ASSESSMENT OF *SALMONELLA* PREVALENCE IN LYMPH NODES OF U.S. AND MEXICAN CATTLE PRESENTED FOR SLAUGHTER IN TEXAS

A Thesis

by

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ABSTRACT

Foodborne salmonellosis has been traced back to undercooked ground beef and other beef products in the past, and due to the widespread presence of lymph nodes (LNs) throughout carcasses, they are a source of *Salmonella* contamination in ground beef products. The objectives of this study were to determine if *Salmonella* prevalence differs (1) between cattle of Mexican and U.S. origins when exposed to the same feedlot environment and (2) between warm and cool seasons. To meet these objectives, paired subiliac LNs (n = 800 LNs) were collected from 100 carcasses per origin (Mexico and U.S.), per season (warm and cool). The paired LNs (left and right sides) were pooled yielding one sample per animal (n = 400 samples), aseptically trimmed of fat, and pulverized before microbiological analysis. Overall, *Salmonella* prevalence in LN samples was 52.0% (208/400). No difference (P = 0.4836) was seen in *Salmonella* prevalence as a function of country of origin, with 54.0% (108/200) and 50.0% (100/200) of LN samples returning *Salmonella*-positive results from cattle of Mexican and U.S. origin, respectively. *Salmonella* prevalence differed (P = 0.0354) between seasons, with 46.5% (93/200) and 57.5% (115/200) *Salmonella*-positive samples from cool and warm seasons, respectively. Serotyping of PCR-confirmed positive samples resulted in fourteen different serovars with Cerro (21.6 %), Anatum (19.7 %), Muenchen (17.8 %), Montevideo (14.4 %), and Kentucky (12.0 %) comprising the majority. These findings dispel previous concerns that Mexican cattle have a higher prevalence rate of

Salmonella than U.S. cattle. These results also suggest that environmental factors may play a large role in the *Salmonella* prevalence rate in bovine LNs, and that additional research is needed to fully understand factors that influence *Salmonella* prevalence in bovine LNs.

DEDICATION

This thesis is dedicated to my parents. To my Mom, for always being my cheerleader, and to my Dad for teaching me the Code of the West.

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NOMENCLATURE

CDC	Centers for Disease Control and Prevention
FSIS	Food Safety and Inspection Service
LN	Lymph node
NVSL	National Veterinary Services Laboratories
PCR	Polymerase Chain Reaction
Salmonella	Non-typhoidal *Salmonella enterica*
SPI1	*Salmonella* pathogenicity island 1
USDA	United States Department of Agriculture

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CHAPTER I

INTRODUCTION

Salmonella is a known contaminant in fresh meat production systems. *Salmonella* is known to cause, on average, an estimated 1.3 million illnesses in the United States every year, resulting in approximately 19,000 hospitalizations and approximately 500 deaths *(13)*. United States Department of Agriculture-Food Safety and Inspection Service (USDA-FSIS)-inspected products (meat, poultry, and egg products) account for approximately 375,000 of these illnesses *(13, 52)*. In 2015, *Salmonella* contamination in food led to 3,944 confirmed cases of salmonellosis *(14)*. Illnesses have been traced to undercooked ground beef and other beef products. In 2015, 3.78% of raw ground beef product tested positive for *Salmonella* spp., whereas raw trim had a 2.07% prevalence *(51)*. Peripheral lymph nodes (LNs) present in the fatty tissues of beef trimmings may be a source of *Salmonella* contamination in ground beef products *(3, 48)*.

In previous studies, researchers demonstrated the ability to isolate *Salmonella* from bovine LNs *(7-9, 23-25, 28, 29, 35, 37)*. Consequently, when the USDA-FSIS released the "S*almonella* Action Plan" in 2013, one of the components of the plan was to "explore the contribution of lymph nodes to *Salmonella* contamination" *(48)*. Peripheral LNs could contribute to the presence of *Salmonella* in ground beef. Research designed to investigate the impact of cattle source and other factors that may impact *Salmonella*

prevalence in cattle will allow researchers and the beef industry to better approach future methods for reducing *Salmonella* in bovine LNs, and ultimately ground beef products.

This project was designed to determine if *Salmonella* prevalence varies between cattle of Mexican or U.S. origin when they are fed in the same South Texas feedlot. Additionally, prevalence during two different seasons, warm (May to August) and cool (December to February), was evaluated. The outcomes of this research can provide insight into how cattle origin and seasonality impact *Salmonella* prevalence in peripheral LNs and can help guide future studies.

CHAPTER II

REVIEW OF LITERATURE

Salmonella. *Salmonella* is a Gram-negative, rod-shaped (non-sporeforming) bacterium that is a member of the *Enterobacteriaceae* family. *Salmonella* is commonly found in nature, with humans and animals being the main reservoirs *(1, 26)*. *Salmonella* is a facultative aerobic microorganism, and its optimal temperature for growth is 37 °C. The optimum pH for *Salmonella* falls between 6.5 to 7.5, although growth can occur outside of this range *(1)*. These conditions allow *Salmonella* to catabolize carbohydrates, which results in gas and acid production *(1)*. The *Salmonella* genus is made up of more than 2400 serovars, being divided into two species, *S. enterica* and *S. bongori.* A vast majority of *Salmonella* serovars are from the *S. enterica* species, which contains six subspecies: *S. enterica* subsp. *enterica* (I), *S. enterica* subsp. *salamae* (II), *S. enterica* subsp. *arizonae* (IIa), *S. enterica* subsp. *diarizonae* (IIb), *S. enterica* subsp. *houtenae* (IV), and *S. enterica* subsp. *indica* (V). These species and subspecies are classified based on differences in their genomic and biochemical makeup. The number one subspecies that contributes to foodborne salmonellosis is *S. enterica* subsp. *enterica* (I) *(47)*.

Non-typhoidal *Salmonella* is known to cause gastrointestinal disease, which is commonly characterized by severe abdominal pain, vomiting, fever, and diarrhea *(16)*. Symptoms typically present after an 8 to 72 hr incubation period, post-consumption of *Salmonella*. Several population subsets are more susceptible to *Salmonella* infection, including the elderly, infants, and immunocompromised individuals *(1)*. *Salmonella* is

classified as an intracellular pathogen, meaning it relies on its host in order to survive *(32)*. When salmonellae make it into the intestinal tract and survive, they colonize within the intestine, and invade the hosts epithelial cells, and causes the inflammation of the intestines *(15)*.

Salmonella's ability to invade host cells has been shown to vary based on the virulence factors present in the microorganism's genome. There are specific operons present within the genome that house virulence factors for the pathogen *(32)*. The *invA* gene found in the *inv* operon in *Salmonella* is one of the genes that allows for the invasion of host cells by the bacterium *(19)*, thus causing illness. The *invA* gene is located on *Salmonella* pathogenicity island 1 (SPI1) *(22)*. Galán et al. *(19)* demonstrated that knocking out the *invA* gene in *Salmonella* made it unable to invade host cells. Galán et al. *(19)* also demonstrated that by combining this knockout version of *Salmonella* with wild-type *Salmonella* it was still capable of invading host cells. SPI1 is a crucial component to *Salmonella*'s pathogenicity. The pathogenesis factors encoded by SPI1 not only help with host invasion but also with inflammatory response of the host *(22)*.

Salmonella infects the intestinal epithelium, which causes the death of the surface layer of cells in the intestine. *Salmonella* is then capable of invading these damaged tissues to penetrate all the way through the intestinal epithelium. After *Salmonella* has invaded, it then infects phagocytes, including dendritic cells and macrophages, and the phagocytes then enter the lymphatic system where they are transported to LNs via lymph fluid *(27)*.

Salmonella is the primary cause of bacterial foodborne illness in the United States, with 1.3 million cases of foodborne salmonellosis per year and more than 500 deaths *(10, 33)*. *Salmonella* outbreaks have been traced back to numerous sources including, beef, poultry, produce, nuts, and pet food *(13)*. It is estimated that 375,000 of these illnesses arise from USDA-FSIS-inspected items of animal origin, including meat, poultry, and egg products *(43, 52)*.

Salmonella occurs naturally in the GI tract of humans and animals, which is why animals are the primary source *(54)*. Since *Salmonella* is naturally occurring in animals, animal-derived food products are typically used for the evaluation of food safety measures *(10, 40)*. Additionally, *Salmonella* has been recovered from the environment of agricultural production systems in soil, where it can survive for weeks at a time, and water samples *(44, 45, 54)*. *Salmonella* contamination in the environment can be facilitated by various species of birds and flies, which can carry *Salmonella (11, 12, 21, 34, 38, 54).* The transmission of *Salmonella* from wild birds to humans has been demonstrated when the *Salmonella* infection of wild birds is mirrored in similarly located human populations *(54)*.

Lymph nodes. A key component of immune response is facilitated by the lymphatic system. This system is comprised of lymph vessels and LNs spread throughout the body. This network of tissues is responsible for the transport and circulation of lymph fluid throughout the entire body *(31)*. Lymph fluid flows to and from LNs via lymph vessels and functions to rid invading microorganisms through phagocytic methods *(2)*. LNs have two primary functions: 1) filtration and 2) to activate

the immune response. Lymphocytes in LNs activate the immune response by monitoring lymph fluid for antigens and then mounting a response against them. LNs filter waste by using macrophages to remove and destroy microorganisms, so that waste cannot enter the blood stream *(31)*.

LNs, tonsils, spleen, and Peyer patches (found in the small intestine) are characterized as "secondary lymphoid organs." The "primary lymphoid organs" consist of red bone marrow and the thymus gland *(41)*. Secondary lymphoid organs house dendritic cells, B cells, and T cells. LNs house macrophages as well as B and T cells which interact with antigens in order to facilitate an immune response *(41)*. Lindgren et al. *(30)* determined that once *Salmonella* has been taken up by macrophages to be phagocytosed, salmonellae are able to survive by replicating and forming vacuoles which in turn kill the macrophage.

Mexican cattle. Approximately 26% of the world's beef is produced in North America *(46)*. Beef cattle production systems in Mexico tend to be less developed than those in the United States and Canada *(20)*. A large portion of cattle produced in Mexico are imported into the United States due to the larger amount of resources available to feed cattle. Over half of the total U.S. cattle imports are from Mexico, and these imported cattle are used in stocker and feedlot operations *(39)*. The majority of these imports come from the Northern region of Mexico where most of these cattle are Continental or British breeds *(39)*. Currently, feedlots do not play a large role in beef cattle production in Mexico, the bulk of cattle are produced through grazing practices.

However, it is predicted that the demand for beef in Mexico will continue to increase and the feedlot sector will grow in kind *(20)*.

Narvaez-Bravo et al. *(36)* examined the prevalence of *Salmonella* in Mexican feedlot environments by collecting a variety of pre-harvest (feces, soil, and water) and post-harvest (hide excisions and carcass swabs) samples. *Salmonella* was isolated from 52.5% of all samples collected. The highest prevalence rate was seen on hide samples and in feces from holding pens, and the lowest prevalence rate was observed in the chilled carcass samples *(36)*.

***Salmonella* in bovine lymph nodes.** Lepovetsky et al. *(28)* first described the ability of microorganisms to colonize within bovine LNs. Different tissues were assessed for overall microbiological content, including LNs, bone marrow, and muscle tissue. While both Gram-negative and Gram-positive microorganisms were isolated from LNs, very few microorganisms were isolated from the bone marrow and muscle tissue samples. With this information, Lepovetsky et al. *(28)* concluded that microorganisms isolated from LNs had proteolytic capabilities which allows them to contribute to "deep spoilage" in beef.

Samuel et al. *(42)* examined the prevalence of *Salmonella* at varying locations along the GI tract, as well as associated mesenteric LNs (those lying between the layers of the mesentery) in both sheep and cattle. Out of 100 cattle surveyed, *Salmonella* was isolated from 77 animals, of those, 61 contained *Salmonella* in at least one mesenteric LN. Thirty-one different serovars of *Salmonella* were isolated from the LNs of these cattle. Sheep had a much lower incidence of positive animals (only 43 of the 100

sampled), with only 14 carrying *Salmonella* in the LNs. Samuel et al. *(42)* suspected the substantial difference in prevalence rates between species was most likely due to differences in management style and age at slaughter.

A study by Arthur et al. *(3)* was the first to examine the prevalence and drug resistance capabilities of *Salmonella* in peripheral LNs, as opposed to mesenteric. Peripheral LNs were used, as they are embedded in fatty tissues associated with beef trimmings used in ground beef. Additionally, Arthur et al. *(3)* compared the prevalence of *Salmonella* in peripheral LNs between cull and feedlot cattle. *Salmonella* was discovered in peripheral LNs at a prevalence rate of 1.60% overall, with a higher prevalence seen in peripheral LNs from cull (2.46%) versus feedlot (0.70%) cattle. The *Salmonella* prevalence was found to be higher in subiliac LNs than in superficial cervical LNs. The highest prevalence rate of *Salmonella* was in the subiliac LNs of cull cattle (3.86%); alternatively, the lowest prevalence rate was seen in the superficial cervical LNs of feedlot cattle (0.61%) *(3)*.

Gragg et al. *(24)* identified a higher prevalence rate of *Salmonella* in peripheral LNs of feedlot cattle when compared to cull cattle, at 14.7 and 1.8%, respectively. Brown et al. *(9)* hypothesized breed type as the predominant factor driving significant differences in *Salmonella* prevalence rates observed between fed and cull cattle. However, when Brown et al. compared beef and dairy cattle, *Salmonella* prevalence rates were 59.7 and 62.1%, respectively, which were not significantly different. *Bos indicus* and *Bos taurus* breed types also were compared. No difference was seen, with *Salmonella* prevalence rates of 97 and 100% for *Bos taurus* and *Bos indicus*,

respectively *(9)*. Differing results between studies indicate that other factors, such as seasonal changes and environmental elements, may contribute to varying rates of *Salmonella* prevalence in peripheral LNs.

The season in which cattle are harvested also has been identified as a factor affecting *Salmonella* within bovine LNs. Li et al. *(29)* developed a risk assessment model to evaluate the contribution of LNs to *Salmonella* contamination of ground beef, by covering multiple factors including seasonal variation, and geographic location, while excluding post-grind interventions. One observation resulting from this model was that the season in which slaughter takes place affected the prevalence of *Salmonella* in peripheral LNs *(29)*. Gragg et al. *(23)* demonstrated differences across seasons for *Salmonella* prevalence in LNs as well, with data collected over an 18-mo span. *Salmonella* prevalence in the peripheral LNs from cattle slaughtered in the initial fall season (September to November) was 8.8%, 1.3% in the winter/spring season (February to March), and 12.0% in the following summer/fall (July to September). In the study by Brown et al. *(9)*, the highest prevalence rate was observed in June and the lowest in May. These findings support a seasonal impact on the prevalence of *Salmonella* in peripheral LNs, with greater prevalence seen in summer months.

Environmental factors have been shown to impact bovine fecal shedding of *Salmonella*. Nesemeier et al. *(37)* found fecal shedding of *Salmonella* to be much higher post-weaning than at finishing or slaughter with 63, 48, and 47% *Salmonella* prevalence rates, respectively. Haneklaus et al. *(25)* evaluated *Salmonella* prevalence in LNs from cattle across seven Texas feeding operations and found *Salmonella* prevalence across

feedlots to vary significantly (0, 4, 24, 40, 40, 42.9, and 88%). Though it is not entirely clear how the environment impacts *Salmonella* presence, differences in prevalence rates between feedlots indicate that environmental factors may play a role in *Salmonella* prevalence in bovine LNs.

Infection of lymph nodes. A few theories have been proposed to explain how *Salmonella* is introduced to bovine LNs. These include inhalation, oral (*Salmonella* is consumed with feed or drinking water), and transdermal routes (*Salmonella* is introduced through some form of skin puncture) of infection. The theory of oral transmission was the first to be heavily studied; mesenteric LNs were initially evaluated because it was believed *Salmonella* and other microorganisms were entering LNs from the intestinal tract *(35, 42)*. The oral transmission route also has been studied in relation to peripheral LNs. Brown et al. *(8)* inoculated cattle via drinking water with small amounts of *Salmonella* over a ten-day period. Intake of *Salmonella* was compared to both a positive control that contained a very high single dose (10^7) of *Salmonella* in drinking water and a negative control of no added *Salmonella*. Both the negative control group and the inoculated water intake group had a prevalence of 12.5%, suggesting consumption in small amounts over time was not contributing to the prevalence of *Salmonella* in peripheral LNs. The positive control group had a prevalence of 62.5%, which suggests it is possible to introduce *Salmonella* into peripheral LNs with an oral dose if at a very high concentration. It should be noted that the concentrations used in the study were much higher than what would be found naturally *(8)*.

Gragg et al. *(24)* suggested a transdermal route of infection based on within-animal diversity of *Salmonella* serovars and regional infections within the animal. Edrington et al. *(17)* developed a *Salmonella* challenge model using three different experiments. In the first, researchers inoculated the legs of a steer with 10^8 CFU/ml of *Salmonella* using needles, and different serovars for each leg. The steers were euthanized and necropsied at 2, 3, and 4 days post-inoculation, and peripheral LNs were evaluated for *Salmonella*. All peripheral LNs sampled contained the same serovar of *Salmonella* that was injected into the leg closest to that LN, except for the subiliac LN, which tested negative for *Salmonella (17)*. In the second part of the study, a 10-microlancet was used for an intradermal method of inoculation, instead of a transdermal inoculation. The expected serovars of *Salmonella* were isolated from LNs associated with the left side of the animal, however, all tests for recovering *Salmonella* from the subiliac LNs on the right side of the animal were negative, and from all LNs *Salmonella* counts were below the level of quantification *(17)*. The third experiment involved two inoculated steers using 10-microlancets with only one *Salmonella* serovar. Legs were inoculated at 2, 4, 6, and 8 days before euthanasia and sample collection. Results were similar to the second phase of the study, with non-quantifiable amounts of the serovar being recovered from most LNs (subiliac LNs were again negative) and no differences seen between days *(17)*. Researchers concluded an intradermal route of inoculation to be predictable and viable for use in challenge models. This supports the hypothesis that *Salmonella* can be introduced transdermally or intradermally, potentially through biting flies or other insects *(34)*.

CHAPTER III

MATERIALS AND METHODS

Study animal source and management. Finished cattle (*n* = 400) were sourced during two seasons (cool = December to February and warm = May to August) from a commercial feedlot in South Texas that routinely manages nearly equal proportions of cattle originating from the U.S. and Mexico. Additionally, it has been established through previous work that *Salmonella* can be consistently recovered from the LNs of cattle managed in this feeding operation *(25)*. Within a given sample collection season (warm or cool), a total of *n* = 200 finished cattle, or *n* = 100 finished cattle per origin (U.S. or Mexico), were transported to a commercial beef processing facility for harvest and subsequent LN collection. All live animal activities were conducted on commercial premises by employees of either the commercial feeding operation or harvest establishment, therefore, Institutional Animal Care and Use Committee approval for this study was not required.

Collection and processing of lymph nodes. A total of 800 LNs (100 carcasses x 2 countries of origin x 2 seasons x 2 subiliac LNs per carcass) were collected. Left and right subiliac LNs from each carcass were pooled in sterile sample bags (*n* = 400 total LN samples), and transported in insulated shipping containers with refrigerant material to the Texas A&M University Food Microbiology Laboratory (College Station, TX). Throughout the project, there were 9 sample collections (5 in the warm season and 4 in the cool season), and for each collection, a single LN from a beef head was inoculated

with *Salmonella enterica* serovar Typhimurium Lileengen Type 2 and used as a positive control.

All LNs were aseptically trimmed of fat using flame-sterilized forceps and a scalpel. Denuded LNs were dipped in ethanol and flame-sterilized to remove any surface contamination, placed into sterile Whirl-Pak filter bags (Nasco, Sandy Springs, GA), and pulverized using a rubber mallet. Pulverized LN samples were stored in refrigerated conditions (~ 4 °C) for no more than 24 h until microbiological analyses were performed.

Positive control preparation. At 48 h before each collection day, the *Salmonella enterica* serovar Typhimurium Lileengen Type 2 culture was propagated by transferring a loop of the stored microorganism from a tryptic soy agar (Fisher Scientific, Waltham, MA) slant to a fresh 10 ml tryptic soy broth (TSB) (Fisher Scientific, Waltham, MA) tube and incubated aerobically at 37 °C for 18 to 24 h. The culture was then transferred by pipetting 0.1 ml into a tube containing 10 ml TSB before incubating for 18 h at 37 °C.

***Salmonella* isolation and confirmation.** *Salmonella* presence/absence was determined by following the USDA-FSIS Microbiological Laboratory Guidebook (MLG) 4.08 *(49)*. Pre-enrichment was performed by using modified tryptone soy broth (mTSB) (Fisher Scientific, Waltham, MA); mTSB was added aseptically to pulverized LN samples at a 4:1 ratio. After the addition of mTSB, the positive control LN was inoculated with 1 mL of *Salmonella enterica* serovar Typhimurium Lileengen Type 2, which was prepared as previously described. All samples were hand-stomached for 60 s

and then incubated for 15 to 24 h at 42 ± 1 °C. After incubation, 0.5 ± 0.05 mL of each sample was transferred aseptically to 10 mL of tetrathionate broth-Hajna (Fisher Scientific, Waltham, MA) and 0.1 ± 0.02 mL of each sample was transferred aseptically to 10 mL of modified Rappaport Vassiliadis Broth (mRV), selective enrichments were incubated at 45 ± 1 °C for 22 to 24 h. Each enrichment then was streaked onto both xylose-lysine-tergitol 4 (Sigma-Aldrich, St. Louis, MO) and Brilliant Green Sulfa agar (Fisher Scientific, Waltham, MA) Petri dishes (VWR International; Randor, PA). Petri dishes then were inverted and incubated at 35 ± 1 °C for 18 to 24 h.

From selective agar plates, three individual colonies representing typical *Salmonella* morphology were chosen per LN sample. Two differential slants (one each of Triple Sugar Iron and Lysine Iron Agar; Sigma-Aldrich) were inoculated with one-half of each selected colony by stabbing the butts and streaking the slants with a sterile needle, generating 6 slants per LN sample. The slants were incubated at 35 ± 2 °C for 18 to 24 h. A sample was considered a presumptive positive if the associated colony yielded positive results, based on the descriptions from MLG 4.08, on both slants *(49)*. The remaining half of each presumptive positive colony then was streaked from the originating selective plate onto a nutrient slant (Fisher Scientific, Waltham, MA). Presumptive positive nutrient slants were incubated for 18 to 24 h at 35 ± 2 °C and stored in refrigerated conditions (~ 4 °C) for no longer than two weeks, until confirmation testing via PCR.

Polymerase chain reaction (PCR). Nutrient slants were transported to the Qualitative and Functional Genomics Laboratory at Texas A&M University (College

Station, TX) for genetic testing to confirm the presumptive positive *Salmonella* isolates. DNA was isolated from each selected colony using an UltraClean® Microbial DNA Isolation Kit (MoBio Laboratories, Inc., Carlsbad, CA). Isolated DNA was frozen and stored at -40 °C until PCR could be conducted. Using methods described by Brandt et al. *(6)*, *Salmonella* was confirmed by the presence of the *InvA* gene located by the primers 5'-GAATCCTCAGTTTTTCAACGTTTC- 3'(forward) and 5'-AGCCGTAACAACCAATACAAATG- 3' (reverse). The PCR conditions used were [(Initial) 94 °C for 120 s, (35 cycles) 94 °C for 30 s, 60 °C for 30 s, 72 °C for 30 s, (Final) 72 ° C for 420 s, (Hold) 4 °C]. Following amplification by PCR, the DNA was run on a 1% agarose gel electrophoresis containing bio red fluorescence at 100 V for approximately 1 h. Fragments indicative of a positive isolate were seen at 678 kDa. Isolates that contained positive *Salmonella* DNA were considered to be from *Salmonella* positive LNs.

***Salmonella* serotyping.** A single representative colony was selected from each positive LN sample, streaked onto a nutrient agar slant, and incubated for 22 to 26 h at 35 ± 2 °C. Following incubation, slants were packaged for shipping following Texas A&M University Environmental Health and Safety Department personnel instructions (IATA packing instruction 650 for shipping by air and DOT Title 49CFR 173.134(a)(ii) and 173.199 for domestic shipments). The slants containing the *Salmonella* isolates were transported to the USDA – Animal and Plant Health and Inspection Service National Veterinary Services Laboratory (Ames, IA) for serotyping.

Statistical analysis. Data were analyzed using JMP Pro software (version 12.0, SAS Institute Inc., Cary, NC). Contingency tables of *Salmonella* prevalence were produced for main effects (season and country of origin), and within-table differences were determined using Fisher's exact test and an $\alpha = 0.05$.

CHAPTER IV

RESULTS AND DISCUSSION

Of the 400 LN samples collected during this project, 208 (52.0%) were positive for *Salmonella*. There was no difference ($P = 0.4836$) in *Salmonella* prevalence between LNs from cattle of Mexican and U.S. origin, with 54.0% (108/200) and 50.0% (100/200) of the samples returning *Salmonella*-positive results, respectively (data not presented in tabular form). These results are in line with results from Brown et al. *(9)*, who compared Holstein steers to beef steers, as well as Brahman cattle to their beef cattle (non-dairy, with less than 25.0% Brahman influence) counterparts. Brown et al. *(9)* ultimately reported no differences in either comparison, indicating that breed does not drive differences in *Salmonella* prevalence in peripheral LNs, but that age, immune function, or environmental factors may be more influential.

Salmonella prevalence differed ($P = 0.0354$) between seasons. Specifically, 46.5% (93/200) of cool and 57.5% (115/200) of warm season samples were positive for *Salmonella* (data not shown in tabular form). The seasonal differences found in the present study are in agreement with previous works *(4, 18, 23)*. These clear seasonal trends and rates of *Salmonella* prevalence in LNs highlight the potential impact of environmental factors.

No difference ($P = 0.6705$) was seen between seasons for samples of Mexican origin, with rates of 52.0% (52/100), and 56.0% (56/100) *Salmonella* prevalence for cool and warm seasons, respectively (Table 1). For samples collected from beef carcasses of

U.S. origin, *Salmonella* prevalence rates of 41.0% (41/100) and 59.0% (59/100) were seen for cool and warm seasons, respectively. Interestingly, *Salmonella* prevalence in samples of U.S. origin differed by season (P =0.0160), unlike those of Mexican origin. *Salmonella* prevalence differences between countries of origin (U.S. or Mexico) within a particular season (warm or cool) also were evaluated. No differences in rates of *Salmonella* prevalence were seen between countries of origin for either the warm (P = 0.7749) or cool (P = 0.1561) season.

TABLE 1. *Prevalence of* Salmonell*a-positive*[a] *lymph nodes*[b] *(LNs) stratified by season*[c] *and origin*

	% (no. positive/no. tested) *Salmonella*-positive LNs	
Season	Mexico	U.S.
Cool	52.0 (52/100) A, X	41.0 (41/100) A, X
Warm	56.0 (56/100) A, X	59.0 (59/100) B, X

A, B: Values within a column lacking a common letter differ ($P < 0.05$).
X, Y: Values within a row lacking a common letter differ ($P < 0.05$).
[a] *Salmonella* was isolated following protocols described by Microbiology Laboratory Guidebook 4.08. Three presumptive positive colonies were selected for confirmation by PCR. One confirmed positive colony for each LN sample was selected for serotyping (n = 208). These colonies were revived in Tryptone Soya Broth and then stored on nutrient agar slants for shipping to NVSL (Ames, IA).
[b] Left and right subiliac LNs (n = 800 LNs) were collected and pooled by animal (n = 400 total samples).
[c] Sample collection seasons were defined as: warm (May to August) or cool (December to February).

Two hundred and eight unique colonies representing all positive samples were sent to NVSL for serotyping. Fourteen serovars were identified, the majority being: Cerro (21.6 %), Anatum (19.7 %), Muenchen (17.8 %), Montevideo (14.4 %), or Kentucky (12.0 %; Table 2). Thirteen serovars were identified among the 108 positive LNs from Mexican cattle, and 11 serovars were seen amongst the 100 positive LNs from U.S. cattle. Eight serovars were observed among the 93 positive LNs from the cool season, and 13 different serovars were observed among the 115 positive LNs from the warm season. It is important to note that the serovar results from this study do not provide a full representation of the possible serovars present as only one colony was selected to represent a LN sample. The serovars identified in this study are similar to those found in previous studies. Anatum and Montevideo have both been found in peripheral LNs at a high frequency *(23, 42)*, and have also been the most commonly found serovars in ground beef *(5)*. Montevideo has been the number one serovar in ground beef since 1998, however, it is not typically tied to human illnesses *(5, 50)*. Meleagridis, Kentucky, Mbandaka, Muenchen, Agona, Lille, and Cerro have all appeared in the LNs of fed cattle, and Brandenburg, Lille, and Mbandaka appeared in the LNs of cull cattle *(23, 42, 53)*. In an evaluation of samples from three South Texas feedlots, Xie et al. *(55)* isolated Montevideo, Anatum, and Muenchen from soil, Montevideo and Anatum from feces, and Anatum from feed components, demonstrating that *Salmonella* in the environment could contribute to the prevalence of *Salmonella* in LNs.

TABLE 1. Salmonella *serovars recovered*[a] *from subiliac lymph nodes*[b] *(LNs) of Mexican and U.S. cattle in two sample collection seasons*[c].

Serovar	All LN (*n* = 208) overall %	Mexico/Cool LN (*n* = 52) relative %	U.S./Cool LN (*n* = 41) relative %	Mexico/Warm LN (*n* = 56) relative %	U.S./Warm LN (*n* = 59) relative %
Cerro	21.63	44.23	4.88	19.64	15.25
Anatum	19.71	23.07	36.59	12.50	11.86
Muenchen	17.79	3.85	21.95	26.78	18.65
Montevideo	14.42	19.23	17.07	10.70	11.86
Kentucky	12.02		12.20	12.50	22.03
Brandenburg	3.85			1.79	11.86
Mbandaka	3.85	9.62		3.57	1.70
Rough_O:gms:-	1.92			1.79	5.09
6,7:g,m,s:e,n,z15	1.44			3.57	1.70
Meleagridis	0.96		4.88		
Rough O:e,h:1,6	0.96		2.43	1.79	
Agona	0.48			1.79	
Lille	0.48			1.79	
Minnesota	0.48			1.79	

[a] *Salmonella* was isolated following protocols described by Microbiology Laboratory Guidebook 4.08. Three presumptive positive colonies were selected for confirmation by PCR. One confirmed positive colony for each LN sample was selected for serotyping (*n* = 208). These colonies were revived in Tryptone Soya Broth and then stored on nutrient agar slants for shipping to NVSL (Ames, IA).

[b] Left and right subiliac LNs (*n* = 800 LNs) were collected and pooled by animal (*n* = 400 total samples).

[c] Sample collection seasons were defined as: warm (May to August) or cool (December to February).

CHAPTER V

CONCLUSION

These results demonstrate that while season did impact *Salmonella* prevalence in LNs, cattle origin did not. To better understand the varying levels of *Salmonella* prevalence, additional research is warranted to investigate how *Salmonella* is being introduced into the LN of feedlot cattle. Once the modes of *Salmonella* infection are determined, appropriate interventions can be developed and implemented to prevent *Salmonella* contamination of LNs that may be incorporated into ground beef products. These data help to dispel a previous concern that cattle of Mexican-origin were a major contributing factor to higher rates of *Salmonella* prevalence in South Texas feedlots.

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