CHALLENGES OF PATHOGEN CONTROL IN BEEF CATTLE PRODUCTION AND PROCESSING IN SOUTH TEXAS

A Dissertation

by

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ABSTRACT

This multi-phase project was designed (1) to evaluate existing post-harvest process controls and intervention strategies used to reduce *Escherichia coli* O157:H7, (2) to evaluate the impacts of cattle source and environmental factors on *Salmonella* prevalence in bovine lymph nodes, and (3) to evaluate sanitary conditions of feedyards in South Texas. The ultimate goal of this project was to identify and implement measures that reduce *E. coli* O157:H7 in beef harvest facilities, and *Salmonella* prevalence in feedyards. To evaluate process control of *E. coli* O157:H7 throughout the beef harvest process, samples were collected from harvest floor processing areas at two commercial beef slaughter establishments, and enumerated for aerobic plate counts, *E. coli/*coliform, and *Enterobacteriaceae*. To survey existing *Salmonella* prevalence, bovine lymph nodes (n = 307) were collected from beef carcasses at a commercial beef processing plant. Lymph nodes were extracted from cattle sourced from seven feedyards. *Salmonella* prevalence in lymph nodes was found to be 0% in cattle sourced from only one of the seven yards. Lymph nodes from cattle sourced from the other feedyards yielded positive samples, with varying prevalence. Of the remaining six feedyards, one feedyard yielded 88.2% prevalence of *Salmonella* in bovine lymph nodes, which was significantly higher than all other feedyards (42.9, 40.0, 40.0, 24.0, and 4.0%). The prevalence of *Salmonella* in the feedlot environment was compared among three feedyards; one yard had 65.0% environmental prevalence of *Salmonella,* which was statistically higher than the other feedyards surveyed. Of the two remaining yards,

one had 0% prevalence of *Salmonella* in fecal and soil samples, which was also the feedyard with 0% prevalence of *Salmonella* in lymph nodes. Findings include (1) the significance of effective sanitary dressing procedures and intervention strategies in a beef harvest environment, (2) that there is clear feedyard-to-feedyard variation with relation to *Salmonella* prevalence in bovine lymph nodes, and (3) that differences in environmental factors existed among feedyards although the reasons remain unclear.

DEDICATION

This dissertation is dedicated to my grandmother, Wanda Kimbell. Her support and love gave me the strength to see this through.

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TABLE OF CONTENTS

Page

ABSTRACT ii

DEDICATION iv

ACKNOWLEDGEMENTS v

TABLE OF CONTENTS vii

LIST OF FIGURES ix

LIST OF TABLES x

CHAPTER I INTRODUCTION 1

CHAPTER II REVIEW OF LITERATURE 4

2.1. Background 4
2.2. Pathogen Prevalence from Feedlot to Harvest 5
2.3. Super Shedders and Pathogen Prevalence 7
2.4. Pathogen Prevalence on Hides 8
2.5. Hide Decontamination Strategies 9
2.6. Hide to Carcass Contamination 13
2.7. *Salmonella* in Bovine Lymph Nodes 14

CHAPTER III EVALUATING THE IMPACT OF POST- HARVEST PROCESS CONTROLS AND INTERVENTION STRATEGIES USED TO REDUCE *ESCHERICHIA COLI* O157:H7 17

3.1. Introduction 17
3.2. Materials and Methods 18
3.3. Results and Discussion 27

CHAPTER IV *SALMONELLA* PREVALENCE IN BOVINE LYMPH NODES DIFFERS AMONG FEEDYARDS 33

4.1. Introduction 33
4.2. Materials and Methods 33
4.3. Results and Discussion 37

CHAPTER V POSSIBLE ENVIRONMENTAL INDICATORS LEADING TO DIFFERENCES IN *SALMONELLA* PREVALENCE IN BOVINE LYMPH NODES AMONG FEEDYARDS 41

5.1. Introduction 41
5.2. Materials and Methods 42
5.3. Results and Discussion 44

CHAPTER VI SUMMARY AND CONCLUSIONS 51

REFERENCES 53

APPENDIX A 66

LIST OF FIGURES

Page

Figure 3.1 Carcass sampling locations 22

Figure 5.1 Cattle gathered under shades in a feedyard 46

Figure 5.2 Water trough design at feedyard A 47

Figure 5.3 Bovine lymphatic organs 50

LIST OF TABLES

Page

Table 3.1 Least-squares means of plate counts (log CFU/cm^2) for APC, *E. coli*/coliforms, and *Enterobacteriaceae* by processing location for Trial #4 30

Table 3.2 Least-squares means of plate counts (log CFU/cm^2) for APC, *E. coli*/coliforms, and *Enterobacteriaceae* by hide sample type for Trial #5 31

Table 4.1 Percentage of *Salmonella*-positive lymph nodes by feedyard for each trip cumulatively 37

Table 5.1 Percentage of *Salmonella* positive environmental samples by feedyard 48

CHAPTER I

INTRODUCTION

Beef processing facilities and researchers have worked tirelessly to develop and implement intervention strategies both pre- and post- harvest to reduce/eliminate the presence of these pathogens in the end product. Some pre-harvest management techniques include reducing the exposure of feeder cattle to wildlife, pests, or contaminated feed/water, and controlling animal density in feedyard pens *(17, 42)*. More novel pre-harvest approaches to controlling pathogens are vaccines, direct fed microbials, and other feed additives *(27, 42, 45, 59)*. Post-harvest intervention strategies are more widely implemented throughout the beef industry as methods to reduce microbiological contamination on finished products. These strategies address hide decontamination *(11)*, carcass sprays *(26, 39)*, and the treatment of subprimals and trimmings *(39)*. FSIS Directive 7120.1 provides guidance to processing facilities by outlining "Safe and Suitable Ingredients Used in the Production of Meat, Poultry, and Egg Products" *(70)*. This directive includes all allowable compounds and associated amounts that can be applied to carcass surfaces, subprimal surfaces, trimmings, or incorporated into various meat products.

Although many processing aids and intervention strategies have been proven to reduce or eliminate both *Escherichia coli* O157:H7 and *Salmonella* on beef hides, carcass surfaces, and products, minimal research is currently available regarding the elimination of *Salmonella* from lymph nodes. *Salmonella* has been identified in bovine

lymph nodes, and due to the nature of beef trimmings, lymph nodes are commonly incorporated into ground beef products. Although studies have been conducted to determine presence of the microorganism *(37, 48, 55)*, little is known regarding the acquisition or elimination of the microorganism by the bovine lymphatic system. As such, this field of research is currently being investigated, with experimental vaccines being evaluated that may help in reducing *Salmonella* prevalence in bovine lymph nodes. Some beef harvest facilities have protocols in place to remove large, easily accessible lymph nodes. However, the generalized nature of the lymphatic system *(54)* does not facilitate the removal of all lymph nodes prior to further processing of beef products. Of special concern, is the threat that *Salmonella* could also be deemed an adulterant in ground beef, which in turn, would imply that lymph glands are adulterated and must be removed from beef products entirely. Therefore, more research in this area is necessary to find ways to reduce/eliminate *Salmonella* in lymph nodes that are commonly present in beef trimmings destined for ground beef.

In an effort to address the knowledge gaps both pre- and post- harvest, the present study was designed to address *Salmonella* in the pre-harvest environment and *E. coli* O157:H7 in the post- harvest environment. We believed that *Salmonella* prevalence would vary among cattle source (i.e. feedyards and/or country of origin). Further, we hypothesized that *Salmonella* prevalence in bovine lymph nodes would be reflected in environmental *Salmonella* prevalence. Finally, with the knowledge that coliforms are known indicators of fecal contamination *(41)*, we wanted to explore the usefulness of such indicators to predict process control during beef harvest. Therefore, we

hypothesized that indicator organisms were present at decreasing levels along the beef slaughter processing line.

This multi-phase project was designed to evaluate existing post-harvest process controls and intervention strategies used to reduce *E. coli* O157:H7, as well as to evaluate the impacts of environmental factors and cattle source on *Salmonella* prevalence in bovine lymph nodes.

CHAPTER II

REVIEW OF LITERATURE

2.1. Background

The Centers for Disease Control and Prevention (CDC) listed *Salmonella* and *Escherichia coli* O157:H7 as two of the six key pathogens in 2010 *(29)*. The number of infections caused by *E. coli* O157:H7 has declined substantially. Healthy People 2010 was a federal initiative designed to reduce the incidence of foodborne illnesses in the United States. Objectives of the 2010 initiative were to reduce the incidence of *Salmonella* and *E. coli* O157:H7 related illnesses to 6.80 and 1.00 cases per 100,000 people, respectively *(28)*. Of nine foodborne illnesses tracked by the CDC, the number of illnesses caused by *E. coli* O157:H7 was the only one to meet the Healthy People 2010 objective. The same data reveal that *Salmonella* infections have actually increased since 2008 *(29)*. New objectives have been set by the United States government to further reduce the number of illnesses caused by these microorganisms. The Healthy People 2020 objectives are 0.60 and 11.40 cases per 100,000 for *E. coli* O157:H7 and *Salmonella*, respectively *(60)*.

In addition to the Healthy People 2010 and 2020 objectives, USDA-FSIS has made additional strides to improve the safety of the U.S. beef supply. Regulations and standards written by USDA-FSIS have had a significant impact on the daily production of beef products. In 1994, *E. coli* O157:H7 was declared an adulterant in ground beef *(62)*, and shortly thereafter, FSIS released performance standards for *Salmonella (61)*.

2.2. Pathogen Prevalence from Feedlot to Harvest

Cattle hides are a known source of microbiological contamination, with *Salmonella* and *E. coli* O157:H7 being commonly identified microorganisms of concern *(6, 21, 24)*. Woerner et al. *(73)* designed a study to determine the prevalence of *E. coli* O157:H7 from the feedlot environment to the end of the beef harvest process. The same study determined that pens with greater than 20% prevalence of *E. coli* O157:H7 in fecal pats from the feedlot floor were associated with 25.5% hide, 51.4% colon, and 14.3, 2.9, and 0.7% positive carcass samples at pre-evisceration, post-evisceration, and after the final intervention, respectively. In this study, pens with fecal pats not greater than 20% showed lower prevalence of *E. coli* O157:H7 with 5.0% hide, 7.5% colon, 6.3% at pre-evisceration, and 0% for both post-evisceration and after the final intervention. With data like these linking pathogen prevalence in the feedlot to the carcasses in a processing facility, one might question the influence transportation has on cattle and the potential for cross-contamination between animals. Past studies have been designed to test for an increase in pathogens on cattle hides after transportation when compared to before. Arthur et al. *(5)* found an *E. coli* O157:H7 prevalence of 50.3% on cattle hides at the feedlot compared to 94.4% prevalence on cattle hides just after stunning at the harvest facility. However, only 29% of the *E. coli* O157:H7 isolates identified prior to transport matched those isolates found post-harvest. In a separate study, Jacob et al. *(38)* found 38.5% prevalence of *E. coli* O157:H7 on cattle hides, followed by 10% on carcasses. The authors of the same study also focused on possible animal and truckload factors that may influence the prevalence indicated previously. Reicks et al. *(51)* evaluated the

influence of trailer cleanliness on prevalence of *Salmonella* and *E. coli* O157:H7 on trailer surfaces and cattle hides before and after transport. A significant difference in *Salmonella* prevalence on trailer surfaces was seen between clean and dirty trailers (3.1 and 43.8%, respectively) prior to cattle shipment. However, *Salmonella* prevalence, although much higher (75.0 and 71.9% for clean and dirty trailers, respectively), was not statistically different following the transport of cattle from the feedyard to the processing plant. Data from the same study showed significant increases of *Salmonella* (53.0 to 83.0% on the midline, and 33.6 to 81.9% on the withers) and *E. coli* O157:H7 (0.3 to 1.3%) prevalence on cattle hides from the feedyard to the harvest facility. In a study conducted by Beach et al. *(13)*, fecal shedding and hide contamination of feedlot and adult pasture cattle were evaluated before and after transport. The authors noted an insignificant increase in fecal shedding of *Salmonella* and *Campylobacter* in feedlot cattle before and after transport. However, a significant increase in fecal shedding of *Salmonella* was identified in adult pasture cattle following transport. Pre-transit, swabs of hides from feedlot cattle had 18.0 and 25.0% prevalence for *Salmonella* and *Campylobacter,* respectively. Post-transit hide swabs for feedlot cattle increased to 56.0% prevalence of *Salmonella,* and decreased to 13% prevalence of *Campylobacter*. The changes in hide prevalence tracked through the transit process of feedlot cattle were statistically significant for both microorganisms evaluated. The only significant change in hide prevalence of adult pasture cattle was an increase in *Salmonella*-positive swabs (19.8% pre-transit to 52.2% post-transit). An insignificant increase in hide prevalence of *Campylobacter* in adult pasture cattle was noted.

2.3. Super Shedders and Pathogen Prevalence

The presence of at least one high shedding animal, or at least one hide that began with high prevalence had a significant impact on subsequent pathogen prevalence on beef carcass surfaces. "High-shedding" or "super-shedding" cattle have been defined as those animals shedding *E. coli* O157:H7 at a level > 10^4 CFU/g of feces *(9)*. While the reasons for "super-shedding" cattle are not entirely understood, this phenomenon has been discussed as a physiological response due to the stress of shipment. However, some researchers have investigated the role of "high-shedding" cattle in a feedlot environment prior to shipping. Arthur et al. *(10)* found that 84.2% of pens containing at least one "high-shedding" individual led to > 20% *E. coli* O157:H7 fecal prevalence in those pens. These authors went on to conclude that pens exhibiting > 20% fecal prevalence usually had > 80% *E. coli* O157:H7 prevalence on hides of cattle in those pens. When studying animal temperament as a factor influences pathogen shedding, Schuehle Pfeiffer et al. *(57)* found calm cattle to shed a higher percentage of *E. coli* O157:H7 than counterparts from other temperament groups. Similar research has evaluated production practices and animal attributes as potential causes of *Salmonella* and *Campylobacter* shedding in feedlot cattle *(13)*. However, of all factors addressed in this study, none were found to impact shedding of *Salmonella* or *Campylobacter* in feedlot cattle. Conversely, these factors did impact hide contamination from both organisms.

2.4. Pathogen Prevalence on Hides

The presence of microorganisms on the hides of incoming cattle presented for slaughter, as well as the distribution of microorganisms on beef carcasses following hide removal have been targeted in past research studies. A study conducted by Bosilevac et al. *(17)* was designed to evaluate the prevalence of *Salmonella* and *E. coli* O157:H7 on hides and carcass surfaces of cattle processed at small harvest establishments. Hide prevalence was documented at 71.0 and 91.0% for *E. coli* O157:H7 and *Salmonella,* respectively. Subsequent prevalence of *E. coli* O157:H7 and *Salmonella* on pre-evisceration carcass surfaces was 33.0 and 58%, respectively. Elder et al. *(34)* indicated a 28% prevalence of *E. coli* O157:H7 in the feces of cattle presented for slaughter, and an 11% prevalence on hides of those cattle. The author also noted a 43% prevalence of *E. coli* O157:H7 on carcass surfaces prior to evisceration, and a 2% prevalence of the same organism on carcasses surfaces post-processing (in the cooler). Although this author did not collect all samples from the same animals, a significant correlation was seen between fecal/hide prevalence and carcass contamination. In addition to determining the prevalence of *Salmonella* on cattle hides, Fegan et al. *(36)* also evaluated the oral cavity, rumen, and fecal material of 100 cattle presented for slaughter. Rates of *Salmonella* prevalence were found to be 29% of oral cavities, 68% of hides, 16% of feces collected after evisceration, 25% of rumen samples, 2% of pre-chill carcasses, 3% of post-chill carcasses, and 48% of feces collected from holding pens. Barkocy-Gallagher et al. *(12)* concluded that the prevalence of *E. coli*, *Salmonella,* and non-O157 STEC varied by season, was lower in feces than on hides, and decreased

during processing and application of antimicrobial interventions. Specifically, *E. coli* O157:H7 was recovered from 5.9% of fecal samples, 60.6% of hides, and 26.7% of carcass samples prior to the pre-evisceration wash. *Salmonella* was recovered from 4.4, 71.0, and 12.7% of fecal, hide, and pre-evisceration wash carcass samples, respectively. Concerning non-O157 *E. coli* serotypes, cells containing the *stx* genes were detected in 34.3, 92.0, 96.6, and 16.2% of fecal, hide, pre-evisceration carcass, and post-evisceration carcass samples, respectively. In addition to research of microbiological contamination of cattle hides, distribution of contamination on hides has also been investigated. In a study by R. G. Bell *(14)*, seventeen hide regions were swabbed for APC and *Escherichia coli* enumeration. The author concluded that the hock, bung, inside leg and flank were the most probable sites for direct or indirect fecal contamination. Antic et al. *(2)* also evaluated various regions of hide-on carcasses for total viable bacterial counts (TVC), *Enterobacteriaceae* counts (EC), generic *E. coli* counts (GEC), and *Salmonella* spp. While all hides presented some level of GEC, *Salmonella* spp. was not isolated from any hides sampled. Distal leg and brisket regions of the hides were found to have significantly more contamination than the rump, flank, or neck regions. These data coincide with those from a study conducted by Reid et al. *(52)* in which the brisket region had substantially higher prevalence of *E. coli* O157:H7 and *Salmonella* than the rump region of hide-on beef carcasses.

2.5. Hide Decontamination Strategies

Since it has been well documented that the hide is a potential source of microbial contamination, much research has been conducted to evaluate hide decontamination

strategies. Mies et al. *(47)* conducted two different trials to evaluate the efficacy of water washes and antimicrobial agents in an automated cattle wash system as potential hide treatments. Data demonstrated that ethanol and 4 to 6% lactic acid treatments were more effective than acetic acid, chorine, and water wash; however, it was noted that the application of these agents to live cattle was not likely to be applied because they could possible create a potential animal welfare issue. In another study *(11)* additional antimicrobial agents, including isopropyl alcohol, hydrogen peroxide, cetylpyridinium chloride, were shown to be effective hide treatments. Arthur et al. *(6)* studied the use of a minimal hide wash cabinet and determined that a 25 to 97 s water wash followed by a 100 to 200 ppm chlorine spray was an effective hide wash intervention strategy for reducing *E. coli* O157:H7. Bosilevac et al. *(18)* determined that a water wash followed by a 1% cetylpyridinium chloride (CPC) treatment was effective in lowering hide prevalence of *E. coli* O157 from 56% to 34%. These authors further discovered that the same combination of water wash and CPC treatment yielded a decrease in preevisceration carcass prevalence of *E. coli* O157 from 23% to 3%. In a separate study, Bosilevac et al. *(21)* compared the use of ozonated and electrolyzed waters to a control water treatment (without antimicrobial properties) for the purposes of decontaminating hides. Control water wash treatment showed no significant reduction of *E. coli* O157:H7 prevalence on hides, whereas both ozonated water and electrolyzed water treatments resulted in a significant reduction in the prevalence of *E. coli* O157:H7 on hides (from 89% to 31% using ozonated water, and from 82% to 35% using electrolyzed water). Ozonated water significantly reduced *Enterobacteriaceae* and aerobic plate counts

(APC) by 3.4 and 2.1 log CFU/100 cm^2, respectively. Significant reductions also were seen following the application of electrolyzed water, resulting in a 3.5 log CFU/cm^2 reduction of APCs and a 4.3 log CFU/cm^2 reduction of *Enterobacteriaceae*. An additional study conducted by Bosilevac et al. *(20)* compared the efficacy of 1.6% sodium hydroxide, 4% trisodium phosphate, 4% chlorofoam, and 4% phosphoric acid as hide decontaminates. Each of these treatments was followed by a rinse of either water or acidified chlorine (200 or 500 ppm). In this study, hide coliform counts were lowered by 1.5 to 2.5-log CFU/100 cm^2 for all treatments listed when paired with a subsequent water rinse. Coliform counts on hides were further reduced by approximately 1.0 and 2.0 log CFU/100cm^2 with the application of 200 ppm and 500 ppm acidified chlorine rinse, respectively. In the second phase of their study, an on-line hide wash cabinet was employed that used a sodium hydroxide wash and a chlorinated (1 ppm) water rinse. When the cabinet was in use, hide prevalence of *E. coli* O157:H7 was significantly reduced from 44% to 17%. Subsequently, a decrease of *E. coli* O157:H7 prevalence of 17% to 2% was observed on preevisceration carcass surfaces. Carlson et al. *(23)* conducted a two-phase study to determine the efficacy of various antimicrobial hide treatments. The first phase of the study included a comparison of 10% acetic acid (at 23 and 55°C), 10% lactic acid (at 23 and 55°C), 3% sodium hydroxide (at 23°C), or 4 and 5% sodium metasilicate (at 23°C). All of these antimicrobials were evaluated in three ways: (1) after being applied alone, (2) being applied following a water rinse, and (3) being applied prior to a water rinse. Treatments followed by a water rinse reduced *E. coli* O157:H7 populations by 0.6 to 2.4 log CFU/cm^2. Treatments applied after a water rinse

reduced *E. coli* O157:H7 by 1.5 to 5.1 log CFU/cm^2. In Phase II of the same study conducted by Carlson et al. *(23)*, hides were treated with either acetic acid, lactic acid, sodium hydroxide, sodium metasilicate, followed by a water rinse. Acetic and lactic acids resulted in the greatest reductions of aerobic plate and total coliform counts when compared to sodium hydroxide and sodium metasilicate treatments. The data from these studies reiterate the importance of implementing antimicrobial hide treatments to reduce incoming microbiological loads, and the potential for hide-to-carcass contamination.

In addition to more the traditional chemical spray interventions, researchers also have explored innovative methods for preventing carcass contamination from the hide. An additional study conducted by Carlson et al. *(24)* compared antimicrobial hide spray treatments (10%, 55°C acetic acid; 10%, 55°C lactic acid; 3%, 23°C sodium hydroxide; or 4%, 23°C sodium metasilicate, or 3%, 23°C sodium hydroxide followed by a high pressure chlorinated water wash), to less traditional hide dehairing treatments (2.4%, 30°C potassium cyanate; or 6.2%, 30°C sodium sulfide). Organic material was removed as a result of all hide spray treatments; however, organic matter and hair were removed as a result of the potassium cyanate and sodium sulfide treatments. Overall, the greatest reductions of *E. coli* O157:H7 were seen with the sodium hydroxide/high pressure wash, potassium cyanate, and sodium sulfide treatments. Further, *Salmonella* reductions were greatest with the sodium hydroxide/high pressure wash, and sodium sulfide treatments. A minimum reduction of 2 log CFU/cm^2 was noted for both *E. coli* O157:H7 and *Salmonella* following hide treatment with acetic acid, lactic acid, and sodium hydroxide. Castillo et al. *(25)* and Nou et al. *(49)* both found chemical dehairing effective in

reducing microbial contamination. However, Schnell et al. *(56)* concluded that chemical dehairing improved the visible appearance of the carcass but had minimal impact on microbial load. Antic et al. *(1)* discovered that a applying a $23 Shellac-in-ethanol solution to experimentally inoculated hides yielded a 2.1 log reduction of *E. coli* O157:H7. While these interventions have been shown to effectively reduce bacterial load, they are more difficult to apply in a commercial setting than a simple chemical spray system.

2.6. Hide to Carcass Contamination

In an effort to address hide contamination prior to slaughter, Van Donkersgoed et al. *(72)* investigated the correlation of tag (mud, bedding, manure) on cattle hides and subsequent carcass contamination. These authors found lowered microbiological counts when tag was shaven off of the hides, and when plant line speeds were slowed; however, reductions in counts were less than 0.5 log CFU/cm^2. Further, a weak correlation between hide surface wetness and *E. coli* counts was noted. Overall, these authors found no consistent relationship between tag scores and carcass contamination. Some studies also have been conducted to identify specific regions of cattle hides that assume the highest levels of contamination. Reid et al. *(52)* conducted a study to determine prevalence of *E. coli* O157:H7, *Salmonella* spp., and *Campylobacter* spp. on three regions of cattle hides. Of the three regions swabbed, the brisket was found to be the most frequently contaminated location on the hide with 22% and 10% prevalence of *E. coli* O157:H7 and *Salmonella* spp., respectively *(52)*. Additional research support the hypothesis that carcass contamination is highest along sites where the hide is cut, or

contact is made between the hide and the carcass surface during the hide removal process *(14)*.

2.7. *Salmonella* in Bovine Lymph Nodes

Lymph nodes are commonly found in lean trimmings destined for ground beef production. Bovine lymphatic tissue, specifically peripheral lymph nodes, has been identified as a potential source of *Salmonella* in trimmings destined for ground beef *(8, 22)*. Traditionally, research involving bovine lymph nodes has been focused on *Salmonella* prevalence in mesenteric lymph nodes *(48, 55)*. Limited studies have focused on the prevalence of *Salmonella (8)* and other bacteria *(43)* in peripheral lymph nodes destined for use in ground product as a component of lean trimmings. The prevalence of *Salmonella* among types of lymph nodes is the primary research target in this field of study rather than the potential influence of cattle origin on *Salmonella* prevalence in lymph nodes.

In a study conducted by Sofos et al. *(58)*, feces, air, and lymph nodes were identified as potential sources of beef carcass contamination during the harvest process. As a result, these samples were evaluated for APCs, total coliform counts, *E. coli* biotype I counts, and presence of *Salmonella.* Microbiological counts for air samples were found to be significantly higher near the hide-pulling station when compared to stations following hide removal. Further, while Sofos et al. *(58)* did identify APC counts of >1,000 CFU/g in 27.9% and 24.3% of cattle in the wet and dry seasons, respectively; *Salmonella* was not identified in any lymph nodes from this study. A study conducted by Arthur et al. *(8)* investigated the prevalence of *Salmonella* in bovine lymph nodes

potentially destined for ground products, and found prevalence to be low (1.6%). In a study conducted by Gragg et al. *(37)*, lymph nodes collected from various locations throughout beef carcasses were analyzed for *Salmonella.* Pulse field gel electrophoresis (PFGE) results indicated that some strains of *Salmonella* were more likely to colonize in different lymph nodes throughout the beef carcass. These data also offer a small glimpse into the current research question regarding the route of entry *Salmonella* takes to enter the lymphatic system of the animal, and eventually colonize within a lymph node. To summarize the findings of Gragg et al. *(37)*, *Salmonella* strains with like PFGE patterns were located in the mesenteric and subiliac lymph nodes within an animal, as well as in the mesenteric lymph nodes and fecal material from the same animals. Similar PFGE patterns were also seen among animals, but only in the mediastinal lymph nodes. The mediastinal lymph glands are known to drain the respiratory organs of the bovine *(54, 63)*. Therefore, the commonality seen among animals may indicate respiratory transmission of *Salmonella.* Route of entry is currently being further investigated by the United States Department of Agriculture – Agricultural Research Service, and some preliminary data regarding this topic were recently presented at a conference regarding pathogen control in beef processing *(44)*. These preliminary findings support the hypothesis that transdermal infection is a viable route of entry for *Salmonella* in the bovine lymphatic system. The transdermal challenge phase of this study was designed to simulate fly bites on the limbs or underline of cattle. *Salmonella* Montevideo was transdermally introduced to the lower forelimbs of cattle, and was subsequently recovered in the superficial cervical lymph nodes. The lower hindlimbs of cattle were

challenged with *Salmonella* Newport, which was later recovered in the popliteal lymph nodes. Finally, *Salmonella* Senftenberg was introduced to the underline of cattle, and recovery of *S.* Senftenberg was later made from the subliliac lymph nodes of the same cattle. An oral challenge was also designed as a component of this study. However, data from the oral challenge phase of this study was not as compelling as those from the transdermal challenge *(44)*. Data such as these, paired with veterinary knowledge of lymphatic drainage, are necessary in fully understanding the route of infection *Salmonella* follows. If route of entry can be determined, pre-harvest control measures can be implemented to reduce the prevalence of *Salmonella* in bovine lymph nodes.

CHAPTER III

EVALUATING THE IMPACT OF POST- HARVEST PROCESS CONTROLS AND INTERVENTION STRATEGIES USED TO REDUCE *ESCHERICHIA COLI* O157:H7

3.1. Introduction

Since the 1992 outbreak associated with *Escherichia coli* O157:H7 contaminated ground beef served at Jack-in-the-Box, the beef industry, researchers, and regulatory agencies have invested a significant amount of money and time trying to protect consumers by preventing and/or reducing *E. coli* O157:H7. While the industry has developed and implemented antimicrobial interventions to be applied at various steps during harvest and fabrication, it still struggles with *E. coli* O157:H7 recalls and outbreaks *(64, 65, 69, 71)*. More recently, USDA-FSIS released verification testing guidance for non-O157 shiga toxin-producing *E. coli* (STEC) and announced that establishments would be required to reassess their HACCP systems to address this hazard should positive test results be found *(68)*. These decisions were driven, in part, by increasing awareness of the non-O157 STEC organisms as a result of outbreak *(30, 31)* and recall *(67)* occurrences. Due to the number of recalls and illnesses associated with *E. coli* O157:H7 over the past couple of years, as well as the increased concern with non-O157 STEC serogroups, it is imperative that a more intensive and coordinated effort be taken to reduce the public health risks associated with beef products. Researchers often focus on a single sector, such as harvest, rather than looking at the total system

from pre-harvest, through harvest and to further processing establishments. Multiple variables (e.g., incoming microbial load, dressing procedures, types of interventions being applied, processing and sanitation practices) may impact the contamination levels on the finished products. The overall goal of this study was to utilize indicator microorganisms to evaluate process control during the beef harvest process. Through a cause-and-effect process, a series of trials were developed in an effort to achieve this goal. The trial objectives were: (1) to use indicator microorganisms validate the determination of microbial reductions by sampling leading and trailing beef sides, before and after an intervention, respectively; (2) to use indicator microorganism to predict pathogen reduction at various steps along the beef harvest process; and (3) to determine the effect of chlorine neutralization on the stability of hide swab samples.

3.2. Materials and Methods

The present study was performed in a series of five trials. Each trial was executed after a detailed meeting between the beef harvest facilities and the study investigators. Each meeting was utilized to discuss issues/questions that should be addressed in relation to the study objectives, sampling techniques, sampling locations (in-plant and carcass surface locations), and other decisions that were made following data analysis from each trial.

3.2.1. Trial #1

3.2.1.1. Planning Phase. After an initial meeting with collaborating investigators, it was determined that Trial #1 of this study would serve as the initial effort to validate the hypothesis that samples taken on leading and trailing beef carcass

sides, before and after an intervention, respectively, can be used to show microbial reductions (this will be referred to as the "leading/trailing reduction hypothesis" from this point forward). To test the leading/leading trailing reduction hypothesis, a single intervention employed at a commercial beef harvest facility was selected. This intervention was an acidified sodium chlorite (ASC) spray cabinet located prior to evisceration. Typical operating parameters for the acid used in this pre-evisceration cabinet were provided to us by management at the beef harvest facility and were defined as: 1200 ppm, 2.5 pH, and approximately 26.7°C (ambient temperature). This step in the processing line was chosen because it follows both hide removal and evisceration. Carcass contamination is known to be highest along sites of opening cuts, or sites where contact is made between the hide and the carcass surface during the hide removal process *(14)*.

3.2.1.2. Sample Collection. Sampling methods were similar to those detailed by Arthur et al. *(7)*. All sponges (3M, St. Paul, MN) were hydrated with 25 ml of buffered peptone water (BPW; BD Diagnostics, Spark, MD). Non-sterile latex gloves were worn at all times, and changed if contact was made with any surface (carcasses, equipment, sample sponge, etc.). Sponges were wrung-out in the bag to remove excess BPW, removed from the bag, and then used to swab the sample surface. When possible, samples were taken along or near the pattern opening lines of the beef carcasses. Sampling locations included the chuck and round both before and after the pre-evisceration cabinet. One sponge per carcass location (chuck and round) was used to swab a 100-cm^2 area near the pattern opening of six carcasses. Samples were taken by

making five horizontal passes with a sponge, flipping the sponge over, and utilizing the opposite side of the sponge to make an additional five vertical passes over the sample surface. Both leading and trailing sides were swabbed before and after the pre-evisceration cabinet (n = 48 total swab samples). To accommodate sample collection, the harvest line was briefly stopped for each sample. We acknowledge that stopping the processing line is not advantageous in any commercial setting, especially when samples are taken frequently or in large quantities. However, we were careful to ensure that the "down-time" on the line was kept at a minimum. Samples were stored in insulated containers with ice packs and transported to the Texas A&M University Food Microbiology Laboratory (College Station) for analysis within 6 h of sample acquisition.

3.2.1.3. Microbiological Analyses. Upon arrival in the laboratory, samples were pummeled by hand for 1 min. Pummeled samples were plated onto APC and *E. coli*/coliform Petrifilm plates (3M, St. Paul, MN) by using appropriate serial dilutions and pipetting 1 ml of sample onto the center of the bottom film. When necessary, a spreader was used over the top film of the *E. coli*/coliform Petrifilm plates to distribute the inoculum over the circular area before gel formed. Plates were incubated for 48 h at 25°C for APC and for 24 h at 35°C for *E. coli*/coliform. Following incubation, plates were counted.

3.2.2. Trial #2

3.2.2.1. Planning Phase. A meeting was held after Trial #1 to discuss methods to improve sample collection. Method improvement was important in order to prevent stopping the production line, and to obtain more useful microbiological counts. One

strategy that was discussed and implemented as part of Trial #2 was to increase the carcass sampling area. Further, an additional sample set was incorporated to determine what microbiological load might be present on the hides of the cattle entering the plant. The final consideration that was incorporated into Trial #2 was the use of Sponge-Sticks (3M) instead of conventional sponges to reduce time lost as a result of contaminated gloves, in turn, minimizing "down-time" on the production line.

3.2.2.2. Sample Collection. Sampling locations included the chuck and round both before and after the pre-evisceration cabinet. One Sponge-Stick (3M) per carcass location (chuck and round) was used to swab a 4,000-cm^2 area near the pattern opening of six carcasses. Samples were taken by making five horizontal passes with a Sponge-Stick (3M), flipping the Sponge-Stick (3M) over, and utilizing the opposite side of the to make an additional five vertical passes over the sample surface. Both leading and trailing sides were swabbed before and after the pre-evisceration cabinet (n = 48 total swab samples). These sampling areas can bee seen in Figure 3.1 *(7)*.

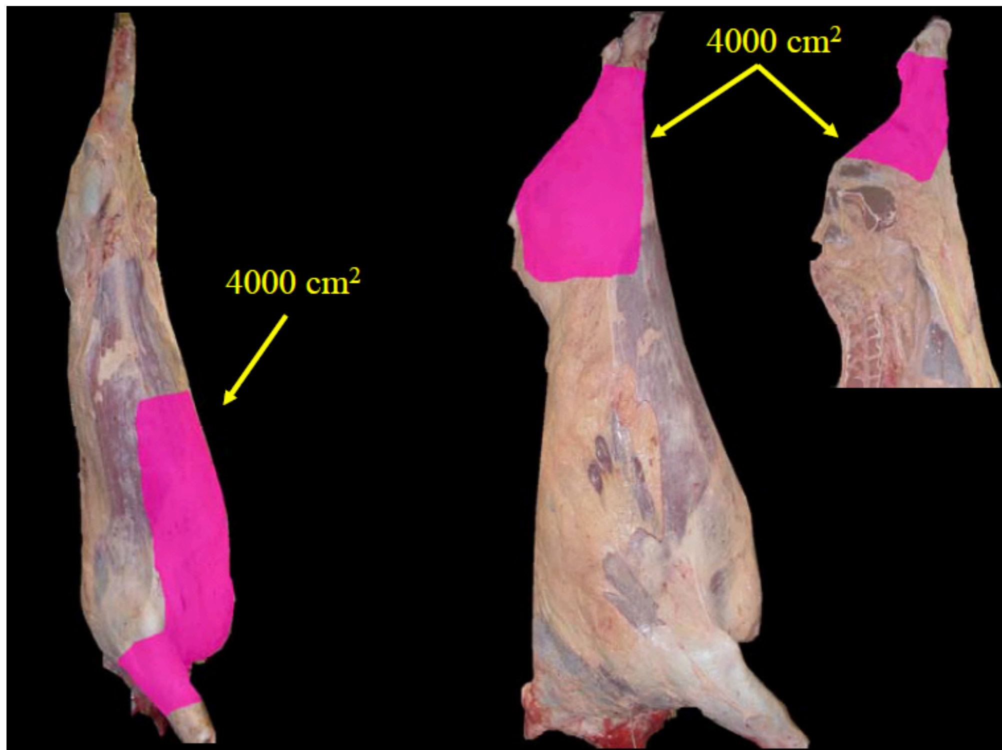

FIGURE 3.1. *Carcass sampling locations.* (Reprinted with permission from the Journal of Food Protection).

The additional set of samples for this trial consisted of 100-cm^2 hide samples (n = 4), taken from the brisket region, between the stunning and sticking steps of the harvest process. All samples were collected using a Sponge-Stick (3M) in the same manner described previously. Sponge-Sticks (3M) were hydrated with 25 ml of BPW (BD Diagnostics). Non-sterile latex gloves were worn at all times, and changed if contact was made with any surface (carcasses, equipment, sample sponge, etc.). Sponge-Sticks (3M) were wrung-out in the bag to remove excess BPW, removed from the bag, and then used to swab the sample surface. Carcass and hide swab samples then were stored in insulated containers with ice packs and transported to the Texas A&M University Food Microbiology Laboratory (College Station) for analysis within 6 h of sample acquisition.

3.2.2.3. Microbiological Analyses. Upon arrival in the laboratory, samples were hand-pummeled for 1 min. Pummeled samples were plated onto APC, *E. coli*/coliform, and *Enterobacteriaceae* Petrifilm plates (3M) by using appropriate serial dilutions and pipetting 1 ml of sample onto the center of the bottom film. When necessary, a spreader was used over the top film of the *E. coli*/coliform and *Enterobacteriaceae* Petrifilm plates to distribute the inoculum over the circular area before gel formed. Plates were incubated for 48 h at 25°C for APC and for 24 h at 35°C for *E. coli*/coliform and *Enterobacteriaceae.* Following incubation, plates were counted.

3.2.3. Trial #3

3.2.3.1. Planning Phase. After much discussion among study collaborators, the decision was made to take this trial in a slightly different direction. It was determined that the primary goal of Trial #3 was to evaluate the fluctuation of indicator microorganism counts on hide and carcass surfaces at various steps of the beef harvest process.

3.2.3.2. Sample Collection. Samples were taken only on the chuck area of five carcasses (n = 55 samples). For hide (n = 15) and carcass surface (n = 40) samples, a 4,000-cm^2 sampling area near the pattern opening was used. Swabs were taking with Sponge-Sticks (3M) in the same manner described previously. Eleven sampling locations within the harvest plant were necessary to execute the objective of this trial. The locations were the following: Before water rinse (between stunning and sticking); before hide-on wash cabinet; after hide-on wash cabinet (calcium hypochlorite); before pre-evisceration cabinet; after pre-evisceration cabinet (ASC); before carcass trimming;

rail-out station; after carcass trimming (zero tolerance); before the lactic acid/hot water cabinet; after the lactic acid/hot water cabinet; and, carcasses in the cooler after a 24 h chill. Operating parameters were collected on all applicable interventions and processing aides. These steps included: lactic acid cabinet, hot water wash, acidified sodium chlorite, and a hide-on calcium hypochlorite wash.

3.2.3.3. Microbiological Analyses. Upon arrival in the laboratory, samples were hand-pummeled for 1 min. Pummeled samples were plated onto APC, *E. coli*/coliform, and *Enterobacteriaceae* Petrifilm plates (3M) by using appropriate serial dilutions and pipetting 1 ml of sample onto the center of the bottom film. When necessary, a spreader was used over the top film of the *E. coli*/coliform and *Enterobacteriaceae* Petrifilm plates to distribute the inoculum over the circular area before gel formed. Plates were incubated for 48 h at 25°C for APC and for 24 h at 35°C for *E. coli*/coliform and *Enterobacteriaceae.* Following incubation, plates were counted.

3.2.4. Trial #4

3.2.4.1. Planning Phase. Following the review of data from Trial #3, Trial #4 was designed to test the "leading/trailing reduction hypothesis," and to quantify indicator microorganisms on hide and carcass surfaces at different steps on the slaughter line. However, a second beef harvest establishment was used for this trial. By sampling at a second establishment, we were able to compare incoming microbiological loads and gather additional data (i.e. amount of time a carcass is exposed to each intervention).

3.2.4.2. Sample Collection. Using the swabbing techniques outline above, Sponge-Sticks (3M) were used to take 4,000cm^2 samples (n = 70) from the chuck and

round areas of five carcasses, near the pattern opening. In addition to collecting leading/trailing carcass samples at the pre-evisceration cabinet, nine sampling locations were utilized at the second establishment for this trial. The nine locations were: hide-on (before the hide puller); at the hide puller (hide split and folded back); before the pre-evisceration cabinet; after the pre-evisceration cabinet (hot water); before split carcass wash; after split carcass wash (room temperature water); after the lactic acid/hot water cabinet; carcasses in the cooler after a 24 h chill; and, the rail-out station. Operating parameters were collected on all applicable interventions and processing aides. These included: lactic acid/hot water cabinet, water wash, and hot water cabinet. All samples were stored in insulated containers with ice packs and transported to the Texas A&M University Food Microbiology Laboratory (College Station) for analysis within 6 h of sample acquisition.

3.2.4.3. Microbiological Analyses. Upon arrival in the laboratory, samples were pummeled by hand for 1 min. Pummeled samples were plated on APC, *E. coli*/coliform, and *Enterobacteriaceae* Petrifilm plates (3M) by using appropriate serial dilutions and pipetting 1 ml of sample onto the center of the bottom film. When necessary, a spreader was used over the top film of the *E. coli*/coliform and *Enterobacteriaceae* Petrifilm plates to distribute the inoculum over the circular area before gel formed. Plates were incubated for 48 h at 25°C for APC and for 24 h at 35°C for *E. coli*/coliform and *Enterobacteriaceae.* Following incubation, plates were counted.

3.2.5. Trial #5

3.2.5.1. Planning Phase. The objective of Trial #5 was to narrow our focus to the hide-on calcium hypochlorite (Accutab, Monroeville, PA) cabinet located at the first beef slaughter establishment, and to neutralize any residual calcium hypochlorite that may have negatively impacted our samples. The compound sodium thiosulfate was selected as the neutralizing agent for this trial since it is a compound known to react with weak acids, and is commonly used to de-chlorinate aquariums and swimming pools. When chlorine is introduced to water, it quickly hydrolyzes to form hypochlorous acid, with which sodium thiosulfate reacts. The neutralizing reaction between sodium thiosulfate and hypochlorous acid yields disodium sulfate, sulfur, and hydrochloric acid. Sodium thiosulfate further reacts with hydrochloric acid to produce the final products: sodium chloride, water, sulfur, and sulfur dioxide gas.

3.2.5.2. Sodium Thiosulfate Preparation. Sodium thiosulfate solution was prepared by dissolving 160 mg sodium thiosulfate to 1 L of deionized water. Following the addition of 25 ml BPW to wet each sponge, the sodium thiosulfate solution was added to each sample bag at one of three levels (0 ml, 0.1 ml, 1.0 ml). Five samples bags were prepared for each level of sodium thiosulfate solution, and each bag was hand-massaged to mix the solutions.

3.2.5.3. Sample Collection. Hides were sampled before the water hose rinse (n = 5; dry hide surface), after the calcium hypochlorite cabinet (n = 15; wet hide surface), and at the hide puller (n = 10; hides were removed, unrolled, then sampled on the skin side). Due to restricted working space, sample surface areas varied. Again, Sponge-

Sticks (3M) were used and samples were stored in insulated containers with ice packs and transported to the Texas A&M University Food Microbiology Laboratory (College Station) for analysis within 6 h of sample acquisition.

3.2.5.4. Microbiological Analyses. Upon arrival in the laboratory, samples were hand-pummeled for 1 min. Pummeled samples were plated on APC, *E. coli*/coliform, and *Enterobacteriaceae* Petrifilm plates (3M) by using appropriate serial dilutions and pipetting 1 ml of sample onto the center of the bottom film. When necessary, a spreader was used over the top film of the *E. coli*/coliform and *Enterobacteriaceae* Petrifilm plates to distribute the inoculum over the circular area before gel formed. Plates were incubated for 48 h at 25°C for APC and for 24 h at 35°C for *E. coli*/coliform and *Enterobacteriaceae.* Following incubation, plates were counted.

3.3. Results and Discussion

3.3.1. Trial #1

Results from pre-intervention samples returned *E. coli/*coliforms counts that were below the level of detection (less than one colony-forming unit (CFU) per plate). APC counts varied widely; however, mean log values for APC counts prior to the ASC application were 1.9 and 1.4 log CFU/cm^2 for round and chuck samples, respectively. After the ASC cabinet, all APC and coliform counts were below the level of detection. Due to the variation in colony counts before the intervention, no determination could be made as to the adequacy of the leading/trailing reduction hypothesis in displaying microbial reductions as a result of using the pre-evisceration cabinet at this beef processing facility.

3.3.2. Trial #2

Like Trial #1, almost all carcass surface counts from Trial #2 were below detectable levels. Again, these data do not allow us to facilitate any discussion on the leading/trailing reduction hypothesis. However, mean hide counts were 6.1, 4.0, 4.1, and 4.2 log CFU for APC, *E. coli*, coliforms, and *Enterobacteriaceae*, respectively. Although hide counts were not as numerous as expected, these data confirm that microorganisms are in fact entering the beef harvesting facility on the hide of the animals. These findings lend themselves to the notion that this particular establishment may have well-practiced sanitary dressing procedures, effective interventions and processing aides, or some combination thereof.

3.3.3. Trial #3

Hide data from Trial #3 reiterate the implication of similar results from Trial #2. APC results were too numerous to count (TNTC) for other hide-on samples taken before the hide-on chlorine cabinet. Following the hide-on chlorine cabinet, APC counts were approximately 4.6 log CFU/cm^2. Most of the carcass surface samples resulted in negative plate counts for all organisms, indicating undetectable counts (less than one CFU per plate). Of the 40 carcass surface samples tested, only two samples had APC counts greater than 1 log CFU/cm^2 (1.7 and 1.9 log CFU/cm^2). This was consistent with the fact that these carcasses were railed out due to fecal contamination on the neck region of the carcass. Hide-on values for *E. coli* were 5.9, 6.2, 3.1 log CFU/cm^2 for pre-hose rinse (dry hide), post-hose rinse (wet hide), and post- calcium hypochlorite cabinet (hide on), respectively. Hide-on values for coliforms were 6.0, 6.3, 3.2 log CFU/cm^2 for

pre-hose rinse, post-hose rinse, and post-chlorine cabinet, respectively. Carcass surface samples produced *Enterobacteriaceae* counts that were below the level of detection. However, hide-on counts were 6.3, 6.5, and 3.5 log CFU/cm^2 for pre-hose rinse, post-hose rinse, and post-calcium hypochlorite cabinet, respectively. These hide data show that while the water hose rinse did not provide a reduction in counts, the calcium hypochlorite cabinet provided roughly a 3 log CFU/cm^2 reduction for *E. coli*, coliforms, and *Enterobacteriaceae*. Although we did not find sufficient bacterial counts to evaluate the distribution of indicator microorganisms along the beef processing line, we were able to see the impact of the calcium hypochlorite cabinet in providing a substantial decrease in the incoming microbiological load on the hides.

3.3.4. *Trial #4*

Microbiological counts from the second establishment provided more insight into the reduction of microorganisms throughout the beef slaughter system (Table 3.1). Although APCs were the only bacterial counts detectable at almost every production step, changes throughout the process can be understood. Like previous trials, these data were not robust enough to provide a clear comparison for the leading/trailing reduction hypothesis. Another aspect of these data is the incoming microbial load seen with the hide-on counts. On the day Trial #4 was conducted, the second establishment had an average incoming hide-on APC load of 8.1 log CFU/cm^2, compared to the first establishment that had 6.1 and 5.9 log CFU/cm^2 APC for Trials #2 and 3, respectively. The first establishment exhibited slightly higher hide-on counts for *E. coli,* coliforms, and *Enterobacteriaceae* during trial #3 than did the second establishment (Table 3.1).

Overall, incoming hide-on microbiological counts at the second establishment were lowered ($P < 0.05$) by hide removal. After all interventions were applied to post hide removal carcass surfaces, microbiological counts were again lowered ($P < 0.05$). An increase ($P < 0.05$) in APC recovery can be noted after carcasses endured a 24 h chill. This increase in APC recovery may be due to carcass handling, carcass contact with establishment surfaces, or slight differences in sampling location.

Table 3.1. *Least-squares means[a] of plate counts (log CFU/cm²) for APC[b],* E. coli/*coliforms, and* Enterobacteriaceae *by processing location for Trial #4.*

	APC	*E. coli*	Coliforms	*Enterobacteriaceae*
Processing Location				
Hide on	8.1 A	4.1 A	4.2 A	5.3 A
At hide puller (rolled hide)	7.7 A	4.1 A	4.2 A	4.8 A
Before pre-evis cabinet	4.5 B	1.9 B	1.9 B	2.1 B
After pre-evis cabinet	4.0 BC	1.0 B	1.0 B	1.2 B
Before split carcass wash	4.0 BC	1.3 B	1.3 B	1.3 B
After split carcass wash	3.5 BC	< 1.0 B	< 1.0 B	< 1.0 B
After lactic/hot water cabinet	< 1.0 D	< 1.0 B	< 1.0 B	< 1.0 B
Cooler	3.1 C	< 1.0 B	< 1.0 B	< 1.0 B
Rail-outs	4.4 BC	1.3 B	1.3 B	2.2 B

[a] Least-squares means within a column lacking a common letter differ ($P < 0.05$).
[b] APC = Aerobic Plate Count

3.3.5. Trial #5

The results from Trial #5 (Table 3.2) display that using sodium thiosulfate to neutralize residual calcium hypochlorite did not alter the recovery of the microorganisms evaluated. This finding is different than anticipated; higher microbiological counts are expected with the use of this neutralizer. Incoming loads (before the water hose rinse) are comparable to other trials at the first establishment. Trial #5 also produced post-calcium hypochlorite cabinet samples that are comparable with other trials at the same

establishment. It appears based on data from this trial and Trials 1-3, that effective interventions/processing aides or sanitary dressing procedures at the first establishment are preventing transfer of microorganisms from the hide to the carcass throughout the slaughter process.

Table 3.2. Least-squares *means*[a] *of plate counts (log CFU/cm*^{2}*) for APC*[b]*,* E. coli/*coliforms, and* Enterobacteriaceae *by hide sample type for Trial #5.*

	APC	*E. coli*	Coliforms	*Enterobacteriaceae*
Hide sample location				
Before water hose rinse[c]	7.7 A	5.3 A	5.4 A	4.6 A
Post hide cabinet[d] – 0 ml $Na_2S_2O_3$[e]	6.7 AB	4.2 AB	4.4 AB	3.7 A
Post hide cabinet – 0.1 ml $Na_2S_2O_3$	5.8 B	3.1 B	3.2 B	3.5 A
Post hide cabinet – 1.0 ml $Na_2S_2O_3$	6.0 B	3.8 AB	3.9 AB	3.5 A
At the hide puller[f]	6.1 B	3.1 B	3.2 B	2.2 A

[a] Least-squares means within a column lacking a common letter differ ($P < 0.05$).
[b] APC = Aerobic Plate Count
[c] Samples taken before the water hose rinse were of dry hides.
[d] Post hide cabinet samples were taken after the hide-on calcium hypochlorite wash cabinet.
[e] $Na_2S_2O_3$ = Sodium thiosulfate; $Na_2S_2O_3$ was used as to neutralize calcium hypochlorite that may have been present after sampling.
[f] At this station the hide was removed, unrolled, and sampled on the skin side.

Data from the present study support the concept that post-harvest practices may impact pathogen contamination on beef carcasses. Data from both establishments demonstrated incoming bacterial loads; however, the resulting carcass surface levels were below detection in only one establishment. Rhoades et al. *(53)* noted *E. coli* O157:H7 prevalence on hides ranged from 7.3 to 76.0%. Subsequently, chilled carcasses surfaces and raw beef products had mean *E. coli* O157:H7 prevalence rates of 0.3 and 1.2%, respectively. Such decreases in prevalence from hide to carcass surfaces further support the effectiveness of sanitary dressing procedures. Based on data from our study, variances in sanitary dressing practices and other in-plant interventions may

have contributed to the differences seen between the two establishments evaluated. Dixon et al. *(33)* compared strict sanitary and conventional procedures for the harvest, dressing, and fabrication of beef cattle. When compared to conventional methods, mean APCs were found to be lower on carcass and subprimal surfaces that were processed using strict sanitary practices. Further, steaks resulting from subprimals handled under strict sanitary conditions were found to be more desirable than those handled using conventional methods. Results from a similar study by Chandran et al. *(32)* demonstrated that while steaks produced under strict sanitary conditions did not differ in APCs when compared to conventional practices, more desirable sensory attributes were noted for steaks produced under strict sanitary practices. These data reiterate the impact and importance of well-practiced sanitary dressing procedures and should encourage all beef processing establishments to evaluate their processes for areas of improvement.

CHAPTER IV

SALMONELLA PREVALANCE IN BOVINE LYMPH NODES DIFFERS AMONG FEEDYARDS

4.1. Introduction

The current study evolved from an effort to identify the possible cause of periodic increases of *Salmonella* prevalence in a commercial beef-processing establishment. After multiple years of tracking data including, carcass mapping, potential environmental factors, weather patterns, and other processing data, management within the establishment speculated that the feedyard source of cattle was related to *Salmonella* prevalence. After monitoring *Salmonella* data over time, and focusing on how these data related to cattle origin, a potential for variation among feedyards was questioned. With limited data available in this field of research, the objective of the present study was designed to determine if *Salmonella* prevalence in bovine lymph nodes varied among feedyards.

4.2. Materials and Methods

4.2.1. Sample collection

Three hundred and seven bovine lymph nodes were obtained from beef carcasses at a commercial beef-processing establishment. Four collections trips were conducted over a three-month span. Each collection trip was designed to obtain lymph nodes from cattle originating from pre-selected feedyards. The superficial cervical (n = 279) and iliofemoral (n = 28) lymph nodes were analyzed for this study. The first collection trip

was organized to obtain a total of 57 bovine lymph nodes (*n* = 29 superficial cervical; *n* = 28 iliofemoral) from four different feedyards. It should be noted that the intent was to collect and analyze 60 lymph nodes; however, three lymph nodes were contaminated and excluded from analysis. Based on internal data collection conducted by management at the beef harvest establishment, one feedyard was identified as the primary feedyard of concern (termed feedyard F for the purpose of this study). As a result, feedyard F was sampled more heavily (*n* = 14 superficial cervical and *n* = 14 iliofemoral) than the other three feedyards chosen at random (feedyard A: *n* = 5 superficial cervical and *n* = 4 iliofemoral; feedyards B and G: *n* = 5 superficial cervical and *n* = 5 iliofemoral, per yard). Superficial cervical lymph nodes were excised from unchilled carcasses that had been transferred from the harvest floor to the blast-chill cooler. Iliofemoral lymph nodes were collected from chilled carcasses during fabrication (approximately 24 to 48 h postmortem). Following excision, fat-encased lymph nodes were placed in labeled Whirlpak bags (Nasco, Modesto, CA) and transported for processing to the Texas A&M University Food Microbiology Laboratory (College Station, TX) in an insulated container with refrigerant packs. Upon arrival in the laboratory, lymph nodes were removed from the insulated container and stored under refrigeration (4°C) until processing.

4.2.2. Sample processing

All lymph nodes (*n* = 307) were aseptically trimmed free of fat and flame-sterilized within 24 h of collection by first immersing the entire fat-encased lymph node in 95% ethanol and flame sterilizing the outside surface. Subsequently, fat was trimmed

using flame-sterilized scalpel and forceps. Following fat removal, the fully exposed lymph nodes were flame-sterilized by dipping in 95% ethanol and flaming as described above. For the set of lymph nodes collected during the first collection trip (*n* = 57), a flame-sterilized scalpel and forceps were used to aseptically pulverize samples by mincing each lymph node to expose the interior node tissue. Because of laboratory constraints, lymph nodes from the first collection trip were the only samples pulverized and analyzed at the Texas A&M University Food Microbiology Laboratory (College Station). All other samples were processed as described above to aseptically extract the fat-encased lymph node, then individually packaged in Whirlpak bags (Nasco) and transported in an insulated container with refrigerant packs to the USDA-ARS Food and Feed Safety Research Center (College Station, TX) for pulverization and analysis. Upon receipt of samples at the USDA-ARS Center, each lymph node was aseptically transferred to a filtered Whirlpak bag (Nasco) and pulverized with a rubber mallet.

4.2.3. Prevalence determination

Lymph nodes excised on the first collection trip were analyzed at the Texas A&M University Food Microbiology Laboratory using a fully automated VIDAS system (bioMérieux, Hazelwood, MO) *(4)*. This system utilizes an enzyme-linked fluorescent assay method based on the specific phage capture technology, and replaces traditional enrichment methods. Both motile and non-motile *Salmonella* can be detected. Briefly, each minced lymph node was placed in a sterile stomacher bag with 225 ml buffered peptone water (Difco, BD, Sparks, MD) and 1 ml of SPT (*Salmonella* Phage Technology) supplement containing brilliant green and novobiocin (bioMérieux).

Samples were pummeled for 1 min using a Stomacher-400 (Tekmar Company, Cincinnati, OH), and subsequently incubated at 41.5°C for 22 to 26 h. A 0.5 ml aliquot of each enriched sample was introduced to a VIDAS SPT *Salmonella* strip (bioMérieux) containing pre-dispensed reagents. Inoculated strips were heated for 5 min at 100°C using a VIDAS Heat and Go unit (bioMérieux) and then allowed to cool for 10 min. Prepared trips then were placed into a VIDAS automated immunoanalyzer (bioMérieux) for analysis within 48 min. Positive samples were those with a test value (TV) ≥ 0.25. Presumptive positive cultures then were confirmed by isolation on ChromID *Salmonella* (bioMérieux) and incubated for 24 h at 37°C. Light pink colonies were confirmed as *Salmonella* according to a USDA Food Safety and Inspection Service method *(66)*, by streaking onto triple sugar iron agar and lysine iron agar (Difco, BD) slants. Tubes were incubated at 35°C for 24 h and those isolates indicating typical *Salmonella* were further confirmed by a combination of biochemical and serological procedures. Serological confirmation of the isolates was tested with polyvalent O antiserum reactive with serogroups A through I and Vi (Difco, BD). Those isolates that were positive for agglutination also were further confirmed biochemically using API 20 E (bioMérieux) strips.

Lymph nodes analyzed at the USDA-ARS Center were prepared by adding 100 ml of tetrathionate broth (Difco, BD) to each filtered Whirlpak bag (Nasco) and hand massaging the mixture for approximately 1 min before incubation (24 h at 37°C). Following incubation, 100 µl of the above enrichment were transferred to 5 ml of Rappaport-Vassiliadis broth (Difco, BD) and incubated an additional 24 h at 42°C. Ten

μl of this enrichment was streaked onto brilliant green agar (Difco, BD) containing 25 μg/ml novobiocin and incubated for 24 h at 37°C. Suspect colonies were picked from the agar and transferred to triple sugar iron slants (Difco, BD) and positive slants were further confirmed as *Salmonella* using slide agglutination with *Salmonella* anti-serum (Difco, BD).

4.3. Results and Discussion

After reviewing the results from the first collection, two interesting findings were noted. Of the four feedyards sampled, cattle from feedyard A returned 0% *Salmonella* positive samples in both the superficial cervical and iliofemoral lymph nodes. Contrastingly, results from cattle out of feedyard B were 100.0% positive for superficial cervical lymph nodes and 80% positive for iliofemoral lymph nodes, for a cumulative percentage of 88.2% positive lymph nodes (Table 4.1). Cattle from the feedyard initially identified by the establishment as the primary source of concern (feedyard F) returned 42.9% positive lymph node samples (Table 4.1).

TABLE 4.1. *Percentage of* Salmonella-*positive lymph nodes by feedyard for each collection trip cumulatively.*

	% (no. positive/no. tested) *Salmonella*-positive lymph nodes				
Feedyard	Collection #1	Collection #2	Collection #3	Collection #4	Total
A	0.0 (0/9)	0.0 (0/25)	0.0 (0/25)	0.0 (0/25)	0.0 (0/84) C[b]
B	90.0 (9/10)	100.0 (25/25)	76.0 (19/25)	88.0 (22/25)	88.2 (75/85) A
C	NC[a]	40.0 (10/25)	NC	NC	40.0 (10/25) B
D	NC	8.0 (2/25)	0.0 (0/25)	NC	4.0 (2/50) C
E	NC	NC	24.0 (6/25)	NC	24.0 (6/25) B
F	42.9 (12/28)	NC	NC	NC	42.9 (12/28) B
G	40.0 (4/10)	NC	NC	NC	40.0 (4/10) B

[a] NC, no lymph nodes were collected from these feedyards on these collection trips.
[b] Percentages within a column lacking a common letter differ ($P < 0.05$).

A second trip was conducted to collect 25 superficial cervical lymph nodes each from cattle out of feedyards A and B, and two additional feedyards (termed “feedyard C” and “feedyard D”). Results from the second trip were again 0% positive for lymph nodes from cattle out of feedyard A and 100.0% positive for lymph nodes from cattle fed in yard B. Feedyards C and D, which were not sampled during the first collection trip, had 40.0% and 8.0% prevalence of *Salmonella* in bovine lymph nodes, respectively. These results emulated those of the first collection trip, providing evidence that there was a clear distinction between feedyards with regards to *Salmonella* prevalence n bovine lymph nodes, although the reason at this time may be unclear.

A third collection was made a month later to determine if the apparent difference in *Salmonella* prevalence of lymph nodes from feedyards could be repeated. Management at the beef harvest establishment pre-selected the feedyards to be sampled. Again, a total of 100 lymph nodes were collected from feedyards A, B, D, and a yard not previously sampled, feedyard E. Samples from cattle fed in yards A and D were found to be 0.0% positive and feedyard B lymph nodes were 76.0% positive for *Salmonella*. With the clear distinction between feedyards A and B being repeated, and an additional yard (feedyard D) exhibiting 0% prevalence of *Salmonella* in lymph nodes of cattle, researchers began to inquire as to what contribution, if any, the country of origin of the cattle may offer.

To address differences in prevalence due to country of origin, a fourth and final collection was made. The final collection consisted of 25 lymph nodes each from cattle fed in yards A and B. This collection focused on cattle solely of Mexican origin,

whereas all other collections were made from cattle of United States origin. Similar results were seen (feedyard A: 0.0% positive; feedyard B: 88.0% positive), further exemplifying the potential influence of feedyard on *Salmonella* prevalence of lymph nodes.

Cumulative totals for percentage of Salmonella positive lymph nodes across collections can be seen in Table 4.1. Salmonella prevalence a feedyards A and D did not differ (P = 0.0735). However, feedyards A and D were found to have a lower (P < 0.05) Salmonella prevalence than all other yards surveyed. It can also be noted in Table 3.1 that feedyard B had a higher (P < 0.05) prevalence of Salmonella in bovine lymph nodes than all other yards.

To date, few researchers have investigated *Salmonella* prevalence in peripheral lymph nodes with minimal focus on fed cattle. Koohmaraie et al. *(40)* collected hide, carcass, peripheral lymph node, and ground beef samples from dairy cattle to determine *Salmonella* prevalence. *Salmonella*-positive test results were obtained from 96.0% of hides, 47.0% of pre-intervention carcass surfaces, 18.0% of lymph nodes, 7.14% of trimmings, and 1.67% of ground beef samples. These hide and carcass data are comparable with those documented by Bosilevac et al. *(17)* in which 91.0% of hides and 58.0% of pre-evisceration carcasses were positive for *Salmonella.* Barkocy-Gallagher et al. *(12)* presented slightly lower prevalence rates of *Salmonella* on hides and pre-evisceration carcasses, at 71.0 and 12.7% positive samples, respectively. Fegan et al. *(36)* noted a decline in *Salmonella* positive samples from 68.0% on hides to 2% on pre-chill carcass surfaces. A similar study by Rhoades et al. *(53)* found 60.0, 1.3, and 3.8%

prevalence of *Salmonella enterica* on hide, chilled carcass, and raw beef product samples, respectively. Gragg et al. *(37)* analyzed peripheral and mesenteric lymph nodes were extracted from cattle at a Mexican slaughter facility and analyzed for *Salmonella* prevalence. Of the lymph nodes extracted that are commonly destined for ground beef applications, 76.5% were found to be positive for *Salmonella.* In a separate study, Bosilevac et al. *(19)* analyzed ground beef samples for *Salmonella* prevalence and found 4.2% positive samples. This result is slightly higher than the 1.67% of *Salmonella* positive samples found more recently by Koohmaraie et al. *(40)*. Although hide prevalence of *Salmonella* is high, substantial decreases are seen throughout beef processing, from harvest onto fabrication and grinding.

The present study provides the basis for a variety of other research questions. Subsequent research has been initiated to investigate the reasons such distinct differences in *Salmonella* prevalence were seen among feedyards. Specific items for consideration may include: cattle type and temperaments, cattle stress levels, pen conditions, feeding regimes, veterinary care, pre-harvest interventions employed, etc. Of greatest importance will be the investigation of practices and environmental factors that may be contributing to the complete absence of *Salmonella* in the lymph nodes of cattle from feedyard A, versus continued presence of this pathogen in cattle from other feedyards.

CHAPTER V

POSSIBLE ENVIRONMENTAL INDICATORS LEADING TO DIFFERENCES IN *SALMONELLA* PREVALANCE IN BOVINE LYMPH NODES AMONG FEEDYARDS

5.1. Introduction

Research related to microbiological baseline data of feedyard environments is limited. Available studies were designed to focus on the presence of anaerobic bacteria *(50)* and *Salmonella (35)* in fecal material of feedlot cattle, not environmental factors surrounding the cattle in those feedlots. Some recent research has been conducted in an effort to determine a relationship between fecal presence of *Salmonella* in feedlot cattle and associated *Salmonella* prevalence in the lymph nodes of those cattle following harvest at a slaughter facility in Mexico *(37)*. Generally speaking, fecal presence of *Salmonella spp.* in the United States feedyard environments is highly variable, but is heavily influenced by comingling of the cattle *(35, 51)*. The impact of comingling on bacterial prevalence has also been evaluated with regards to transportation of feedlot cattle to harvest facilities *(51)*. The current study was conducted in two phases; the first phase involved surveys conducted by face-to-face interviews with the management personal and visual inspections of feedyards. Designed as subsequent research to those data presented in the previous chapter of this document, feedyards A, B, D, and F were selected for the interview phase of the current study. The second phase involved sampling and testing the feedyard environment to determine the prevalence of

Salmonella prevalence in the feedyard environment, as well as to provide some insight on available routes of infection.

5.2. Materials and Methods

5.2.1. Phase I

Interviews were conducted in a verbal question-and-answer format between Texas A&M University faculty and management personnel at each of four feedyards (A, B, D, F). A Texas A&M University staff member transcribed interview responses from feedyard management onto a survey form (Appendix A). The survey form contained questions regarding incoming cattle, cattle management practices, feed composition, pen management, pest control programs, and unique feedyard attributes. The questions on the survey form were designed to identify differences in cattle type, care, and management among yards. Following each face-to-face interview, feedyard management provided researchers with a tour of the feedlot. The tours allowed researchers to visually assess cattle condition, pen condition, feed bunk and water trough configuration, and prevalence of flies. This type of information was needed to fully understand what environmental challenges or benefits cattle may face prior to arrival at each yard, and during their feeding period.

5.2.2. Phase II

5.2.2.1. Sample Collection. A total of sixty environmental samples were obtained from three feedyards located in southern Texas. Upon arrival at each feedyard, five pens were selected for sampling. These pens contained finished cattle that were being prepared for shipment to a commercial harvest facility within a week of sample

collection. From each pen, one sample each of feed, water, feces, and soil was collected. All samples were collected in sterile 50-ml centrifuge tubes (VWR, Radnor, PA). Feed (n = 5) samples were collected directly from the feed bunks located at the front of each pen. Water samples (n = 5) were obtained by submerging a closed centrifuge tube under water with gloved hands. The tube was then opened, allowed to fill, and re-sealed under water. This method was used to ensure that water samples did not contain only water from the surface of each trough. Soil samples (n = 5) were collected by filling one centrifuge tube with soil from four random locations within each pre-selected pen. Finally, fecal samples (n = 5) were obtained by monitoring each pen of cattle; when defecation was observed, the fecal material was immediately collected in a centrifuge tube. Following collection, all environmental samples (n = 60) were transported in an insulated container with refrigerant packs to a commercial laboratory (San Antonio, TX) for analysis.

5.2.2.2. Microbiological Analysis. All samples were analyzed using Assurance GDS for *Salmonella* (BioControl Systems, Bellevue, WA) *(3)*. This system utilizes automated nucleic acid amplification, and single enrichment media to provide results within 21 h. Briefly, in a sterile stomacher bag, each pulverized lymph node was diluted 1:10 in BPW and incubated at 35 to 38°C for 18 to 24 h. A 1.0 ml aliquot of each enriched sample was transferred in a sample block well containing Sample Concentrating Reagent (BioControl) and the sample block was agitated at 600 rpm for 600 s. Using a PickPen (BioControl) for immunomagnetic separation, samples were transferred through a Wash Solution (BioControl) to a suspension plate. For each

sample, 20 µl of the washed bead-bacteria then was transferred to an amplification tube containing 10 µl of polymerase buffer solution. Prepared amplification tubes were loaded in the Assurance GDS Rotor-Gene (BioControl). Upon completion of the assay, the Rotor-Gene program identified each test sample as positive, negative, or “no amp”. “No amp” readings indicated that amplification did not occur, and must be repeated.

Prior to cultural confirmation, enrichment of presumptive positive samples was conducted by incubation in BPW for 20 to 24 h at 35°C. A 0.1 ml aliquot of each enriched sample was transferred to 10 ml of Rappaport-Vassiliadis (RV) broth and subsequently incubated in a water bath at 42°C for 18-24 h. Ten µl of this enrichment was streaked onto brilliant green agar (Difco, BD) containing 25 µg/ml novobiocin and incubated for 24 h at 37°C. Suspect colonies were picked from the agar and transferred to triple sugar iron slants (Difco, BD) and positive slants were further confirmed as *Salmonella* using slide agglutination with *Salmonella* anti-serum (Difco, BD).

5.3. Results and Discussion

The primary goal of this phase was to identify any major management differences between feedyards A and B, to aide us in understanding the extremely low (feedyard A) and high (feedyard B) prevalence of *Salmonella* in lymph nodes from cattle sourced from these yards. Generally, management practices in south Texas feedyards were found to be similar, with calves entering each of the feedyards weighing approximately 113.4 to 272.2 kg. In most cases, these calves were vaccinated and dewormed within two days of arrival; they were then re-vaccinated in seven to ten days, and turned out in a grass pasture. Calves remained in a grass pastures with a grain

supplement for 30 to100 d depending on incoming weight. Cattle then were moved to a feedyard pen where they are housed for the remainder of their time in the feedyard (200 to 280 d). Depending on the feedyard, cattle were limit fed until a body weight of 294.8 to 340.2 kg was achieved. Once calves weighed approximately 340.2 kg, they were placed on full feed with a finishing ration. Some minor differences were noted between yards with regards to fly populations, cattle type, feed ingredients, etc.

More noticeable differences were seen in feedyard size, pen crowding, and shades available to the cattle. All of the feedyard managers quoted similar feedyard capacity (10,000 to 15,000 head) during the interview phase of this study. Throughout the duration of our tours of the feedyards, and during environmental sample collection on a second visit, it was noted that feedyard D was substantially smaller than the other yards in terms of geographical square-footage. As a result, cattle at this feedyard seemed much more crowded than cattle at other yards. Another unique attribute of feedyard D was the complete absence of shades available to the cattle. Management informed us that they have increased issues with pen conditions and crowding when the shades are installed (Figure 5.1). Studies have shown that over-crowding, lack of shade, and heat stress is detrimental to both the cattle and to the profitability of the feedyards *(16, 51)*.   Therefore, the shades at feedyard D were removed while new shades were being designed to optimize space and minimize crowding. Other evident differences between feedyards were found in the designs and cleaning procedures of the water troughs at each yard.

FIGURE 5.1. *Cattle gathered under shades in a feedyard.*

None of the feedyards we visited utilized treated water, although all had some form of a trough-cleaning program in place. Feedyard A had the most well-established and consistently implemented trough-cleaning program, in addition to a unique trough design. Troughs at feedyard A (Figure 5.2) were shallow, and as a result were constantly re-filling with fresh water. Water troughs at this yard also were designed with a lever allowing each trough to easily be rotated and fully dumped for cleaning. Further, these troughs were only installed on the perimeter of each pen, preventing cattle from trying to climb, wallow, or congregate in and/or around the water troughs.

FIGURE 5.2. *Water trough design at feedyard A.*

At other feedyards, placement of the troughs within the pen varied, but all troughs were some variation of a stationary cement water receptacle. Water from Feedyard A was visibly cleaner than water samples from the other two feedyards; however, when water samples were analyzed, *Salmonella* prevalence in water from feedyards A and D, while low, did not differ (Table 5.1). Prevalence of *Salmonella* in water from feedyard F was the highest (Table 5.1).

TABLE 5.1. *Percentage of* Salmonella-*positive environmental samples by feedyard.*

Feedyard	% (no. positive/no. tested) *Salmonella*-positive environmental samples				
	Soil	Water	Feed	Feces	Overall
A	0.0 (0/5)	20.0 (1/5)	80.0 (4/5)	0.0 (0/5)	25.0 (5/20) B[a]
D	80.0 (4/5)	20.0 (1/5)	20.0 (1/5)	20.0 (1/5)	35.0 (7/20) B
F	100.0 (5/5)	80.0 (4/5)	20.0 (1/5)	60.0 (3/5)	65.0 (13/20) A
Total	60.0 (9/15)	40.0 (6/15)	40.0 (6/15)	26.7 (4/15)	41.7 (25/60)

[a] Percentages within a column lacking a common letter differ ($P < 0.05$).

Environmental sample collection was to be conducted at the same feedyards (feedyards A, B, D, F) we visited previously for interview purposes; however, we were unable to enter feedyard B due unforeseen circumstances. Therefore, environmental results for feedyards A, D, and F can be seen in Table 5.1. Feedyards A and D did not differ ($P > 0.05$) in total *Salmonella*-positive environmental samples. Feedyard F had more ($P < 0.05$) *Salmonella*-positive environmental samples than the other two yards. Based on data presented in the preceding chapter of this document, these findings are not surprising. However, more compelling findings can be noted when assessing *Salmonella* prevalence by sample type. Samples obtained from feedyard A that produced positive test results only included feed and water samples.

Such results are perplexing as cattle from this particular feedyard are ingesting the *Salmonella* with the feed and do not seem to be shedding the organism in their feces. One plausible explanation may be that the level of *Salmonella* in the feed is not sufficient to be identified in the feces. Feed has traditionally been identified as a vector for transmission of *Salmonella* in swine production. However, when *Salmonella* serotypes are identified in the animal, they rarely correlate with the serotypes found in the feed *(15)*. A recent study further supports the need to determine possible routes of infection *(37)*. Gragg et al. *(37)* found like serotypes of *Salmonella* in the mesenteric

and subiliac lymph nodes, and among feces and mesenteric lymph nodes. The same researchers also found similar serotypes in the mediastinal lymph nodes among animals. Such findings may suggest respiratory transmission among animals, in addition to possible oral and transdermal infection of individual animals *(44)*. This information may partially explain the relationship between the presence of *Salmonella* in feces and soil at feedyards D and F, and cattle from those yards having superficial cervical lymph nodes containing *Salmonella* (data presented in preceding chapter). The superficial cervical lymph nodes drain the areas of the forelimb in the same manner that the subiliac lymph nodes drain the areas of the hind limb *(54, 63)*. These lymph nodes can be observed in Figure 5.3 *(46)*. Transdermal infection of the lower limbs due to wallowing, cuts, scrapes, or insect bites could trigger an immune response in the superficial cervical and subiliac lymph nodes *(44)*. Although the full implications of these data may be unknown at this time, these findings warrant additional research in an effort to gain knowledge regarding environmental contributions to *Salmonella* presence or absence in bovine lymph nodes.

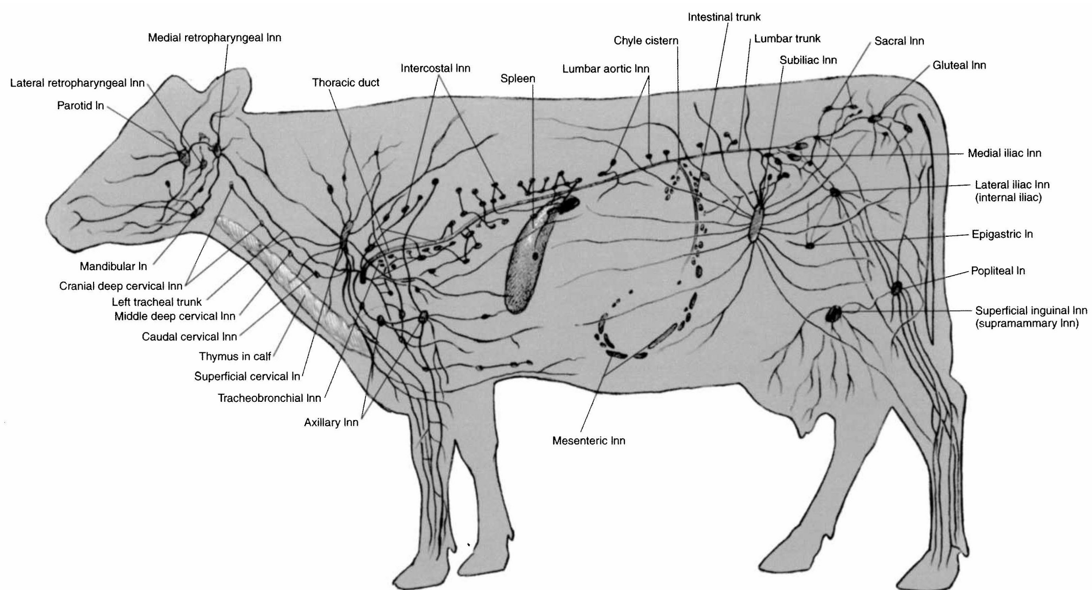

FIGURE 5.3. *Bovine lymphatic organs.* (Reprinted with permission from Wiley Publishing).

CHAPTER VI

SUMMARY AND CONCLUSIONS

Data from the present study support the concept that post-harvest practices may impact pathogen contamination on beef carcasses. Data from both establishments demonstrated incoming bacterial loads; however, the resulting carcass surface levels were below detection in only one establishment. Other researchers have evaluated process control with similar findings in decreased prevalence of indicator microorganisms and pathogens on cattle hides, carcass surfaces, and steaks. Results from the current and past studies support the need for effective sanitary dressing in maintaining process control during beef harvest and processing. Beef processing establishments could benefit by evaluating their current practices and implementing improved sanitary methods for harvest, dressing, fabrication, and further processing.

Data show a clear difference in *Salmonella* prevalence among feedyards, specifically feedyards A and B. The complete absence of *Salmonella* in the lymph nodes of cattle from feedyard A versus continued presence of this pathogen in cattle from other feedyards is certainly a novel finding. However, the reason for such a trend is not yet known. The initiation of additional research is crucial to the understanding of such distinct differences in *Salmonella* prevalence. Many aspects of *Salmonella* prevalence in bovine lymph nodes remain unknown. Route of entry and duration of *Salmonella* infection must be understood before preventative measures for managing the prevalence of this organism in lymph nodes destined for ground beef products would be possible.

Data such as these may help determine the possible influence of feedyard management practices and environmental factors that may be contributing to differences in *Salmonella* prevalence. The ultimate objective of this research would be to identify feedyard management practices that reduce *Salmonella* prevalence, and implement those practices industry wide.

Additional environmental samples from feedyards need to be collected before conclusive statements can be made regarding the data presented in Chapter V. Nonetheless, trends among environmental samples that tested positive for *Salmonella* spp. are visible. While the full implication of these findings not yet understood, additional research is warranted in an effort to assist the beef industry in producing the safest product possible.

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APPENDIX A

FEEDYARD SURVEY FORM

Date: ____________ Yard Name: _______________ Yard Location: ______________

I. Incoming Cattle

Type: Stocker Sale Barn Mexican Other: __________________

Distance traveled (miles): _________________ Travel duration: ___________________

Receiving program: ____

____

____

____

____

II. Cattle Management

Time in feedlot: ____

Implants: ____

Vaccinations: ____

____

Sub-therapeutic antibiotic use: ______________________________________

____

Other routine vet care: ____

____

Sorting Methods: ____

____

Prep for shipment: ____

____

Pathogen interventions: ____

____

Practices unique to this yard: ______________________________________

____

Treatment of sick cattle (handling, meds): ______________________

____

____

III. Feed

Typical rations: ______________________

____

____

Typical ingredients: ______________________

____

____

Seasonal ration adjustments: ______________________

____

Seasonal ingredient adjustments: ______________________

____

Feed additives used (i.e. β-agonists, ionophores): ______________________

____

IV. Pens

Feedlot capacity: ______________________

# of head/pen: ______________________

Mud/Dust levels: ______________________

____

Manure management: ______________________

____

Drainage: ______________________

____

Feeder/Waterer configurations (potential for contamination): ______________________

____

Water source/treatments: ____

____

General Condition: ____

____

____

V. Flies, Birds, Rodents

Fly control program: ____

____

____

Bird control issues/program: ____

____

____

Other notes on pests and rodents: ____

____

____

VI. Other

Unique yard attributes: ____

____

____

Practices unique to this yard: ____

____

____

Management challenges unique to this yard: ____

____