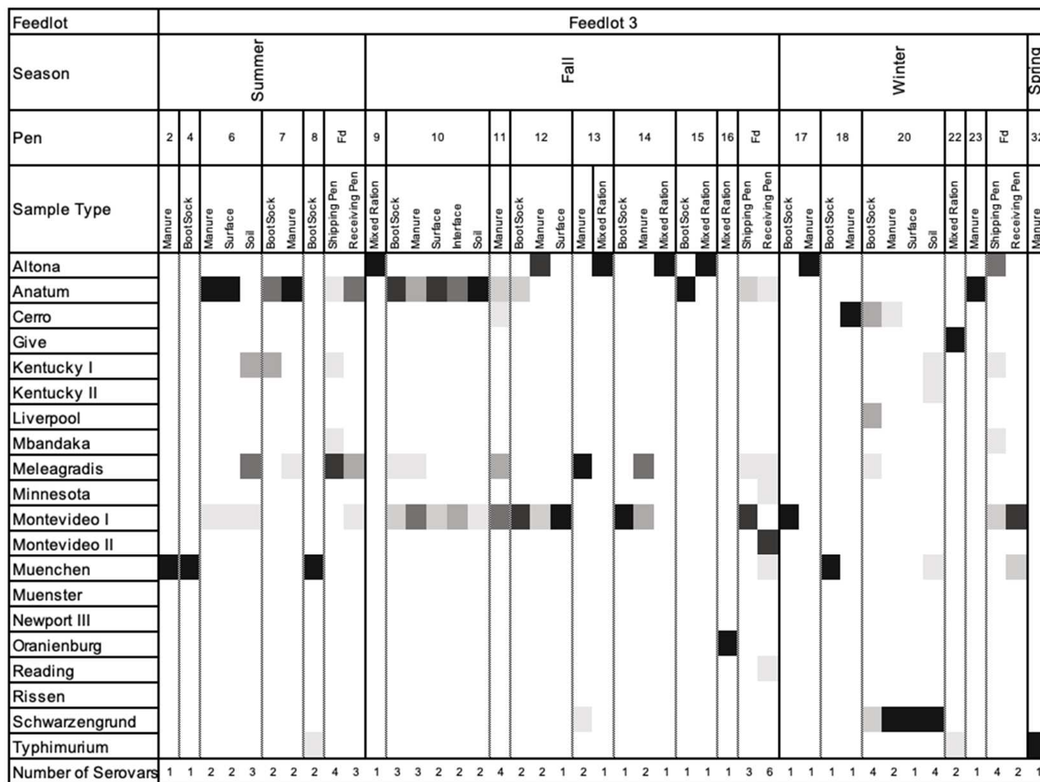
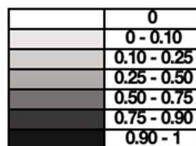
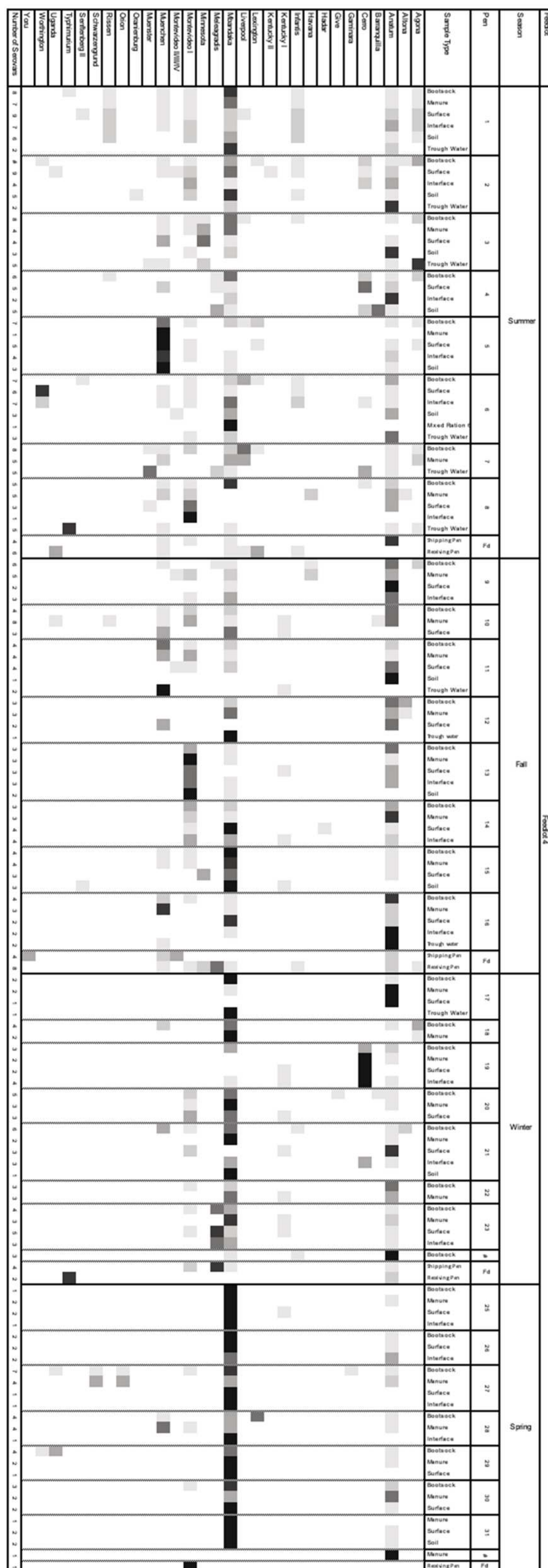


Figure 7: Relative frequency of serovars recovered from Feedlot 3

A heatmap indicating the relative frequency of serovars found in positive samples from Feedlot 3. The absence of a serovar is indicated by white, following a gradient of increasingly darker shades to indicate a higher relative frequency of serovars within in a sample.





	0
	0 - 0.10
	0.10 - 0.25
	0.25 - 0.50
	0.50 - 0.75
	0.75 - 0.90
	0.90 - 1

Figure 8: Relative frequency of serovars recovered from Feedlot 4

A heatmap indicating the relative frequency of serovars found in positive samples from Feedlot 4.

The absence of a serovar is indicated by white, following a gradient of increasingly darker shades to indicate higher relative frequency of serovars within in a sample.

	Serovar	Frequency	%Majority	%Minority
Feedlot 1	Anatum	25	80.00	20.00
	Montevideo I	19	26.32	73.68
	Muenchen	15	60.00	40.00
	Newport III	8	25.00	75.00
Feedlot 2	Anatum	42	50.00	50.00
	Montevideo I	33	51.52	48.48
	Muenchen	26	38.46	61.54
	Mbandaka	17	11.76	88.24
	Yoruba	11	45.45	54.55
	Muenster	6	50.00	50.00
	Kentucky I	5	0.00	100.00
	Kentucky II	5	40.00	60.00
Feedlot 3	Montevideo I	19	42.11	57.89
	Anatum	17	64.71	35.29
	Meleagradis	12	33.33	66.67
	Altona	7	100.00	0.00
	Muenchen	7	57.14	42.86
	Kentucky I	5	0.00	100.00
	Schwarzengrund	5	60.00	40.00
Feedlot 4	Mbandaka	109	51.38	48.62
	Anatum	106	30.19	69.81
	Montevideo I	59	16.95	83.05
	Muenchen	44	20.45	79.55
	Agona	18	5.56	94.44
	Infantis	16	0.00	100.00
	Cerro	14	35.71	64.29
	Kentucky I	14	0.00	100.00
	Meleagradis	11	45.45	54.55
	Lexington	7	14.29	85.71
	Minnesota	7	0.00	100.00
	Rissen	7	0.00	100.00
	Liverpool	6	16.67	83.33
	Altona	5	0.00	100.00
	Montevideo II	5	0.00	100.00

Table 2: Relative frequency of each serovar by Feedlot

	Serovar	Frequency	%Majority	%Minority
Summer	Anatum	75	44.00	56.00
	Montevideo I	54	16.67	83.33
	Muenchen	46	32.61	67.39
	Mbandaka	41	39.02	60.98
	Agona	15	6.67	93.33
	Infantis	14	0.00	100.00
	Meleagradis	12	16.67	83.33
	Cerro	9	11.11	88.89
	Muenster	9	44.44	55.56
	Newport III	8	25.00	75.00
	Lexington	6	0.00	100.00
	Liverpool	6	16.67	83.33
	Rissen	6	0.00	100.00
	Typhimurium	6	33.33	66.67
	Yoruba	6	33.33	66.67
	Kentucky II	5	40.00	60.00
	Minnesota	5	0.00	100.00
Fall	Anatum	70	52.86	47.14
	Montevideo I	51	43.14	56.86
	Mbandaka	36	25.00	75.00
	Muenchen	33	36.36	63.64
	Kentucky I	11	0.00	100.00
	Meleagradis	9	33.33	66.67
	Altona	7	71.43	28.57
	Montevideo II	5	20.00	80.00
	Yoruba	5	60.00	40.00
Winter	Anatum	29	37.93	62.07
	Mbandaka	29	44.83	55.17
	Montevideo I	18	38.89	61.11
	Kentucky I	10	0.00	100.00
	Muenchen	10	30.00	70.00
	Cerro	9	66.67	33.33
	Meleagradis	7	57.14	42.86
	Schwarzengrund	5	80.00	20.00
Spring	Mbandaka	24	83.33	16.67
	Anatum	16	12.50	87.50
	Montevideo I	7	28.57	71.43

Table 3: Relative frequency of each serovar by Season

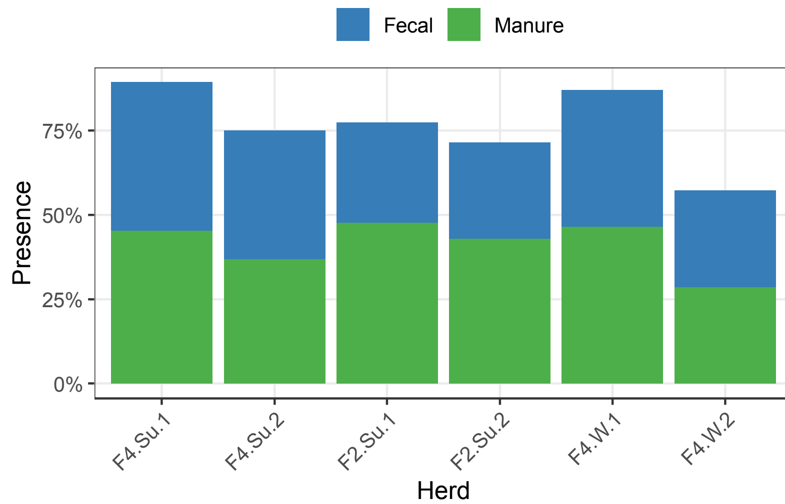


Figure 9. *Salmonella* prevalence in fresh feces versus manure pad samples. Samples are separated by pen (x-axis), and positive fecal samples are shown in blue and manure pad samples in green. F2=Feedlot 2; F4=Feedlot 4; Su=summer; W=winter; 1=pen 1; 2=pen 2. Herd = pen. Figure generated by Christian Gunning.

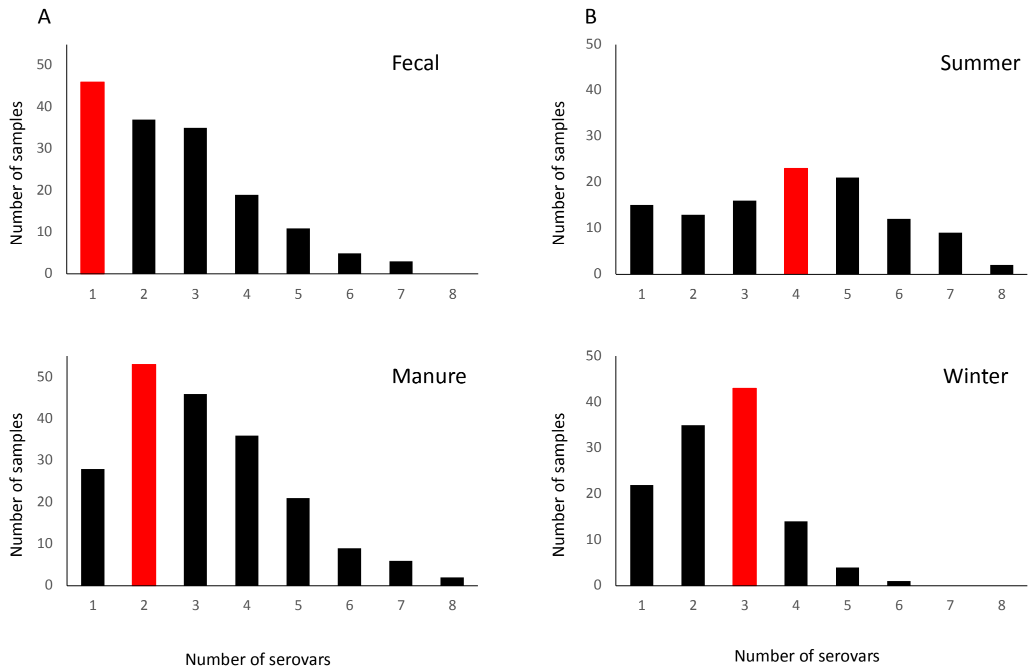


Figure 10. ***Salmonella*** **population complexity**

The bar graphs indicated the frequency serovar populations were found based on samples types fecal and manure (A) and by season (B). With the red bar indicating the serovar population that occurred most frequently.

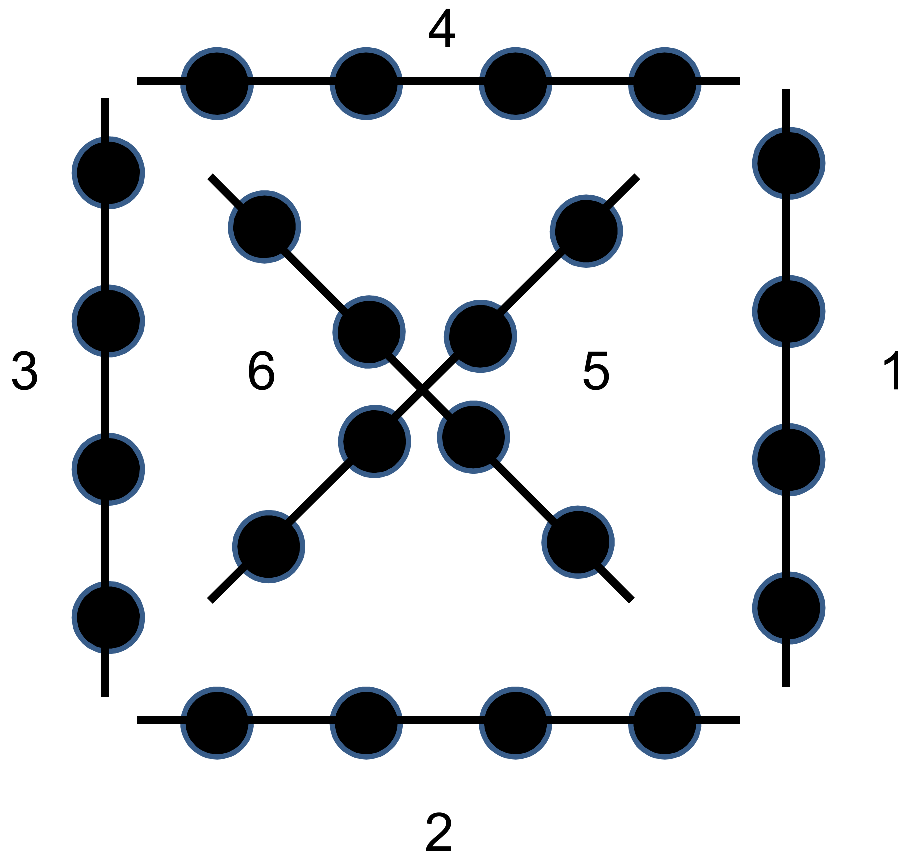


Figure 11. Sampling schematic for collection of fecal and manure samples

Each line in the box with two diagonals, indicates the path walked in bootsocks to collect manure samples. The circles represent the four fecal samples collected on the same path to create six fecal composite sample. For a total of six fecal samples and six manure samples, totaling 12 samples per pen.

Feedlot	Feedlot 2															
Season	Summer															
Pen #	Pen 1															
Day	0	15	30	45	60	90	124	0	15	30	45	60	90	124		
Sample ID	Fecal Pad Composite							Manure Layer Composite							Fecal Grab	Hide Swab
Agona																
Altona																
Anatum																
Enteritidis																
Havana II																
Infantis																
Kentucky I																
Kentucky II																
Mbandaka																
Montevideo I																
Muenchen I																
Newport III																
Poona																
Typhimurium																
Number of serovars	4	4	3	3	2	1	2	5	5	5	3	3	3	2	4	11

Figure 12. Serovar profiles in Manure and Fecal samples per day in Feedlot 2 Pen 2 in the Summer Placement

Six samples were taken per sampling period, all six samples were combined to show the total pen diversity. Each serovar is color coded, to indicate the serovar presence. White boxes indicate the absence of presence.

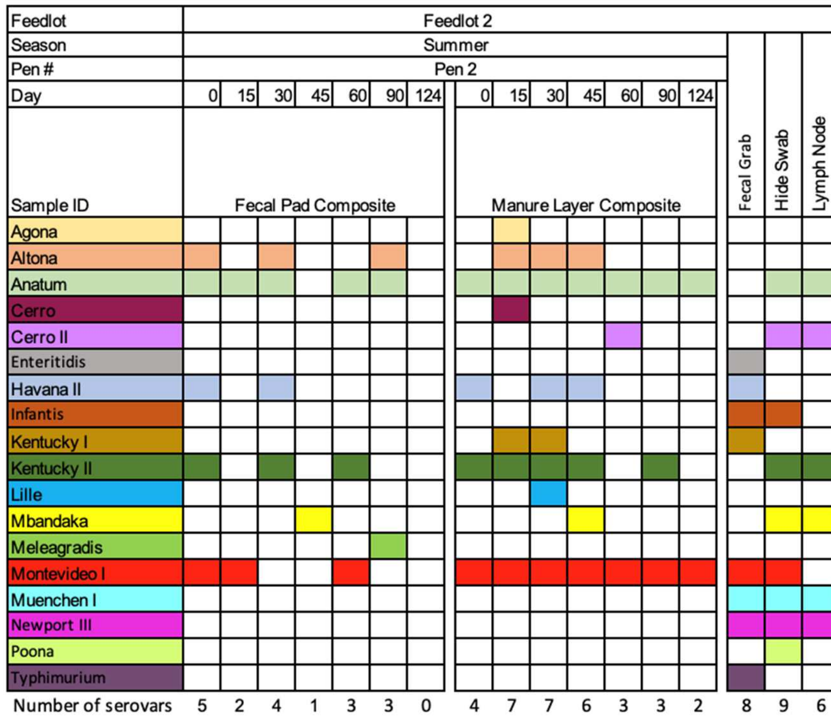


Figure 13. Serovar profiles in Manure and Fecal samples per day in Feedlot 2 Pen 2 in the Summer Placement

Six samples were taken per sampling period, all six samples were combined to show the total pen diversity. Each serovar is color coded, to indicate the serovar presence. White boxes indicate the absence of presence.

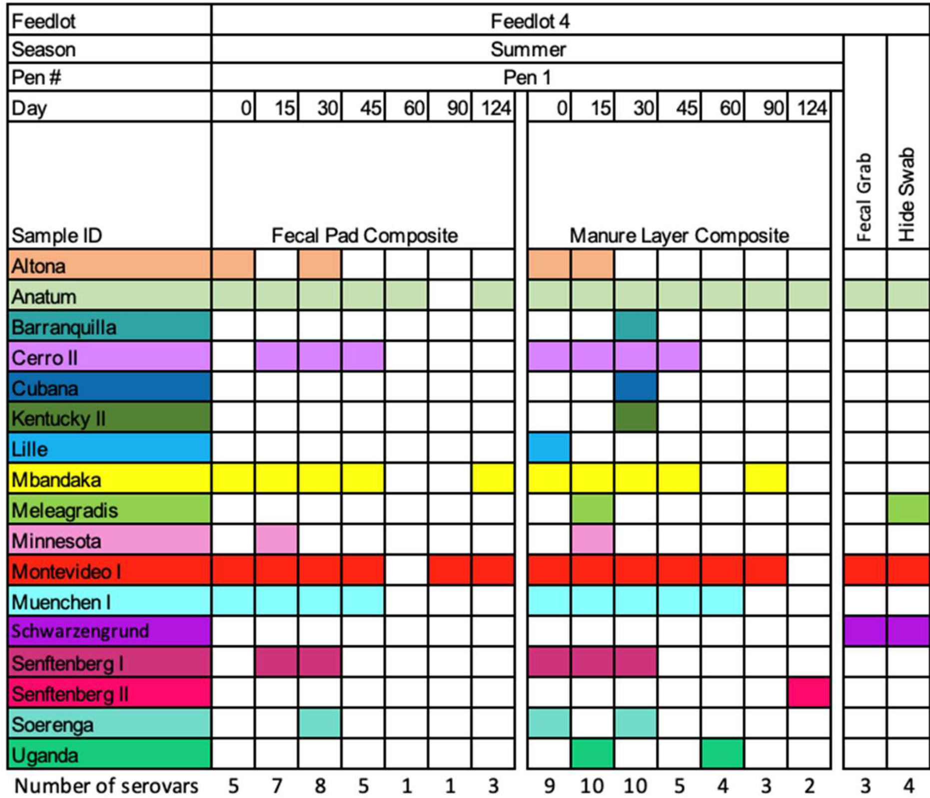


Figure 14. Serovar profiles in Manure and Fecal samples per day in Feedlot 4 Pen 2

Summer Placement

Six samples were taken per sampling period, all six samples were combined to show the total pen diversity. Each serovar is color coded, to indicate the serovar presence. White boxes indicate the absence of presence.

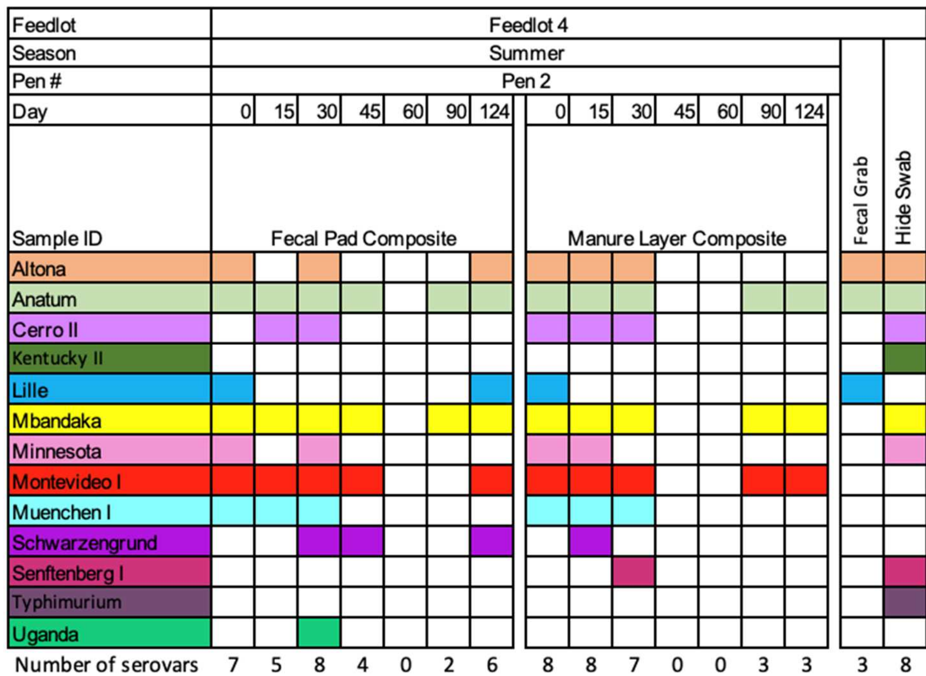


Figure 15. Serovar profiles in Manure and Fecal samples per day in Feedlot 4 Pen 2

Summer Placement

Six samples were taken per sampling period, all six samples were combined to show the total pen diversity. Each serovar is color coded, to indicate the serovar presence. White boxes indicate the absence of presence.

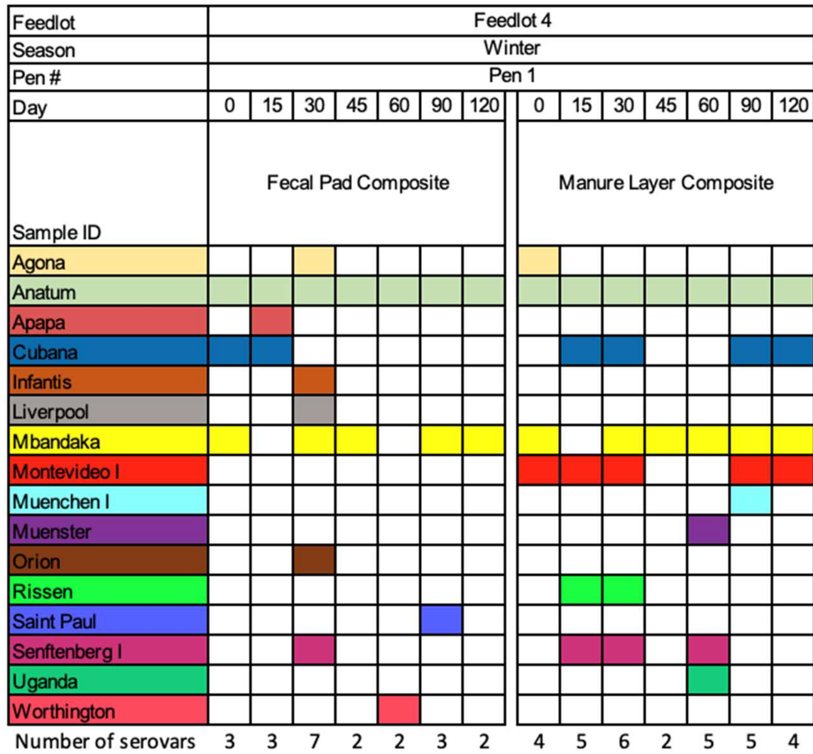


Figure 16. Serovar profiles in Manure and Fecal samples per day in Feedlot 4 Pen 1 Winter placement

Six samples were taken per sampling period, all six samples were combined to show the total pen diversity. Each serovar is color coded, to indicate the serovar presence. White boxes indicate the absence of presence.

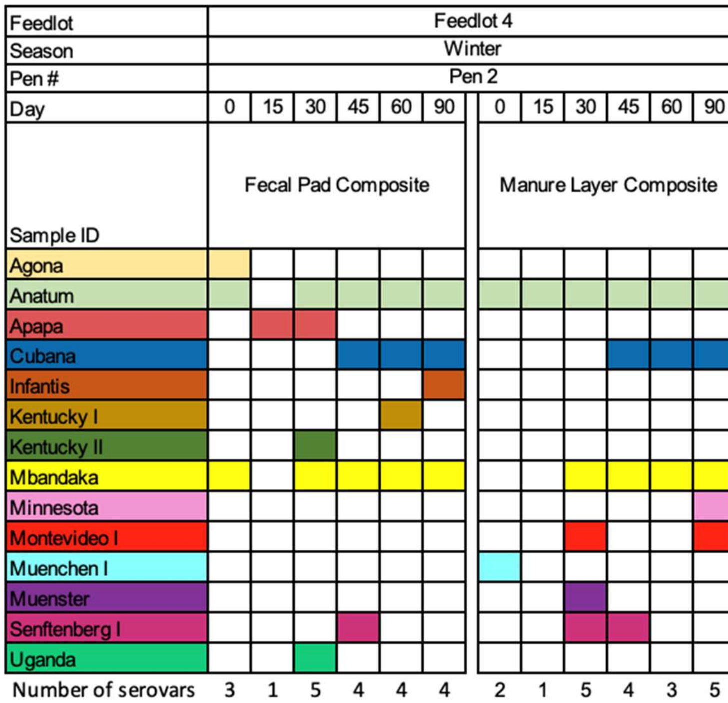


Figure 17. Serovar profiles in Manure and Fecal samples per day in Feedlot 4 Pen 2 Winter placement

Six samples were taken per sampling period, all six samples were combined to show the total pen diversity. Each serovar is color coded, to indicate the serovar presence. White boxes indicate the absence of serovar presence within a sample.

DISCUSSION

The cattle industry has examined *Salmonella* survival at slaughter, prevalence on the hide, in the lymph nodes, and in cattle feces (Bosilevac et al., 2005; Brichta-Harhay et al., 2008; Buncic & Sofos, 2012; Gragg et al., 2013; Gutiérrez et al., 2020; Haneklaus et al., 2012; Samuel et al., 1979; Stephens, Loneragan, Thompson, et al., 2007; Webb et al., 2017). Rates of fecal shedding and potential vectors for *Salmonella* have also been examined to reduce *Salmonella* on feedlots (Carlson et al., 2020; Gopinath et al., 2012; Olafson et al., 2014; Sargeant et al., 2021). Detailed examination of *Salmonella* serovar populations over time has not been examined in cattle. Studies have looked at *Salmonella* prevalence before transport to slaughter, in the lairage pen, and at slaughter (Arthur et al., 2008; Buncic & Sofos, 2012; Fegan et al., 2004; Miller et al., 2008). This study aimed to examine *Salmonella* serovar populations by sampling various locations across four feedlots in Texas. In the planning of this study, all feedlots were chosen based on their historical *Salmonella* prevalence. Two feedlots had a high historical prevalence, and two had a low prevalence of *Salmonella*. The first study, "Bio-mapping *Salmonella* Populations Across Four Commercial Feedlots" examined serovar populations from various areas within feedlots to better understand population differences. The second study, "Evaluation of *Salmonella* serovar populations in two cattle feedlots over two placements" aimed to investigate the changes in *Salmonella* serovar populations over the course of cattle placement on the feedlot.

By sampling in various locations across the feedlot, areas with higher diversity and prevalence can be found. In this study, 32 pens were sampled per season, each with varying *Salmonella* prevalence. *Salmonella* prevalence varied based on the season (Barkocy-Gallagher et

al., 2003; Edrington et al., 2008; Frankel et al., 2012; Holley et al., 2006; Wottlin et al., 2022). The findings in this study about *Salmonella* prevalence agree with previous studies showing that *Salmonella* prevalence decreases in the colder months. *Salmonella* prevalence was highest in the summer (91/228), then the fall (89/228), followed by a decrease in prevalence in both winter (50/228) and spring (31/228). All feedlots showed a seasonal reduction, with feedlot 4 having the highest *Salmonella* prevalence each season, with 42 positive samples in the summer, 35 in the fall, and 26 in the winter and spring. Colder temperatures were expected to correlate to a decrease in *Salmonella* prevalence across all feedlots equally. Previous studies have shown that fecal shedding of *Salmonella* is reduced in colder months (Edrington et al., 2008; Holley et al., 2006; Wottlin et al., 2022). Our study shows a high *Salmonella* prevalence in the summer and fall, followed by a decrease in prevalence in the winter and spring.

In the Second study, examining *Salmonella* prevalence in two feedlots during two seasonal placements showed decreased prevalence in the winter. Feedlot 4 had positive samples in both the winter and summer placements. This is unexpected due to a typical decrease in *Salmonella* prevalence in the colder months (Edrington et al., 2008). In the first part of the study, feedlot 4 was observed to have a continually high prevalence through all four seasons. Feedlot 2 in the winter placement recovered too few positive samples (10/168); these samples were excluded for further analysis. The reduction is partially thought to be due to the lack of heat stress in the colder months (Cummings, Warnick, Alexander, Cripps, Gröhn, McDonough, et al., 2009; Edrington et al., 2008). Temperature has been shown to affect the ability of *Salmonella* to survive in manure at colder temperatures (Phan-Thien et al., 2020; Tran et al., 2020). The lack of positive samples in the winter aligns with results from the previous study and current literature (Edrington et al., 2008; García et al., 2010; Holley et al., 2006).

Salmonella prevalence varied by sample location; *Salmonella* has been found frequently on cattle hides and feces but less frequently in the lymph nodes and other organs (Dodd et al., 2011; Haneklaus et al., 2012; Im et al., 2016; Mcevoy et al., n.d.; Stephens, Loneragan, Thompson, et al., 2007). Gragg et al. (2013) showed that *Salmonella* prevalence within lymph node samples varied from 3% to 90% prevalence, with fecal and hide samples having 94% and 100% *Salmonella* prevalence. Our data agrees with previous studies (Brichta-Harhay et al., 2008; Gragg et al., 2013; Stephens, Loneragan, Thompson, et al., 2007), demonstrating a higher recovery rate of *Salmonella* from hide samples than fecal and lymph node samples.

The shipping/receiving pens showed the most serovars found per sample, averaging 3.9 serovars. This might be due to the fact that all cattle are brought into that pen before leaving or entering the feedlot. Cattle shed more often when stressed, and *Salmonella* was found to become airborne during the loading or unloading of cattle (Miller et al., 2008). Transportation and heat stress are known factors affecting shedding (Gronstol et al., 1974). The stress from transport increasing the rate of fecal shedding along with dust generation during unloading cattle into the shipping pen could be one explanation for the higher serovar diversity in the shipping/receiving pens across all feedlots and increased recovery of *Salmonella* from the hide. Therefore, it is not surprising that we found that the shipping/receiving pen have the highest number of serovars. Complex serovar populations have been found in cattle, water samples, and animal feed (Deaven et al., 2021; Shariat et al., 2022; Siceloff et al., 2021). Cattle pens have unique serovar populations (Levent et al., 2019), and the change in pens obscured observations of the transmission. Cattle source has also been shown to affect serovar populations (Levent et al., 2021). Another study by Levent et al. (2019) showed that *Salmonella* populations observed on day 0 or 7 differ from the populations found in the feces, hide, and lymph nodes at slaughter;

however, this change could have been due to treatment with antibiotics. Only one cohort remained in the same pen for the sampling duration, and no characteristic trends about how *Salmonella* dynamics change over time could be observed.

Similarly, the second study showed, that in both feedlots, some serovars were only found from fecal and hide swabs collected at slaughter. From feedlot 2, serovars Poona and Typhimurium appeared at slaughter. In feedlot 4 pen 2, serovars Kentucky II and Typhimurium only appeared on the hide swabs. Poona could have been found on the hide due to the dust generated from loading the cattle. The previous study showed that the shipping/receiving pens had the most diversity. While the shipping/receiving pens were not sampled in this study, serovar Poona could be from the shipping pen, and both cohorts would move through the shipping pen, allowing serovar Poona to colonize the hide. This study showed that *Salmonella* serovar populations in cattle feces and from the pen environment differ. It also showed that serovar populations within feedlots, on average, have 2.9 serovars per sample. It also showed that serovar populations varied based on sample type.

The first study showed multiserovar populations in 76% of samples, ranging from 1-10 serovars per sample. Another deep serotyping study involving cattle showed that 60% of samples had multiserovar populations (Siceloff et al., 2021). Other deep serotyping studies have shown that 80% of water samples and 56% of animal feed samples have multiserovar populations (Deaven et al., 2021; Shariat et al., 2022; Siceloff et al., 2022). Similarly, in this study, we found that population complexity varied based on the sampling location; not all pens had equal *Salmonella* prevalence. While large populations seem to exist rarely, 11 serovars have also been found via CSS in the broiler breeder environment and blood meal samples (Shariat et al., 2022; Siceloff et al., 2022). The longitudinal study found as many as eight serovars in an individual

sample, with an average of 2.9 serovars per sample.. This study for a maximum of 8 serovars within a single sample; the previous study showed a maximum of 10 serovars.

Gutema et al. (2019) showed 17 serovars that appear most often in North American cattle. Our study found that serovars Anatum, Montevideo I, and Mbandaka appeared most often, which were also found in that study. This study found that 23 different serovars appeared multiple times (in more than five samples), and 37 serovars occurred overall. Serovars were classified as in the “majority” based on the relative frequency within a sample; the serovar with the largest relative frequency was said to be in the majority, as opposed to serovars not found in the majority of a sample “minority”. Nine of the top 23 serovars appeared less than ten times, which due to the small sample size, might not accurately reflect how these serovars appear in all *Salmonella* populations. Serovars scored in the minority might not be found via traditional culturing methods. This study shows that *Salmonella* populations in cattle feedlots are complex, commonly having multiple serovars in a sample; serovars commonly found in the minority could be overlooked in traditional *Salmonella* culturing.

Fresh fecal samples were collected to show *Salmonella* colonizing the cattle, whereas manure samples represented *Salmonella* in the environment, though linked to *Salmonella* shed in fecal samples. *Salmonella* is commonly shed in cattle feces, and despite the limited persistence in soil, *Salmonella* prevalence was higher in the manure samples collected via bootsocks (Bardsley et al., 2021; Gopinath et al., 2012; Holley et al., 2006). *Salmonella* shed in cattle feces is one vector that allows *Salmonella* to enter the environment (Baloda et al., 2001; García et al., 2010; Phan-Thien et al., 2020). *Salmonella* can survive for 40 days in manure-amended soils (Tran et al., 2020); therefore, *Salmonella* could be surviving in the soil on the feedlot. The pen environment is open to numerous vectors that can introduce *Salmonella* to the feedlot, including

wild birds, flies, and interactions with other animals such as dogs, horses, and deer (Carlson et al., 2015, 2020; Hamilton et al., 2021; Machtinger et al., 2021; Olafson et al., 2016; Tizard, 2004; U.S. Department of Agriculture National Animal Health Monitoring System, 2013). A USDA survey showed that in 90% of feedlots, the cattle had some interaction with other animals, including dogs, wild p, and horses (USDA, 2020).

One major constraint that affected the goal of this study was that 5/6 cohorts changed pens midway on various days throughout the study. Due to changes in the pen, *Salmonella* transmission between cattle feces and the environment could not be observed.

This study showed that *Salmonella* serovar populations in cattle feces and from the pen environment differ. It also showed that serovar populations within feedlots, on average, have 2.9 serovars per sample. It also showed that serovar populations varied based on sample type.

This was the first study to examine *Salmonella* serovar populations in commercial feedlots. Further examination of serovar populations and their transmission in the feedlot environment is needed to understand the dynamics occurring in feedlots. Next, examining serovar populations and survival in feedlot neighboring pens to see transmission effects between neighboring cattle pens.

CONCLUSIONS

This was the first study examining *Salmonella* serovar populations in a commercial feedlot environment. The goals of this study were to survey the complexity of *Salmonella* populations and understand changes in *Salmonella* populations across the feedlot environment. *Salmonella* was primarily found in the upper soil layers, with decreasing diversity as soil depth increased. *Salmonella* presence was decreased as soil depth increased. The most diverse populations were found in the shipping/receiving pen, the only pen all cattle enter and leave through. Cattle are known to shed *Salmonella* at higher rates due to stress related to transportation; so, shedding is likely increased in the shipping/receiving pen. *Salmonella* prevalence and diversity are pen dependent, showing that *Salmonella* is not ubiquitous across the feedlot, but the populations change per pen. Serovar complexity varied based on the sample, with the shipping/receiving pens having the most diversity at an average of 3.9 serovars. The complexity varied along with the season; overall, the serovar complexity was the highest in the summer, with an average of 3.6 serovars. The frequency that a serovar appears in the minority varied based on the feedlot. Establishing a baseline for *Salmonella* dynamics within feedlots and the seasonal change in *Salmonella* prevalence will create a starting point for further research. Examining serovar populations between neighboring cattle pens to see the influence of cattle on the neighboring environment.

<u>Evaluation of *Salmonella* serovar populations in two cattle feedlots over two placements</u>

The goals of this study were to observe *Salmonella* transmission between cattle feces and the pen environment. *Salmonella* shed from cattle can survive in the soil environment; *Salmonella* in the environment can infect and colonize the cattle. These interactions were expected to reach equilibrium, resulting in identical populations. To test this, we sampled two

pens in two feedlots during two placement periods. Due to the cattle shifting pens during placement, no trends could be observed in the transmission of *Salmonella* between the feces and the environment. However, we observed that dried manure samples had higher serovar complexity than the fecal samples, likely because the manure samples were taken via bootsock sampling over a larger area. The higher serovar complexity within the dried manure samples could indicate that *Salmonella* is being introduced to the environment via vectors other than shedding in cattle feces. The dried manure samples had more samples (86%; 173/201) containing two or more serovars than the fecal samples (70%;110/156). This study examined the different *Salmonella* populations in cattle feces and the dried manure, this showed that the manure samples contained higher serovar complexity than the fecal samples. Indicating that fecal samples are not sufficient to understand the *Salmonella* populations on beef cattle feedlots.

REFERENCES

Andrews, W. H., Wang, H., Jacobson, A., Ge, B., Zhang, G., & Hammack, T. (2022). *Bam Chapter 5_ Salmonella _ Fda*.

Arthur, T. M., Bosilevac, J. M., Brichta-Harhay, D. M., Kalchayanand, N., King, D. A., Shackelford, S. D., Wheeler, T. L., & Koohmaraie, M. (2008). Source Tracking Of Escherichia Coli O157:H7 And *Salmonella* Contamination In The Lairage Environment At Commercial U.S. Beef Processing Plants And Identification Of An Effective Intervention †. In *Journal Of Food Protection* (Vol. 71, Issue 9). http://meridian.allenpress.com/jfp/article-pdf/71/9/1752/1681415/0362-028x-71_9_1752.pdf

Arthur, T. M., Bosilevac, J. M., Brichta-Harhay, D. M., Kalchayanand, N., Shackelford, S. D., Wheeler, T. L., & Koohmaraie, M. (2007). Effects Of A Minimal Hide Wash Cabinet On The Levels And Prevalence Of Escherichia Coli O157:H7 And *Salmonella* On The Hides Of Beef Cattle At Slaughter †. In *Journal Of Food Protection* (Vol. 70, Issue 5). http://meridian.allenpress.com/jfp/article-pdf/70/5/1076/1681365/0362-028x-70_5_1076.pdf

Asakura, H., Yamamoto, S., Sasaki, Y., Okada, Y., Katabami, S., Fujimori, A., Munakata, K., Shiraki, Y., Nishibu, H., Hisamoto, C., Kawase, J., Ojima, Y., Kiyoshima, A., & Shiroma, K. (2022). Bacterial Distribution And Community Structure In Beef Cattle Liver And Bile At Slaughter. *Journal Of Food Protection*, *85*(3), 424–434. https://doi.org/10.4315/JFP-21-288

Baloda, S. B., Christensen, L., & Trajcevska, S. (2001). Persistence Of A *Salmonella* Enterica Serovar Typhimurium DT12 Clone In A Piggery And In Agricultural Soil Amended With *Salmonella*-Contaminated Slurry. *Applied And Environmental Microbiology*, *67*(6), 2859–2862. https://doi.org/10.1128/AEM.67.6.2859-2862.2001

Bardsley, C. A., Weller, D. L., Ingram, D. T., Chen, Y., Oryang, D., Rideout, S. L., & Strawn, L. K. (2021). Strain, Soil-Type, Irrigation Regimen, And Poultry Litter Influence *Salmonella* Survival And Die-Off In Agricultural Soils. *Frontiers In Microbiology*, *12*(March). https://doi.org/10.3389/fmicb.2021.590303

Barkocy-Gallagher, G. A., Arthur, T. M., Rivera-Betancourt, M., Nou, X., Shackelford, S. D., Wheeler, T. L., & Koohmaraie, M. (2003). Seasonal Prevalence Of Shiga Toxin-Producing Escherichia Coli, Including O157:H7 And Non-O157 Serotypes, And *Salmonella* In Commercial Beef Processing Plants †. In *Journal Of Food Protection* (Vol. 66, Issue 11). http://meridian.allenpress.com/jfp/article-pdf/66/11/1978/1675817/0362-028x-66_11_1978.pdf

Bosilevac, J. M., Nou, X., Osborn, M. S., Allen, D. M., & Koohmaraie, M. (2005). Development And Evaluation Of An On-Line Hide Decontamination Procedure For Use In A Commercial Beef Processing Plant †. In *Journal Of Food Protection* (Vol. 68, Issue 2).

Brichta-Harhay, D. M., Guerini, M. N., Arthur, T. M., Bosilevac, J. M., Kalchayanand, N., Shackelford, S. D., Wheeler, T. L., & Koohmaraie, M. (2008). *Salmonella* And Escherichia Coli O157:H7 Contamination On Hides And Carcasses Of Cull Cattle Presented For Slaughter In The United States: An Evaluation Of Prevalence And Bacterial Loads By Immunomagnetic Separation And Direct Plating Methods. *Applied And Environmental Microbiology*, *74*(20), 6289–6297. https://doi.org/10.1128/AEM.00700-08

Buncic, S., & Sofos, J. (2012). Interventions To Control *Salmonella* Contamination During Poultry, Cattle And Pig Slaughter. *Food Research International*, *45*(2), 641–655. https://doi.org/10.1016/j.foodres.2011.10.018

Carlson, J. C., Chandler, J. C., Bisha, B., Lejeune, J. T., & Wittum, T. E. (2020). Bird-Livestock Interactions Associated With Increased Cattle Fecal Shedding Of Ciprofloxacin-Resistant

Escherichia Coli Within Feedlots In The United States. *Scientific Reports*, *10*(1). https://doi.org/10.1038/s41598-020-66782-4

Carlson, J. C., Hyatt, D. R., Bentler, K., Mangan, A. M., Russell, M., Piaggio, A. J., & Linz, G. M. (2015). Molecular Characterization Of *Salmonella* Enterica Isolates Associated With Starling-Livestock Interactions. *Veterinary Microbiology*, *179*(1–2), 109–118. https://doi.org/10.1016/j.vetmic.2015.03.015

Chong, A., Cooper, K. G., Kari, L., Nilsson, O. R., Hillman, C., Fleming, B. A., Wang, Q., Nair, V., & Steele-Mortimer, O. (2021). Cytosolic Replication In Epithelial Cells Fuels Intestinal Expansion And Chronic Fecal Shedding Of *Salmonella* Typhimurium. *Cell Host And Microbe*, *29*(7), 1177-1185.E6. https://doi.org/10.1016/j.chom.2021.04.017

Cummings, K. J., Warnick, L. D., Alexander, K. A., Cripps, C. J., Gröhn, Y. T., James, K. L., Mcdonough, P. L., & Reed, K. E. (2009). The Duration Of Fecal *Salmonella* Shedding Following Clinical Disease Among Dairy Cattle In The Northeastern USA. *Preventive Veterinary Medicine*, *92*(1–2), 134–139. https://doi.org/10.1016/j.prevetmed.2009.07.002

Cummings, K. J., Warnick, L. D., Alexander, K. A., Cripps, C. J., Gröhn, Y. T., Mcdonough, P. L., Nydam, D. V., & Reed, K. E. (2009). The Incidence Of Salmonellosis Among Dairy Herds In The Northeastern United States. *Journal Of Dairy Science*, *92*(8), 3766–3774. https://doi.org/10.3168/jds.2009-2093

Deaven, A. M., Ferreira, C. M., Reed, E. A., Chen See, J. R., Lee, N. A., Almaraz, E., Rios, P. C., Marogi, J. G., Lamendella, R., Zheng, J., Bell, R. L., & Shariat, N. W. (2021). *Salmonella* Genomics And Population Analyses Reveal High Inter- And Intra- Serovar Diversity In Freshwater. *Applied And Environmental Microbiology*, AEM.02594-20. https://doi.org/10.1128/aem.02594-20

Dodd, C. C., Renter, D. G., Shi, X., Alam, M. J., Nagaraja, T. G., & Sanderson, M. W. (2011). Prevalence And Persistence Of *Salmonella* In Cohorts Of Feedlot Cattle. *Foodborne Pathogens And Disease*, *8*(7), 781–789. https://doi.org/10.1089/fpd.2010.0777

Edrington, T. S., Ross, T. T., Callaway, T. R., Martinez, C. H., Hume, M. E., Genovese, K. J., Poole, T. L., Anderson, R. C., & 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

Fabre, L., Zhang, J., Guigon, G., Le Hello, S., Guibert, V., Accou-Demartin, M., De Romans, S., Lim, C., Roux, C., Passet, V., Diancourt, L., Guibourdenche, M., Issenhuth-Jeanjean, S., Achtman, M., Brisse, S., Sola, C., & Weill, F.-X. (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

Fegan, N., Vanderlinde, P., Higgs, G., & 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

Franz, E., Van Diepeningen, A. D., De Vos, O. J., & Van Bruggen, A. H. C. (2005). Effects Of Cattle Feeding Regimen And Soil Management Type On The Fate Of Escherichia Coli O157:H7 And *Salmonella* Enterica Serovar Typhimurium In Manure, Manure-Amended Soil, And Lettuce. *Applied And Environmental Microbiology*, *71*(10), 6165–6174. https://doi.org/10.1128/AEM.71.10.6165-6174.2005

García, R., Bælum, J., Fredslund, L., Santorum, P., & Jacobsen, C. S. (2010). Influence Of Temperature And Predation On Survival Of *Salmonella* Enterica Serovar Typhimurium And

Expression Of Inva In Soil And Manure-Amended Soil. *Applied And Environmental Microbiology*, *76*(15), 5025–5031. https://doi.org/10.1128/AEM.00628-10

Gopinath, S., Carden, S., & Monack, D. (2012). Shedding Light On *Salmonella* Carriers. In *Trends In Microbiology* (Vol. 20, Issue 7, Pp. 320–327). https://doi.org/10.1016/j.tim.2012.04.004

Gragg, S. E., Loneragan, G. H., Nightingale, K. K., Brichta-Harhay, D. M., Ruiz, H., Elder, J. R., Garcia, L. G., Miller, M. F., Echeverry, A., Ramírez Porras, R. G., & Brashears, M. M. (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

Grimont, P., & Weill, F. (2007). Antigenic Formulae Of The *Salmonella* Serovars. *WHO Collaborating Centre For Reference And Research On Salmonella*, *9th Editio*.

Gronstol, H., Osborne, A. D., & Pethiyagoda, S. (1974). Experimental *Salmonella* Infection In Calves. 1. The Effect Of Stress Factors On The Carrier State University Of. In *J. Hyg., Camb* (Vol. 72).

Gutiérrez, T. P., Lozano, M. S. R., Suárez, E. J. D., Guzmán, N. R., Ramos, O. S., Pérez, C. F. H., & Medina, R. D. M. (2020). Lymph Nodes And Ground Beef As Public Health Importance Reservoirs Of *Salmonella* Spp. *Revista Mexicana De Ciencias Pecuarias*, *11*(3), 795–810. https://doi.org/10.22319/RMCP.V11I3.5516

Gutierrez-Bañuelos, H., Pinchak, W. E., Min, B. R., Carstens, G. E., Anderson, R. C., Tedeschi, L. O., Krueger, W. K., Krueger, N. A., Lancaster, P. A., & Gomez, R. R. (2011). Effects Of Feed-Supplementation And Hide-Spray Application Of Two Sources Of Tannins On Enteric

And Hide Bacteria Of Feedlot Cattle. *Journal Of Environmental Science And Health, Part B*, *46*(4), 360–365. https://doi.org/10.1080/03601234.2011.559419

Haley, B. J., Kim, S. W., Pettengill, J., Luo, Y., Karns, J. S., & Van Kessel, J. A. S. (2016). Genomic And Evolutionary Analysis Of Two *Salmonella* Enterica Serovar Kentucky Sequence Types Isolated From Bovine And Poultry Sources In North America. *Plos ONE*, *11*(10). https://doi.org/10.1371/journal.pone.0161225

Hamilton, A. M., Paulsen, D. J., Trout Fryxell, R. T., Orta, V. E., Gorman, S. J., Smith, D. M., Buchanan, J. R., Wszelaki, A. L., & Critzer, F. J. (2021). Prevalence Of *Salmonella* Enterica In Flies On A Diversified Cattle And Fresh Produce Farm Across Two Growing Seasons. *Journal Of Food Protection*, *84*(6), 1009–1015. https://doi.org/10.4315/JFP-20-339

Haneklaus, A. N., Harris, K. B., Griffin, D. B., Edrington, T. S., Lucia, L. M., & 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

Hekman, W. E., Heijnen, C. E., Burgers, S. L. G. E., Van Veen, J. A., & Van Elsas, J. D. (1994). Transport Of Bacterial Inoculants Through Intact Cores Of Two Different Soils As Affected By Water Percolation And The Presence Of Wheat Plants. *Microbial Ecology*, *16*, 143–158.

Holley, R. A., Arrus, K. M., Ominski, K. H., Tenuta, M., & Blank, G. (2006). *Salmonella* Survival In Manure-Treated Soils During Simulated Seasonal Temperature Exposure. *Journal Of Environmental Quality*, *35*(4), 1170–1180. https://doi.org/10.2134/jeq2005.0449

Ifsac. (2022). *Foodborne Illness Source Attribution Estimates For 2020 For Salmonella, Escherichia Coli O157, And Listeria Monocytogenes Using Multi-Year Outbreak Surveillance Data, United States*. https://www.cdc.gov/foodsafety/ifsac/projects/index.html.

Im, M. C., Seo, K. W., Bae, D. H., & 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

Levent, G., Schlochtermeier, A., Ives, S. E., Norman, K. N., Lawhon, S. D., Loneragan, G. H., Anderson, R. C., Vinasco, J., Den Bakker, H. C., & Scott, H. M. (2021). High-Resolution Genomic Comparisons Within *Salmonella* Enterica Serotypes Derived From Beef Feedlot Cattle: Parsing The Roles Of Cattle Source, Pen, Animal, Sample Type, And Production Period. *Applied And Environmental Microbiology*, *87*(12), 1–20. https://doi.org/10.1128/AEM.00485-21

Levent, G., Schlochtermeier, A., Ives, S. E., Norman, K. N., Lawhon, S. D., Loneragan, G. H., Anderson, R. C., Vinasco, J., & Scott, H. M. (2019). *Population Dynamics Of Salmonella Enterica Within Beef Cattle Cohorts Followed From Single-Dose Metaphylactic Antibiotic Treatment Until Slaughter*. https://www.ers.usda.gov/topics/animal-products/cattle-beef/sector-at-a-glance/

Machtinger, E. T., Gerry, A. C., Murillo, A. C., & Talley, J. L. (2021). Filth Fly Impacts To Animal Production In The United States And Associated Research And Extension Needs. *Journal Of Integrated Pest Management*, *12*(1). https://doi.org/10.1093/jipm/pmab026

Mcevoy, J. M., Doherty, A. M., Sheridan, J. J., Blair, I. S., & Mcdowell, D. A. (2003). *The Prevalence Of Salmonella Spp. In Bovine Faecal, Rumen And Carcass Samples At A Commercial Abattoir*.

Miller, M. F., Loneragan, G. H., Harris, D. D., Adams, K. D., Brooks, J. C., & Brashears, A. M. M. (2008). Environmental Dust Exposure As A Factor Contributing To An Increase In Escherichia Coli O157 And *Salmonella* Populations On Cattle Hides In Feedyards. In *Journal Of*

Food Protection (Vol. 71, Issue 10). http://meridian.allenpress.com/jfp/article-pdf/71/10/2078/2204450/0362-028x-71_10_2078.pdf

Ng, H., Bayne, H. G., & Garibaldi, J. A. (1969). Heat Resistance Of *Salmonella*: The Uniqueness Of *Salmonella* Senftenberg 775W. In *APPLIED MICROBIOLOGY*.

Nielsen, L. R., Schukken, Y. H., Gröhn, Y. T., & Ersbøll, A. K. (2004). *Salmonella* Dublin Infection In Dairy Cattle: Risk Factors For Becoming A Carrier. *Preventive Veterinary Medicine*, *65*(1–2), 47–62. https://doi.org/10.1016/j.prevetmed.2004.06.010

Olafson, P. U., Brown, T. R., Lohmeyer, K. H., Harvey, R. B., Nisbet, D. J., Loneragan, G. H., & Edrington, T. S. (2016). Assessing Transmission Of *Salmonella* To Bovine Peripheral Lymph Nodes Upon Horn Fly Feeding. *Journal Of Food Protection*, *79*(7), 1135–1142. https://doi.org/10.4315/0362-028X.JFP-15-414

Olafson, P. U., Lohmeyer, K. H., Edrington, T. S., & Loneragan, G. H. (2014). Survival And Fate Of *Salmonella* Enterica Serovar Montevideo In Adult Horn Flies (Diptera: Muscidae). *Journal Of Medical Entomology*, *51*(5), 993–1001. https://doi.org/10.1603/ME13217

Phan-Thien, K., Metaferia, M. H., Bell, T. L., Bradbury, M. I., Sassi, H. P., Van Ogtrop, F. F., Suslow, T. V., & Mcconchie, R. (2020). Effect Of Soil Type And Temperature On Survival Of *Salmonella* Enterica In Poultry Manure-Amended Soils. *Letters In Applied Microbiology*, *71*(2), 210–217. https://doi.org/10.1111/lam.13302

Porwollik, S., Genovese, K., Chu, W., Loneragan, G. H., Edrington, T., & Mcclelland, M. (2018). Neutral Barcoding Of Genomes Reveals The Dynamics Of *Salmonella* Colonization In Cattle And Their Peripheral Lymph Nodes. *Veterinary Microbiology*, *220*(April), 97–106. https://doi.org/10.1016/j.vetmic.2018.05.007

Samuel, J. L., O'Boyle, D. A., Mathers, W. J., & Frost, A. J. (1979). Isolation Of *Salmonella* From Mesenteric Lymph Nodes Of Healthy Cattle At Slaughter. *Research In Veterinary Science*, 238–241.

Sargeant, J. M., Totton, S. C., Plishka, M., & Vriezen, E. R. (2021). *Salmonella* In Animal Feeds: A Scoping Review. In *Frontiers In Veterinary Science* (Vol. 8). Frontiers Media S.A. https://doi.org/10.3389/fvets.2021.727495

Shariat, N., & Dudley, E. G. (2014). Crisprs: Molecular Signatures Used For Pathogen Subtyping. *Applied And Environmental Microbiology*, *80*(2), 430–439. https://doi.org/10.1128/AEM.02790-13

Shariat, N. W., Feye, K. M., Richards, A. K., Booher, B., Flores, Z., Rubinelli, P. M., Olson, E. G., & Ricke, S. C. (2021). Incidence Of *Salmonella* Serovars Isolated From Commercial Animal Feed Mills In The United States And Serovar Identification Using CRISPR Analysis. *Journal Of Applied Microbiology*, *130*(6), 2141–2146. https://doi.org/10.1111/jam.14933

Shariat, N. W., Larsen, B. R., Schaeffer, C., & Richardson, K. E. (2022). Animal Feed Contains Diverse Populations Of *Salmonella*. *Journal Of Applied Microbiology*. https://doi.org/10.1111/jam.15525

Siceloff, A. T., Ohta, N., Norman, K. N., Loneragan, G. H., Norby, B., Morgan Scott, H., & Shariat, N. W. (2021). Antimicrobial Resistance Hidden Within Multiserovar *Salmonella* Populations. *Antimicrobial Agents And Chemotherapy*, *65*(6). https://doi.org/10.1128/AAC.00048-21

Siceloff, A. T., Waltman, D., & Shariat, N. W. (2022). Regional *Salmonella* Differences In United States Broiler Production From 2016 To 2020 And The Contribution Of Multiserovar

Populations To *Salmonella* Surveillance. *Applied And Environmental Microbiology*, *88*(8). https://doi.org/10.1128/aem.00204-22

Stephens, T. P., Loneragan, G. H., Thompson, T. W., Sridhara, A., Branham, L. A., Pitchiah, S., & Brashears, A. M. M. (2007). Distribution Of Escherichia Coli O157 And *Salmonella* On Hide Surfaces, The Oral Cavity, And In Feces Of Feedlot Cattle. In *Journal Of Food Protection* (Vol. 70, Issue 6). http://meridian.allenpress.com/jfp/article-pdf/70/6/1346/1680993/0362-028x-70_6_1346.pdf

Stevik, T. K., Aa, K., Ausland, G., & Hanssen, J. F. (2004). Retention And Removal Of Pathogenic Bacteria In Wastewater Percolating Through Porous Media: A Review. In *Water Research* (Vol. 38, Issue 6, Pp. 1355–1367). Elsevier Ltd. https://doi.org/10.1016/j.watres.2003.12.024

Tack, D. M., Ray, L., Griffin, P. M., Cieslak, P. R., Dunn, J., Rissman, T., Jervis, R., Lathrop, S., Muse, A., Duwell, M., Smith, K., Tobin-D'angelo, M., Duc, ;, Vugia, J., Joanna, ;, Kufel, Z., Beverly, ;, Wolpert, J., Tauxe, ; Robert, & Payne, D. C. (2020). *Preliminary Incidence And Trends Of Infections With Pathogens Transmitted Commonly Through Food — Foodborne Diseases Active Surveillance Network, 10 U.S. Sites, 2016–2019*. https://www.cdc.gov/mmwr/mmwr_continuingEducation.html

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 CRISPR-Seroseq FOOD MICROBIOLOGY Crossm Downloaded From. *Aem.Asm.Org 1 Applied And Environmental Microbiology*, *84*, 1859–1877. https://doi.org/10.1128/AEM.01859-18

Tizard, I. (2004). Salmonellosis In Wild Birds. *Seminar In Avian And Exotic Pet Medicine*, *13*(2), 50–66.

Touchon, M., & Rocha, E. P. C. (2010). The Small, Slow And Specialized CRISPR And Anti-CRISPR Of Escherichia And *Salmonella*. *Plos ONE*, *5*(6). https://doi.org/10.1371/journal.pone.0011126

Tran, D. T. Q., Bradbury, M. I., Van Ogtrop, F. F., Bozkurt, H., Jones, B. J., & Mcconchie, R. (2020). Environmental Drivers For Persistence Of Escherichia Coli And *Salmonella* In Manure-Amended Soils: A Meta-Analysis. *Journal Of Food Protection*, *83*(7), 1268–1277. https://doi.org/10.4315/0362-028X.JFP-19-460

U.S. Department Of Agriculture National Animal Health Monitoring System. (2013). *Part I: Management Practices On U.S. Feedlots With A Capacity Of 1,000 Or More Head*. http://www.aphis.usda.gov/nahms.

Uzzau, S., Brown, D. J., Wallis, T., Rubino, S., Leori, G., Bernard, S., Casadesus, J., Platt, D. J., & Olsen, J. E. (2000). REVIEW Host Adapted Serotypes Of *Salmonella* Enterica. In *Epidemiol. Infect* (Vol. 125).

Vosik, D., Tewari, D., Dettinger, L., M’ikanatha, N. M., & Shariat, N. W. (2018). CRISPR Typing And Antibiotic Resistance Correlates With Polyphyletic Distribution In Human Isolates Of *Salmonella* Kentucky . *Foodborne Pathogens And Disease*, *15*(2), 101–108. https://doi.org/10.1089/fpd.2017.2298

Webb, H. E., Brichta-Harhay, D. M., Brashears, M. M., Nightingale, K. K., Arthur, T. M., Bosilevac, J. M., Kalchayanand, N., Schmidt, J. W., Wang, R., Granier, S. A., Brown, T. R., Edrington, T. S., Shackelford, S. D., Wheeler, T. L., & Loneragan, G. H. (2017). *Salmonella* In

Peripheral Lymph Nodes Of Healthy Cattle At Slaughter. *Frontiers In Microbiology*, *8*(NOV), 1–10. https://doi.org/10.3389/fmicb.2017.02214

Wottlin, L. R., Edrington, T. S., & 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

Yin, Z., Liu, J., Du, B., Ruan, H. H., Huo, Y. X., Du, Y., & Qiao, J. (2020). Whole-Genome-Based Survey For Polyphyletic Serovars Of *Salmonella* Enterica Subsp. Enterica Provides New Insights Into Public Health Surveillance. *International Journal Of Molecular Sciences*, *21*(15), 1–19. https://doi.org/10.3390/ijms21155226

You, Y., Rankin, S. C., Aceto, H. W., Benson, C. E., Toth, J. D., & Dou, Z. (2006). Survival Of *Salmonella* Enterica Serovar Newport In Manure And Manure-Amended Soils. *Applied And Environmental Microbiology*, *72*(9), 5777–5783. https://doi.org/10.1128/AEM.00791-06

CHAPTER 4

SUMMARY

Salmonella is a leading cause of foodborne illness in the United States; beef products are the sixth largest contributor to salmonellosis cases (IFSAC, 2022). The majority of *Salmonella* mitigation methods in beef products are aimed toward slaughter. Surveying and understanding *Salmonella* dynamics in cattle feedlots prior to slaughter has yet to be examined.

In this first study, we looked at *Salmonella* in four beef cattle feedlots once per season to evaluate *Salmonella* prevalence within pens and the feedlot environment; this was done by sampling eight pens each season. In each feedlot, eight bootsock, manure, surface, interface, soil, mixed rations, and water trough samples. Further samples were collected from the shipping and receiving pen, for a total of 232 samples collected per season. CRISPR-SeroSeq was then used to examine serovar populations and showed individual serovar populations per pen. *Salmonella* prevalences were highest in feedlot 4 (76%), with a reduction in prevalence in the colder months. *Salmonella* serovar populations can differ based on the cattle pen (Levent et al., 2019). Samples from the different parts of the soil column showed a reduction in prevalence as soil depth increased (surface 30.5%; interface 21.9%; soil 19.5%).

Serovar diversity varied based on both feedlot and season; feedlot 4 showed the most diversity with 30 serovars, 20 serovars in feedlot 3, and 19 serovars in both feedlots 1 and 2. Across all feedlots, the most abundant serovars were Anatum, Montevideo I, and Mbandaka, with the most abundant serovar changing per season.

In the second study, fecal and dried manure samples were collected, from eight pens, two pers per feedlot, per placement period, for a total of 672 samples collected. In the winter placement of feedlot 2 only (10/168) samples were *Salmonella* positive. Feedlot 4 showed the

highest prevalence in both the summer (82.1%) and winter (84.5%) placement. There was an overall average of 2.9 serovars per sample. In both fecal and manure samples, serovars sporadically appeared in each pen. There are many vectors that can introduce *Salmonella* to feedlots such as wild birds, animal feed, flies, and water trough contamination (Elser et al., 2019; LeJeune et al., 2001; Olafson et al., 2016; Sargeant et al., 2021; Shariat et al., 2022). Indicating that potentially some other vector was introducing *Salmonella* into the environment.

Salmonella prevalence in the lymph nodes, hide, and feces have shown varying prevalence (Gragg et al., 2013). Serovars found from samples collected at slaughter showed the most diversity within hide samples, with a maximum of 12 serovars. In feedlot 2 serovars appeared on the hide that had not been previously found in either the feces or manure.

To conclude, *Salmonella* serovar populations are complex; both studies showed an average of more the 2 serovars per sample. Serovar populations varied between feedlots, sample type, and season. *Salmonella* serovars in cattle feces and the dried manure of the pen were different, but no conclusions on the increase or decrease in similarity can be made because 5/6 cattle herds changed pens during the course of this study.

REFERENCES

Elser, J. L., Adams Progar, A. L., Steensma, K. M. M., Caskin, T. P., Kerr, S. R., & Shwiff, S. A. (2019). Economic and livestock health impacts of birds on dairies: Evidence from a survey of Washington dairy operators. In *PLoS ONE* (Vol. 14, Issue 9). Public Library of Science. https://doi.org/10.1371/journal.pone.0222398

Gragg, S. E., Loneragan, G. H., Nightingale, K. K., Brichta-Harhay, D. M., Ruiz, H., Elder, J. R., Garcia, L. G., Miller, M. F., Echeverry, A., Ramírez Porras, R. G., & Brashears, M. M. (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

IFSAC. (2022). *Foodborne illness source attribution estimates for 2020 for Salmonella, Escherichia coli O157, and Listeria monocytogenes using multi-year outbreak surveillance data, United States*. https://www.cdc.gov/foodsafety/ifsac/projects/index.html.

LeJeune, J. T., Besser, T. E., Merrill, N. L., Rice, D. H., & Hancock, D. D. (2001). Livestock drinking water microbiology and the factors influencing the quality of drinking water offered to cattle. *Journal of Dairy Science*, *84*(8), 1856–1862. https://doi.org/10.3168/jds.S0022-0302(01)74626-7

Levent, G., Schlochtermeier, A., Ives, S. E., Norman, K. N., Lawhon, S. D., Loneragan, G. H., Anderson, R. C., Vinasco, J., & Scott, H. M. (2019). *Population Dynamics of Salmonella enterica within Beef Cattle Cohorts Followed from Single-Dose Metaphylactic Antibiotic Treatment until Slaughter*. https://www.ers.usda.gov/topics/animal-products/cattle-beef/sector-at-a-glance/

Olafson, P. U., Brown, T. R., Lohmeyer, K. H., Harvey, R. B., Nisbet, D. J., Loneragan, G. H., & Edrington, T. S. (2016). Assessing transmission of Salmonella to bovine peripheral lymph nodes upon horn fly feeding. *Journal of Food Protection*, *79*(7), 1135–1142. https://doi.org/10.4315/0362-028X.JFP-15-414

Sargeant, J. M., Totton, S. C., Plishka, M., & Vriezen, E. R. (2021). Salmonella in Animal Feeds: A Scoping Review. In *Frontiers in Veterinary Science* (Vol. 8). Frontiers Media S.A. https://doi.org/10.3389/fvets.2021.727495

Shariat, N. W., Larsen, B. R., Schaeffer, C., & Richardson, K. E. (2022). Animal feed contains diverse populations of Salmonella. *Journal of Applied Microbiology*. https://doi.org/10.1111/jam.15525