Salmonella White Paper

Beef Industry Food Safety Council
(BIFSCo)

January 2020

Revised Fall 2019

Original Publication, March 2016

Original Authors:

Devin L. Hanson, PhD, Josh J. Ison, PhD, Katelyn S. Malin, MS, and Hattie E. Webb, PhD

Executive Summary

Salmonella enterica is a member of the Enterobacteriaceae family and is closely related to *Escherichia co*li. *Salmonella* has long been known as a pathogen of humans and animals and was named after a U.S. veterinary microbiologist, Dr. Daniel E. Salmon. In humans, *Salmonella* causes two general forms of disease: typhoidal salmonellosis characterized by systemic disease following fecal-oral transmission and non-typhoidal characterized by acute gastroenteritis following consumption of contaminated food. Of relevance to the food industry are non-typhoidal *Salmonella* and these constitute the vast majority of salmonellosis cases in the U.S. Of concern in the U.S., the incidence of salmonellosis has not meaningfully changed over the past 20 years. Of all salmonellosis cases, approximately one-third are attributable to food produced under inspection by the Food Safety and Inspection Service of the United States Department of Agriculture (USDA-FSIS). Within this category, poultry is the primary vehicle of exposure. Overall, beef products account for approximately 10% of foodborne *Salmonella* cases.

Not only is *Salmonella* a pathogen of humans, it is also a pathogen of animals. While this zoonotic pathogen can result in high morbidity and animal wastage, much of the time, carriage among populations of food-producing animals can be asymptomatic. Moreover, in the southern high plains of the U.S., herd-level prevalence approaches 100% and animal-level prevalence is often greater than 50% compared to the northern high plains, where animal-level prevalence is frequently <1%.

Historically, the assumed route of carcass (and by extension, ground beef) contamination with *E. coli* O157:H7 and *Salmonella* was primarily through the hide. As such, pathogen reduction plans built on the principles of Hazard Analysis and Critical Control Points (HACCP) were designed to reduce hide-to-carcass contamination (as well as other sources of contamination), prevent cross-contamination, and reduce or, where possible, eliminate contamination on the surface. These plans effectively reduced surface and ground beef contamination with *E. coli* O157 by more than 90%. Moreover, the human incidence of *E. coli* O157 has also shown a decline temporally associated with the reduction of *E. coli* contamination in ground beef. Yet while surface contamination of *Salmonella* has similarly declined, the extent of reduction in ground beef contamination has not matched that observed for *E. coli* O157:H7. Moreover, the incidence of human disease has not meaningfully declined over time despite concerted efforts to affect change.

Recent work emerging from a number of universities, government, and private laboratories indicates that *Salmonella* in peripheral lymph nodes (PLNs) may be to blame for the discordant results between *E. coli* O157:H7 and *Salmonella* contamination levels in ground beef. Harborage in PLNs effectively protects *Salmonella* from surface decontamination efforts and, based on recently published risk assessment, appears to largely account for *Salmonella* contamination of ground beef.

While beef is a relatively uncommon source of salmonellosis in humans, recent findings of its harborage in PLNs point to the need for alternative approaches – potentially involving pre-slaughter strategies – to more effectively reduce ground beef contamination with this pathogen.

Table of Contents

List of Figures

List of Tables

List of Abbreviations

AMS	Agricultural Marketing Services
APC	aerobic plate count
CAPF	commercial agricultural production facility
CDC	Centers for Disease Control and Prevention
CFU	colony forming units
DFM	direct-fed microbial
E-beam	electron beam
EO	electrolyzed oxidized water
FDA	U.S. Food and Drug Administration
FOOD	Foodborne Outbreak Online Database
FoodNet	Foodborne Diseases Active Surveillance Network
FSIS	Food Safety Inspection Services
GIT	gastrointestinal tract
HACCP	Hazard Analysis and Critical Control Point
LOQ	limit of quantification
MDR	multi-drug resistant
NARMS	National Antimicrobial Resistance Monitoring System for Enteric Bacteria
NMV	non-mammalian vectors
NTW	not trimmed but washed
NTNW	not trimmed not washed
PLNs	peripheral lymph nodes
QALY	quality adjusted life years
SRP	siderophore receptors and porins
TMR	total mixed ration
TNW	trimmed but not washed
TW	trimmed and washed
USDA	United States Department of Agriculture
UV	ultraviolet

1 Introduction

Sa*lmonella* remains a persistent public health concern both in the U.S. and abroad. The majority of nontyphoidal salmonellosis cases are associated with foodborne vehicles. In foods of animal origin, poultry and eggs are invariably the most commonly implicated source of human exposure. Beef, in comparison a relatively uncommon source of exposure, is nevertheless occasionally attributed as the food source for various sporadic cases and outbreaks of disease. Since the implementation of pathogen reduction plans based on the principles of Hazard Analysis and Critical Control Points (HACCP) in the mid-1990s, the contamination of carcasses and ground beef with *Escherichia coli* O157:H7 has drastically declined. Yet, while the contamination of the surface of carcasses with *Salmonella* has similarly declined, the extent of reduction in ground beef contamination has not matched that observed for *E. coli* O157:H7. Moreover, the incidence of human disease has not meaningfully declined over time despite concerted efforts to affect change. Clearly more needs to be done but maybe not simply more of the same. The purpose of this white paper is to provide an update on *Salmonella* carriage in cattle and people, *Salmonella* control in slaughter establishments, and likely routes by which ground beef is contaminated, with the goal of focusing attention on those approaches that meaningfully reduce *Salmonella* in ground beef.

2 *Salmonella* and Public Health

2.1 *Background*

An estimate of the burden of disease associated with foodborne pathogens, such as *Salmonella enterica* (hereafter referred to as *Salmonella*), is crucial to a description of the magnitude of the associated public health concern. Moreover, such estimates can aid in the development of intervention strategies to reduce the incidence of salmonellosis in the human population. *Salmonella* has long been recognized as an important pathogen in human public health and is known for causing millions of cases of foodborne illness globally each year (Baird-Parker, 1990; Mead et al., 1999; Rodrique et al., 1990). The characteristic symptoms of salmonellosis in humans include diarrhea, fever, and abdominal cramps, which typically develop 12 to 72 hours after infection and last for four to seven days (CDC, 2014d).

Current estimates indicate that exposure to *Salmonella* results in 93.76 million illnesses worldwide each year (Majowicz et al., 2010). In the US, it is estimated that *Salmonella* is responsible for 1.028 million illnesses, 19,300 hospitalizations, and nearly 400 deaths annually (Scallan et al., 2015). USDA's Economic Research Service (ERS) estimates that the mean total annual cost of foodborne illness from *Salmonella* is approximately $3.7 trillion for all cases and outcomes (USDA-ERS, 2013). Despite these figures, the Centers for Disease Control and Prevention (CDC) reported only 42,000 laboratory-confirmed clinical cases of salmonellosis (CDC, 2012). This difference between estimates and reported cases is mainly the result of individuals that experience mild symptoms and forego medical care. It is estimated that approximately 85% of all human salmonellosis cases can be attributed to the consumption of contaminated foods (Doyle, 2013). Extensive laboratory confirmation of isolates, an accurate case definition, comprehensive case reporting, and

epidemiological modeling is necessary to produce more accurate estimates of foodborne illness for a specific pathogen within a population.

2.2 *Salmonella Surveillance Methods*

The CDC oversees a broad collection of surveillance systems designed to monitor the burden of many diseases within the U.S. population. One such monitoring system is The Foodborne Diseases Active Surveillance Network (FoodNet). FoodNet is a collaborative effort among CDC, 10 state health departments, the USDA-FSIS, and the U.S. Food and Drug Administration (FDA). FoodNet monitors approximately 15% of the U.S. population by collecting surveillance data for nine major pathogens commonly transmitted through food, including *Salmonella* (CDC, 2014a).

In a recent edition of the Morbidity and Mortality Weekly Report, Tack et al. (2019) reported the 2018 FoodNet findings citing 25,606 laboratory-confirmed cases of foodborne illness, which led to the hospitalization of 5,893 individuals and 120 deaths. In the 2018 FoodNet data, *Salmonella* was the second most commonly reported pathogen with 9,084 cases of illness (18.3 cases per 100,000 individuals), ranked behind *Campylobacter* which had 9,723 cases of illness reported*.* This was a 9% change in incidence rate compared with 2015-2017 data. The most commonly reported *Salmonella* serotypes by case were *Salmonella* serotype Enteritidis (*S*. Enteritidis: 2.6 per 100,000 individuals), *Salmonella* serotype Newport (*S*. Newport: 1.6), and *Salmonella* serotype Typhimurium (*S*. Typhimurium: 1.5) (Tack et al., 2019).

As with any surveillance system, it is important to assess possible limitations while interpreting and reporting results. A series of events must transpire for a case of salmonellosis to be confirmed and reported. This involves seeking medical care, clinical diagnosis, submission of a specimen for further laboratory testing and confirmation, and the eventual reporting of actual cases. There are several factors that may prevent individuals from seeking medical care which limit the number of reported cases of infection (e.g., severity of illness, socioeconomic status, and access to healthcare) (Scallan et al., 2006). It is also possible for confirmed cases to go unreported and, quite commonly, individuals seek medical care without submitting a specimen for laboratory analysis. These challenges lead to the underreporting of actual cases of human illness and what is referred to as a surveillance pyramid, in which the number of reported cases is much smaller than the true population value (Tauxe et al., 2010). Another possible limitation of FoodNet data, specifically, is that a portion of the total reported cases may be attributed to sources other than foodborne infection, e.g., human-to-human or animal-to-human transmission of disease (CDC, 2014a).

In addition to the FoodNet surveillance data, the CDC also collects national *Salmonella* surveillance data from clinical diagnostic laboratories through passive surveillance (CDC, 2011). In this system, clinical diagnostic laboratories submit both human (i.e., clinical) and animal (i.e., clinical and non-clinical) *Salmonella* isolates to state and regional public health laboratories that are then responsible for confirming, serotyping, and the final reporting of the results (i.e., demographic information, serotype, and source of specimen) to the CDC (CDC, 2011). In line with the most recent FoodNet data, the annual report of the national *Salmonella* surveillance

data, published in 2016, also named the top three illness-causing serotypes as *S*. Enteritidis (16.8%), *S*. Newport (10.1%), and *S*. Typhimurium (9.8%).

These surveillance systems are maintained by the CDC in order to monitor the pulse of foodborne illness in the U.S. This provides a means to compare yearly trends (e.g., morbidity and mortality for known pathogens) and identify emerging pathogens that pose a considerable hazard to human health. Researchers may use data generated from FoodNet and other monitoring systems to make inferences about common food safety practices, assess food safety initiatives, and evaluate interventions currently in place (ODPHP, 2014). The results of these pathogen-monitoring systems also provide an opportunity for further analyses through the comparison of data from surveys, other surveillance efforts, and research projects based on specific population parameters to provide realistic estimates of the burden of disease while taking various factors into consideration (Scallan and Mahon, 2012). These estimates then lead to the development of future research objectives and drive food safety regulation through implementation of food safety standards and directives set forth by regulatory agencies such as USDA-FSIS (CDC, 2013).

2.3 *Economic Impact of Salmonella*

In addition to establishing the overall estimates of foodborne illness, the resulting estimated economic burden (i.e. the monetary measurement of foodborne illness) is a useful means to further realize the consequence of human illness within a population. The economic impact associated with foodborne *Salmonella* infection is of great importance and, therefore, for multiple reasons makes appraising the total economic burden of this foodborne pathogen a priority (Buzby and Roberts, 2009). Illustrating the magnitude of the financial burden inflicted on the economy by foodborne salmonellosis is necessary to justify intensifying surveillance efforts. Comparing the cost of illness between pathogens is necessary in order to determine an immediate course of action but can also be beneficial in determining the proper allocation of government funding for research into specific areas focusing on the prevention of the more prevalent foodborne pathogens (Mangen et al., 2010).

When determining the total economic cost per illness, there are several factors that must be considered. The basic cost-of-illness model accounts for the costs of diagnosis, medical care, and treatment as well as losses in productivity due to time away from work and illness-related mortality when applicable. This model has been used to estimate the economic losses associated with foodborne illness for various pathogens, including *Salmonella* (Scharff, 2012). When employed by Scharff (2012), the basic cost-of-illness model projected that a typical case of non-typhoidal *Salmonella* should cost approximately $4,312 (90% confidence interval; $1,558 to $10,042). The total cost per case may differ due to the severity and duration of the illness which fluctuates among individuals based on their immune status upon exposure and the serotype of *Salmonella* contracted.

The enhanced cost-of-illness model takes the basic cost-of-illness model one step further by including estimates of pain, suffering, and functional disability measures into the model in place of productivity loss due to illness. This enhanced model uses quality-adjusted life years (QALY) to estimate the total cost per

case while including the economic cost associated with pain and suffering (130). These models can be utilized periodically when the most pertinent estimates become available. For instance, prior to the Scallan et al. (2011) publication, estimates provided by Mead et al. (1999) were used in cost-of-illness models to project the economic burden of foodborne illnesses (Scharff et al., 2009). Improvements in methods and estimation of under-reporting and under-diagnosis may account for a portion of the differences in parameter estimates between the two publications; therefore, it is not clear if the apparent reduction in total illnesses reported by Scallan et al. (2011) from the estimates previously provided by Mead et al. (1999) is the result of a change in the actual burden of disease (Scharff, 2012) or the result of differing methods in estimation, or a combination of both. Regardless, estimates from the CDC FoodNet program indicate that the incidence of reported salmonellosis cases has not changed over time.

The cost-of-illness estimates described by Scharff (2012) address economic losses for individuals affected by domestically acquired foodborne illness. It is important to acknowledge that the cost of foodborne illness to industry and public health agencies were not included in these models. Therefore, the total economic burden on society estimated by Scharff is, presumably, an underestimate of the actual value. For instance, Sockett and Roberts (1991) reported the costs of investigating salmonellosis that included time and resources devoted by local public health authorities, in addition to medical care, treatment, and productivity losses. This survey incorporated 1,482 confirmed cases of salmonellosis reported in England and Wales over an eight-month period (Sockett and Roberts, 1991). Public health costs associated with health department investigation and laboratory testing accounted for 16% of the total cost estimated by the survey.

Utilizing multiple source attribution methods when determining the health burden of *Salmonella* has been shown to be a useful tool. Batz et al. (2012) examined the burden on public health for 14 major pathogens (e.g., *Norovirus*, *Salmonella*, and *Campylobacter* spp.) and 12 broad food categories (e.g., poultry, pork, beef, eggs, and complex foods). For this study, the authors combined publicly available outbreak data from the CDC's Foodborne Outbreak Online Database (FOOD) and expert elicitation for food attribution estimates (Batz et al., 2012). In terms of total number of illnesses, *Salmonella* spp. ranked below *Norovirus*, but ranked highest among all bacterial pathogens in hospitalizations (19,336) and deaths (378), which are likely to be the driving factors for the highest loss of QALY at 16,782 and total annual cost of illness of $3.31 million (Batz et al., 2012). Interestingly, when the 14 pathogens were ranked by their burden of illness (i.e., the average rank in QALY losses and number of illnesses), the stratified pathogen food pairings indicated that *Salmonella* ranked 4th, 6th, 8th, and 10th when paired with poultry, complex foods, produce, and eggs, respectively.

2.4 *Important Serotypes in Public Health*

More than 2,500 *Salmonella* serotypes are recognized to date. Many are known to cause illness in humans, yet most human illnesses are attributed to a relatively few serotypes. *Salmonella* serotypes vary considerably in terms of invasiveness and rates of illness. Various serotypes have been associated with causing mild to severe illness, depending on virulence factors and the immune status of the individual. Current research shows that a select few serotypes can cause severe illness in relatively few infected persons (e.g., *Salmonella* serotype

Dublin and *Salmonella* serotype Choleraesuis), while others (e.g., *S.* Typhimurium, *S.* Enteritidis, and *S.* Newport) are responsible for a larger proportion of the total salmonellosis cases (Jones et al., 2008). Examining *Salmonella* infection by serotype adds another important level of understanding to the current epidemiological knowledge of this pathogen.

Salmonella Enteritidis is the most common serotype identified in outbreaks of foodborne illness and can be isolated from a variety of hosts, although it is most commonly associated with eggs and poultry products. *Salmonella* Enteritidis is known to asymptomatically infect hen ovaries leading to the internal contamination of eggs (Guard-Petter, 2001). Since eggs are frequently consumed raw or undercooked, creating an efficient vehicle for human infection, they are the most commonly identified source of foodborne *S.* Enteritidis outbreaks (Braden, 2006). For the few outbreaks of *S.* Enteritidis not associated with eggs, a wide variety of foods have been implicated such as poultry, raw milk, alfalfa sprouts, raw almonds, pork, and beef (CDC, 2014c).

Salmonella Typhimurium is the third most prevalent serotype isolated from food, accounting for 9.8% of laboratory-confirmed cases of salmonellosis (CDC, 2018). *Salmonella* Typhimurium is also one of the top serotypes isolated from food-producing animals and retail meats (Louden et al., 2012). In a six-year span from 2007 to 2013, 61 outbreaks of *S.* Typhimurium were recorded for animal contact (e.g., frogs, hedgehogs, and turtles) and a variety of food sources such as beef, cantaloupe, lettuce, chicken, and eggs (CDC, 2014c).

Most of the information currently available for foodborne pathogens and associated illness comes from previous outbreak investigations. An outbreak is characterized by two or more laboratory-confirmed cases of foodborne illness that must have been acquired from a common (i.e., epidemiologically linked) source. Outbreak investigations provide a unique opportunity to learn more about foodborne pathogens and contribute to the control and prevention of future illnesses (Bean and Griffin, 1990). The information gleaned can identify secondary risk factors that contribute to outbreaks of foodborne illness (e.g., temperature abuse, raw materials, inadequate handling, and environmental factors) (Tirado and Schmidt, 2001). Paniselloa et al. (2000) demonstrated the use of retrospective analysis of foodborne illness outbreak data and its value to maintain and further develop HACCP systems by establishing critical control points along the food production chain.

2.5 *Disease Attribution*

Although feces are most likely the source of *Salmonella* exposure for humans, several important routes of transmission bring humans in contact with *Salmonella*. According to Doyle (2013), the routes of infection for humans are:

1) Direct Contact – where individuals are exposed to a human or animal shedding the pathogen. This can be human-to-human contact in clinical settings, households, and other institutions or animal-to-human contact in an animal husbandry setting (e.g., livestock handling) or petting zoos;

2) Contaminated Food – most commonly foods of animal origin (e.g., eggs, poultry, pork, and beef) are implicated as sources of *Salmonella*. Produce is also a common source of *Salmonella* outbreaks where contaminated soil or runoff water is the source of contamination. Complex foods are also an important source of *Salmonella* infection, likely as a result of improper handling and the inclusion of implicated ingredients such as eggs or ground meats; and

3) Contaminated Water – waterborne outbreaks of salmonellosis are more common in developing countries where water sources become contaminated with human or animal feces as a result of water runoff.

Since there are many possible routes of infection for *Salmonella*, source attribution is an appropriate measure to assess each route of infection. Source attribution for enteric bacteria is recognized as the estimation of the proportion of human illness cases for a specific disease (e.g., non-typhoidal *Salmonella* infection) that can be attributed to a specific animal reservoir, food product, or ingredient (Doyle, 2013; Greig and Ravel, 2009). By determining the proportion of illnesses attributed to a common source, strides can be made toward reducing the incidence of human illness associated with that source.

Cattle, swine, and poultry are known to harbor and shed *Salmonella* capable of causing disease in humans; thus, these species are considered important reservoirs for this pathogen. Furthermore, it has been shown that the classification of *Salmonella* serotypes among animal reservoirs has proven to be informative as some serotypes are associated with different reservoirs and, therefore, may have differing vehicles for human exposure (Greig and Ravel, 2009). For example, *Salmonella* serotypes commonly associated with cattle include: *S.* serotype Anatum, *S.* Montevideo, *S.* Dublin, and *S.* Infantis (Doyle, 2013). Interestingly, however, the serotypes recovered from ground beef differ, in that *S.* Montevideo, *S.* Dublin, *S.* serotype Cerro, *S.* Newport, *S.* Anatum, *S.* serotype Muenster, and *S.* Mbdanka are the most prevalent (Bosilevac et al., 2009; Doyle, 2013). Alternatively, the same serotypes associated with chickens are commonly found in ground chicken, namely *S.* Kentucky, *S.* Enteritidis, *S.* Heidelberg, *S.* serotype I 4,5,12:i:-, and *S.* Typhimurium (Doyle, 2013). Attribution data provided for reservoirs and food vehicles associated with each *Salmonella* serotype are valuable to inform future research, risk management, and aid in the development of pathogen inhibition in the food production chain to limit human illness (Pires et al., 2009).

Exploring source attribution among various food products provides another means to assess the public health impact of *Salmonella*. The food products commonly implicated in *Salmonella* outbreaks are eggs, chicken, pork, beef, fruit, and turkey (Jackson et al., 2013). When assessing attribution data for outbreaks and number of cases, a figure in Doyle (2013) reported that meat, eggs, and fresh produce accounted for 29, 27, and 13% of outbreaks and 25, 25, and 15% of total cases, respectively. Further stratification of meat-related outbreaks and cases demonstrated that 34, 25, and 16% of outbreaks and 29, 21, and 19% of the total cases were attributable to chicken, pork, and beef, respectively (Doyle, 2013).

Source attribution can be achieved using a variety of methods including the analysis of outbreak surveillance data, case-control studies, microbiological subtyping analysis, comparative exposure assessments, and by using expert elicitation (Pires, 2013). Each method for determining source attribution has limitations and

advantages that, depending on the nature of the objective and data available, could affect the outcome. For instance, the use of outbreak data to estimate source attribution does not account for sporadic illnesses. Since the CDC estimates that 95% of salmonellosis cases are sporadic, applying outbreak data to estimate source attribution may not accurately represent the entire scope of *Salmonella* infections or sources of exposure. Another current limitation of source attribution research is the lack of common categories to describe foods and food commodities (Batz et al., 2012). Establishing a convention for categorizing food products will likely maximize the utility of source attribution data by allowing the results to be compared among attribution studies that employ different methods and data.

Estimating the burden of foodborne illness by determining the total number of illnesses, hospitalizations, and deaths, as well as the associated economic cost, is necessary to illustrate the significance of foodborne salmonellosis in humans. These estimates of the public health burden, along with source attribution data, can be used to inform risk assessments for animal reservoirs and assess the efficacy of food safety interventions. The burden associated with the harborage of *Salmonella* in cattle populations, as well as some of the potential pre- and post-harvest interventions, are more thoroughly discussed in the following sections. This review is intended to highlight the current knowledge of the implications of *Salmonella*.

2.6 *Antimicrobial Resistance and Salmonella*

Antimicrobial resistance in *Salmonella* is a pertinent public health issue that has been a contentious topic of discussion for several decades (Cherubin, 1981; Threlfall et al., 2000). In terms of antimicrobial resistance, an isolate is typically considered resistant if it can grow in the presence of an antimicrobial at a concentration greater than the defined minimum inhibitory concentration (MIC) or less than some distance from a source of an antimicrobial (zone of inhibition). Infections with resistant organisms may be associated with poorer clinical outcomes in comparison with phylogenetically-related susceptible strains (Aarestrup, 2006). Some observational evidence suggests that antimicrobial resistant *Salmonella* infections may be more severe than typical salmonellosis and are likely to result in more invasive bloodstream infections (Varma et al., 2005). Therefore, it is possible that resistant strains might be more virulent than pansusceptible strains (i.e., susceptible to all clinically relevant antimicrobials).

Ongoing discussions about the administration of antimicrobial compounds to food-producing animals and its contribution to antimicrobial resistance in foodborne pathogens continue (Aarestrup and Pires, 2009; Acar and Moulin, 2006; Cherubin, 1981; *Helke, 2017). The threat that antimicrobial resistance poses to the public warrants further investigation and continued monitoring of problematic pathogens and antimicrobial agents. In order to better understand the emergence, persistence, and spread of antimicrobial-resistant bacteria, the National Antimicrobial Resistance Monitoring System for Enteric Bacteria (NARMS) was established in 1996. This program is a collaborative effort among CDC, FDA, and USDA to track changes in the susceptibility of enteric bacteria isolated from ill people, retail meats, and food-processing animals to antimicrobial agents that are of high importance in human and veterinary medicine (FDA, 2012). The NARMS retail meat

surveillance program monitors the prevalence and trends in antimicrobial resistance for *Salmonella*, *Campylobacter*, *Enterococcus*, and *E. coli* isolated from chicken, ground turkey, ground beef, and pork chops (FDA, 2011). NARMS began monitoring *Salmonella* in food animals in 1997 and retail meats in 2002 (Karp et al., 2017).

In 2011, *Salmonella* was recovered from 12, 12.3, 2.1, and 0.7% of chicken, ground turkey, pork chop and ground beef samples, respectively. Overall, *S.* Typhimurium, *S.* serotype Kentucky, and *S.* serotype Heidelberg accounted for 48% of the *Salmonella* isolates recovered through the retail meat program due to the high prevalence of these serotypes in poultry samples. Of the nine *Salmonella* isolates from ground beef, *S.* serotype Kentucky, *S.* serotype Infantis (Acar and Moulin, 2006), *S.* serotype Mbandaka, *S.* serotype Montevideo, and *S.* serotype Litchfield (Aarestrup, 2006) were observed. The most multidrug-resistant (MDR) *Salmonella* isolates, defined as resistant to three or more antimicrobial classes, were recovered from poultry with 44.9% of chicken isolates and 50.3% of ground turkey isolates being MDR. On the other hand, only 11.1% of ground beef and 28.6% of pork isolates were classified as MDR (FDA, 2011). Currently, there is low prevalence of antimicrobial resistant bacteria on final carcasses and products. These low numbers indicate that current interventions in processing facilities are effective in reducing potential risk of contamination (Schmidt et al., 2015).

3 Pre-Harvest Overview

The source of a *Salmonella* infection among cattle is among the most difficult to fully comprehend. Due to the abundance of vehicles, pathogens are often widely disseminated with the original source of infection being unknown. Potential sources of contamination on commercial agricultural production facilities (CAPF) consist of incoming cattle, the environment, feces, feed, water, rodents, wild animals, flies, and birds (Adhikari et al., 2009; Callaway et al., 2014; Daniels et al., 2003; Greig et al., 2014; LeJeune et al., 2001b; Olafson et al., 2014). It is the diverse and constant interaction among cattle and these vehicles of exposure that elevates the prevalence of this bacterium in the cattle industry in the U.S. Fecal *Salmonella* shedding among cattle can persist for extended durations following clinical disease (Callaway et al., 2008), potentially resulting in the widespread environmental contamination and increasing the risk of within-herd transmission.

3.1 *Salmonella in the Beef Animal*

It has been well documented that ruminants make excellent hosts for *Salmonella* and thus it can be easily disseminated in the feces (Callaway et al., 2010; Fedorka-Cray et al., 1998; Kunze et al., 2008). *Salmonella* are pathogens capable of residing as transient members of the intestinal microbial population within bovine species (Callaway et al., 2008). Although the prevalence of *Salmonella* within CAPFs is relatively high, especially in the southern U.S. (Blau et al., 2005; Dagartz et al., 2003; Dodd et al., 2011; Kunze et al., 2008; Wells et al., 2001), the incidence of salmonellosis does not reflect this in mature cattle (Cummings et al., 2009; Edrington et al., 2008). Young animals are frequently colonized by *Salmonella* and are most likely to experience salmonellosis within 2-4 weeks of age (House et al., 2001). A large proportion of mature cattle in the south are infected but show no clinical signs of *Salmonella* infection leading to a high number of

asymptomatic carriers (Dodd et al., 2011). Thus, reliance on overt clinical indicators of illness is not an effective indicator of *Salmonella* colonization, as infected animals may appear healthy (Callaway et al., 2008).

Reasons behind the absence of clinical signs of *Salmonella* infections in cattle are currently uncertain. House et al. (House et al., 2001) discovered it was a brief interval (<24 hours) from birth to detection of *Salmonella* in fecal samples of dairy calves. Thus, relatively quick fecal shedding of *Salmonella* in calves was attributed to the immediate exposure of the calf to the pathogen within the environment (House et al., 2001). However, recent data has shown that rather than acquiring this pathogen after birth, animals may be infected in utero (Hanson et al., 2016). These data indicate a vertical (transplacental) infection from dam to fetus without noticeably affecting viability. Immediately after parturition, *Salmonella* was recovered from multiple lymphatic-associated tissues as well as tissues in the gastrointestinal tract in 50% (10/20) of calves sampled. The observed prevalence of *Salmonella* by each calf tissue type tested is shown in **Figure 1** (Hanson et al., 2016). Multiple serogroups were present with the primary serogroups consisting of C1, C2, E1, and other, 42, 30, 15, and 13%, respectively (Hanson et al., 2016). If the pathogen infiltrates the fetus prior to immune maturation, it's feasible to hypothesize that these animals don't recognize *Salmonella* from an immunological perspective. This novel discovery warrants further investigation into disrupting the transmission dynamics of these pathogens on CAPFs.
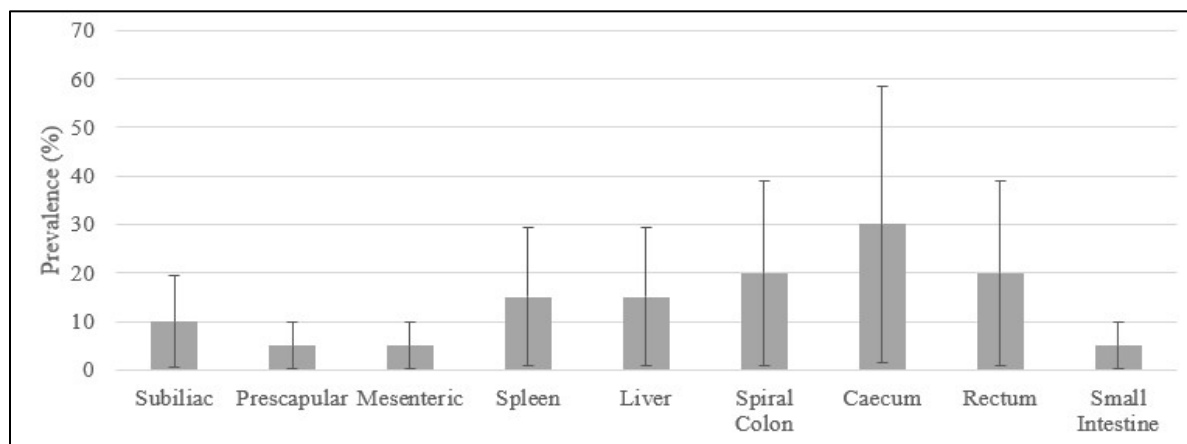
Figure 1. Prevalence of Salmonella by sample type collected from 20 full-term calves (adapted from Hanson et al., 2016).

Empirical evidence has shown the prevalence of *Salmonella* varies significantly due to both season and region (Blau et al., 2005; Cummings et al., 2009; Dargatz et al., 2003; Dodd et al., 2011; Kunze et al., 2008; Van Donkersgoed et al., 2001; Wells et al., 2001) and is apparent when evaluating the prevalence of fecal *Salmonella* in CAPFs (Edrington et al., 2004; Kunze et al., 2008). Estimating the prevalence of *Salmonella* in the animals within a facility is often conducted by sampling feces and/or hides (Arthur et al., 2008b; Brichta-Harhay et al., 2011; Callaway et al., 2005; Stephens et al., 2007b). Each sample type is unique in that individually the samples provide meaningful insight for evaluating the prevalence of *Salmonella* on the herd level as well as the individual level. The prevalence of *Salmonella* has shown oscillating cycles across seasons and is typically the highest during the summer and fall, and lowest during the winter and spring (Alam et al., 2009; Dodd et al., 2011). Rather than being a function of the season, this is primarily reflective of the temperature within seasons (Kendrovski et al., 2011), *Salmonella* typically thrives in warm weather and is

suppressed in cold weather (Kendrovski et al., 2011; Losinger et al., 1997). It is currently uncertain if *Salmonella* completely dissipates in the environment during these colder months or is reduced to a concentration below the limit of detection of current microbiological methods. Regional differences may be described as *Salmonella* being ubiquitous in the southern regions of the U.S. (Edrington et al., 2008) and herd-specific in the northern regions (Cummings et al., 2009; Rao et al., 2010). Research agrees that cattle obtained from confined animal feeding operations in the southern region of the US would be more likely to introduce *Salmonella* into a herd (Cummings et al., 2009; Dargatz et al., 2003; Dodd et al., 2011; Kunze et al., 2008; Van Donkersgoed et al., 2009; Wells et al., 2001).

3.2 *Fecal Prevalence*

The prevalence of *Salmonella* in feces has been intensely investigated during the last 20 years using a multitude of sampling schemes (Blau et al, 2005; Callaway et al., 2006; Dodd et al., 2011; Edrington et al., 2004; Kunze et al., 2008; Loneragan et al., 2012; Stephens et al., 2007b; Wells et al., 2001). Edrington et al. (2004) sampled 60 healthy lactating dairy cattle on each of four CAPFs in the southwest U.S. during August 2001, January 2002, and August 2002 (60 cows per farm, per sampling; n=720 total samples). *Salmonella* prevalence on one of the farms ranged from 1.7% in January 2002 to 92% in August 2002 (n=60). Kunze et al. (2008) sampled multiple

CAPFs located in the southwest once during each of the four seasons and recovered *Salmonella* from 30.3% (n=600) of samples. In this study, the authors reported no significant difference in prevalence between seasons. Wells et al. (2001) collected samples from 19 states, including 91 dairies and 97 cull cow markets, between the months of February and July. *Salmonella* was recovered from 10% (n=6,595) of total samples with a higher portion of positive samples collected from facilities in the southern U.S. (45% of dairies culture positive). However, Callaway et al. (2006) collected feces from four CAPFs across four different states between the months of June and September and only recovered *Salmonella* from 9.96% (n=960) of samples, with the largest proportion of positive samples (37%) coming from farms in the northeast. While 37% (n=240 from the northeast region farms) is relatively low (Callaway et al., 2006) compared to fecal prevalence previously reported from the southern region (Dodd et al., 2011; Kunze et al., 2008), it is, however, substantially greater than other studies conducted in the northern portions of North America (Rao et al, 2010; Van Donkersgoed et al., 2009).

Most recently, a longitudinal evaluation of fecal prevalence across three different CAPFs was conducted over a 12-month period in 2013-2014. Data collection was limited to CAPFs located within 1.5 km of each other that specialize in rearing either dairy or beef cattle. The overall prevalence for each farm was 82.4 (n=1202), 73.4 (n=1125), and 78.9% (n=919), with the largest variation across months within a single farm being 8 to 100% as observed in **Figure 2** (Renter et al., 2014 unpublished) and the increased prevalence was in the warmer months of the year. Likavec et al. (2016) reported results that validate the higher prevalence in warmer temperatures with an increase in prevalence in dairy pens exposed to higher temperatures (>25°C) over pens that were exposed to lower temperatures (< 25°C).

Dargatz et al., (2016) observed the prevalence and antimicrobial resistance of *Salmonella* from cattle feces in up to three pens of cattle in each of 68 feedlots in 12 states. All isolates were evaluated for susceptibility to a panel of 15 antimicrobial drugs. When tested, 74.4% of isolates were susceptible to all antimicrobial drugs. The most common resistance was to tetracycline or sulfisoxazole and less than 10% of the isolates were resistant to any other antimicrobials in the panel. This study indicates that a low occurrence of antimicrobial resistance, with the exception of tetracycline and sulfisoxazole, exists when *Salmonella* is present in feed lot cattle. (Dargatz et al., 2016).

Figure 2. Prevalence of Salmonella in feces by commercial agricultural production facilities (CAPF) and month of sample collection post-enrichment (adapted from Renter et al., 2014 unpublished).

3.3 *Hide Prevalence*

Pathogen prevalence on hides may reflect several sources of contamination, which accurately reflects the pathogen load of the environment (Arthur et al., 2008b) in addition to the load of the individual animal (Rhoades et al., 2009). Feces from one animal can contaminate multiple hides, and hides can be contaminated with feces from multiple animals, so these samples widely reflect both pen level and individual contamination (Barkocy-Gallagher et al., 2003). Despite management practices employed by facilities, cattle activity and frequent movement throughout the day result in pulverization and subsequent aerosolization of pen floor material (McEachran et al., 2015). Facilities near one another have the capability to share bacteria due to fugitive dust generated within pens (Von Essen and Auvermann, 2005). Arid environments are commonly plagued with copious amounts of wind, thereby accommodating the dispersion of microorganisms, not only into multiple ecological niches, but also into biological niches such as the hides of cattle (McEachran et al., 2015). Due to the intermittent grooming of cattle by themselves and each other, hide contamination often serves as an additional vehicle for a *Salmonella* infection (Arave and Albright, 1981).

To track the source of *Salmonella* contamination in ground beef, Koohmaraie et al. (2012) reported 96% (n=100) of hides were *Salmonella*-positive in dairy cattle at the time of harvest. This estimated prevalence is similar to findings of Brichta-Harhay et al. (2008; 2011), where the mean prevalence of hide contamination was 89.6% (n=3,040) across six different abattoirs in four geographically distinct regions of the U.S. Although it has been thoroughly documented that hide contamination is high in cattle prior to harvest, a portion of this contamination may be attributed to cross-contamination that occurs among animals during transportation and lairage (Reicks et al., 2007; Wheeler et al., 2014).

3.4 *Salmonella in Water*

It has been shown that water troughs in CAPFs are reservoirs for *Salmonella* and have the potential to infect many animals within a herd (Edrington et al., 2008; LeJeune et al., 2001b). Edrington et al. (2008) reported high concentrations of *Salmonella* in water troughs with seasonal fluctuations in the prevalence of *Salmonella*. This is a significant concern due to the increased exposure when considering the number of cattle that consume water from the same trough (LeJeune et al., 2001a). The prevalence of *Salmonella* in the water throughout the collection period was sporadic; prevalence ranged from 0 to 75% with a mean prevalence of 38% over the nine-month period (Edrington et al., 2008). Unlike fecal samples, water prevalence didn't follow a consistent trend of being elevated during warmer months and suppressed during the colder months as shown in **Figure 3** (Edrington et al., 2008, Renter et al., unpublished). The varying level of pathogens in the water could either reflect the prevalence of *Salmonella* in the animals within the environment, extended exposure to sun light, the ambient water temperature across the sampling period, or the amount of time between cleanings of the troughs (LeJeune et al., 2001b; Pachepsky et al., 2014; Smith et al., 2008).
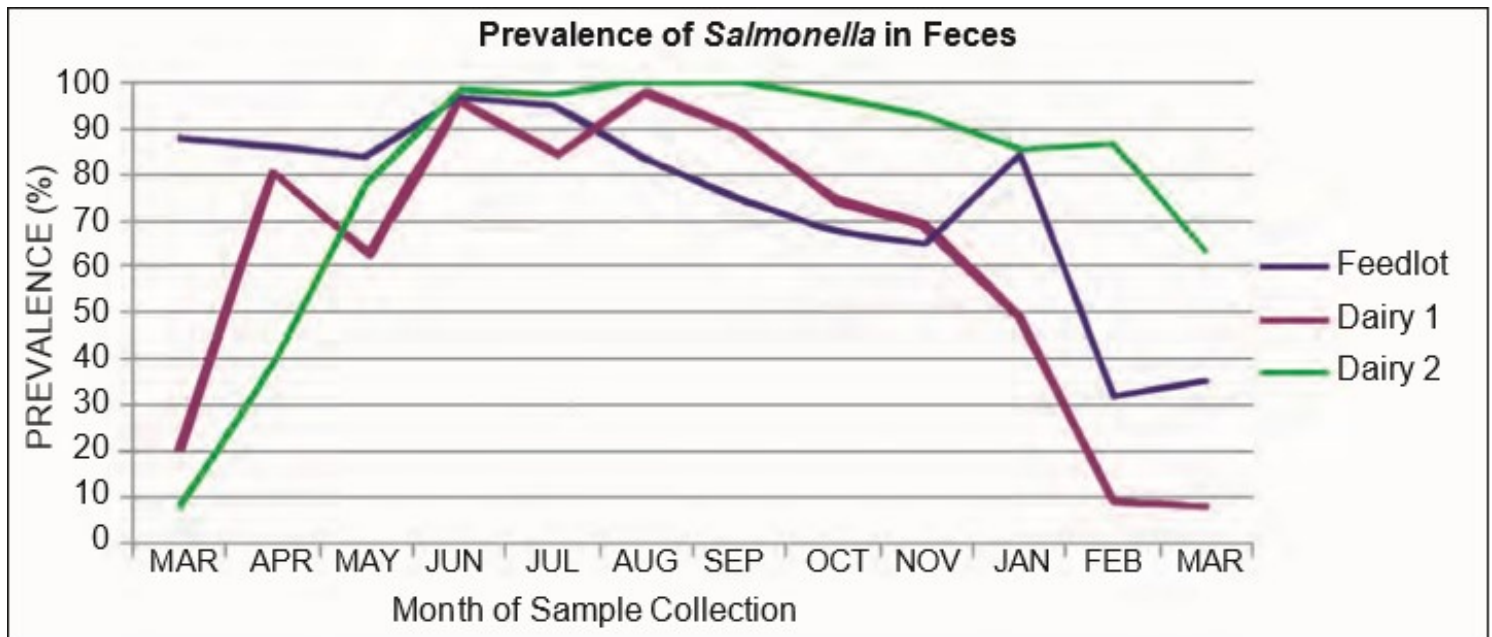

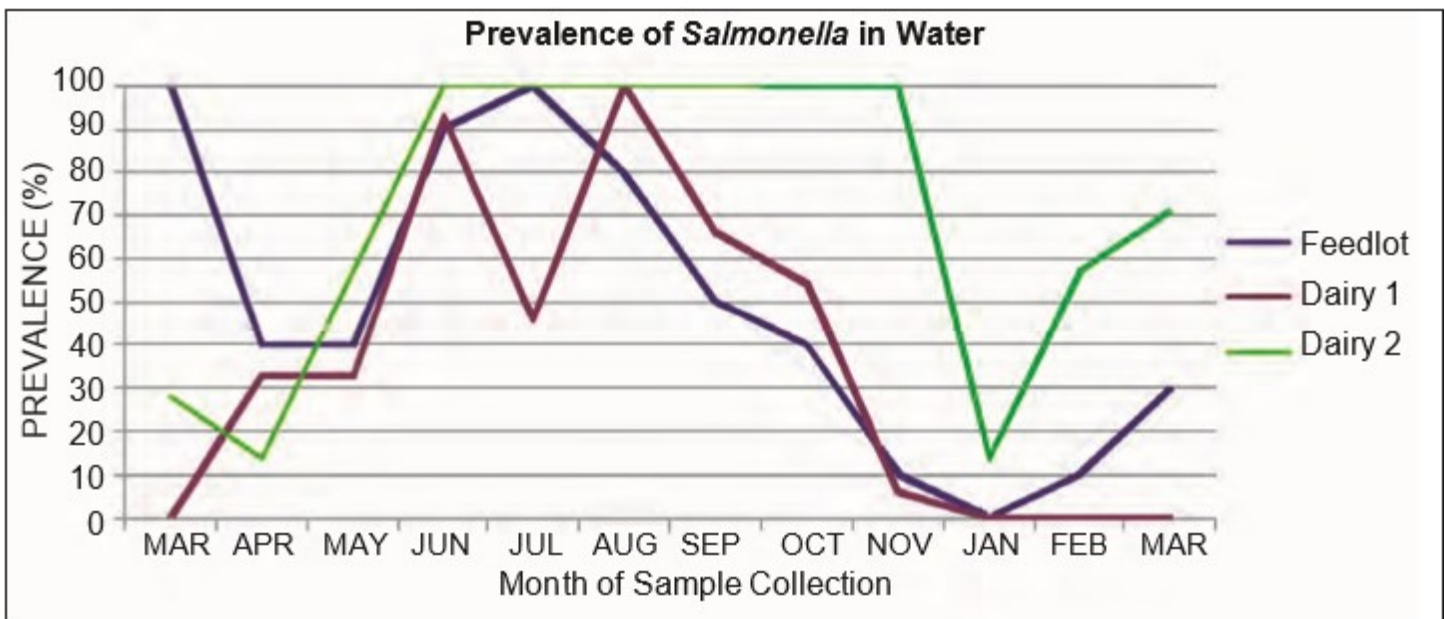**Figure 3**. Prevalence of Salmonella in water by commercial agricultural production facilities (CAPF) and month of sample collection post-enrichment (adapted from Renter et al., unpublished).

The exact source of water contamination is unknown; however, it could be a multitude of different vehicles. The initial water holding tank could be contaminated with *Salmonella* leading to contamination in every

trough. Additional potential methods of contamination include cattle contaminating the water troughs via feed and/or fecal matter (LeJeune et al., 2001b), or non-cattle-related possibilities such as birds and flies (Pedersen et al., 2006). These non-cattle related vectors are routinely found in CAPFs and typically consume water, in addition to defecating or bathing in water sources, which is a likely mode of contamination in troughs (Carlson et al., 2011). Cattle routinely consume water after visiting the feed bunk and often drop feed particles into the water; therefore, if the particles are infected this could account for the contamination (LeJeune et al., 2001b; Stephens et al., 2007b). Direct contamination from the animal's oral cavity is also a plausible route of infection; Stephens et al. (2007b) recovered *Salmonella* from oral swabs in 94% (n=50) of animals enrolled in a study in West Texas.

3.5 *Salmonella in Feed*

Animal feed or forage may be the source of a limited number of infections for farm animals that could in theory lead to human illness (Jones et al., 1982; Queiroz et al., 2018). The role of contaminated feeds in the non-clinical presence of *Salmonella* in animals is largely unknown (Dargatz et al., 2005). The hazard to human health from animal feed is reliant on vegetative bacterial cells or other microorganisms colonizing the animal following consumption of the feed and contaminating the foods for humans derived from the animals consuming the contaminated feeds (Hinton, 2000). It is unclear if feed ingredients become contaminated with fecal bacteria prior to delivery to the feedlot and/or after arrival (Dargatz et al., 2005). Contamination of feeds could occur while growing, in storage, during transport, or during handling for processing into mixed feeds (Dargatz et al., 2005; Davies and Wray, 1997; Losinger et al., 1997).

The first potential source of contamination of feedstuffs is through fertilizing and irrigation of crops. Utilizing farm animal excreta as fertilizer is a valuable resource for replenishing nutrients into crop lands, either prior to or while growing forages, and serves as an effective method of waste disposal (Hinton, 2000). However, the use of animal manure as a nutrient source for crops and irrigation with water contaminated by animal waste has been implicated in several pathogen outbreaks (Doyle and Erickson, 2008). In a 2011 study by Toth et al. (2011) it was discovered that *Salmonella* could survive in irrigation water and farm soil under typical conditions for 137 and 276 d, respectively. Fertilizing with manure and sewage that has not been properly treated may lead to *Salmonella* contamination of forages that are routinely fed to cattle (Dargatz et al., 2005; Hinton, 2000; Krytenburg et al., 1998). Therefore, composting is a practice often utilized to reduce pathogens in manure that will be used to fertilize cropland.

Secondly, multiple pests inhabit CAPFs and are known carriers of many human and cattle pathogens including *Salmonella* (Callaway et al., 2014; Carlson et al., 2011; Clark and McLean, 2003; Meerburg and Kijlstra, 2007; Pedersen et al., 2006). Avian species typically congregate in large roosting groups and exploit abundant and highly palatable food sources (Feare et al., 1992; LeJeune et al., 2008). It has been shown that birds are capable of infecting feedstuffs in the following ways: 1) mechanical transmission of contaminated cattle feces from the pens to the feed; and 2) defecation directly onto the feedstuffs (Carlson et al., 2011). In addition,

rodents commonly inhabit CAPFs, similarly exploiting food sources and transmitting pathogens to feedstuffs (Meerburg and Kijlstra, 2007).

Another potential source of contamination of feedstuffs is through horizontal transmission via the wind. CAPFs are often open-air facilities, which facilitate environmental dispersal of particulate matter via wind (McEachran et al., 2015). As mentioned above, pulverized feces are easily dispersed by the frequent wind in the southwest U.S. Sprinklers are often utilized to mitigate the occurrence of blowing dust on CAPFs; however, data are mixed regarding this technique's efficacy in reducing the incidence of fecal shedding of pathogenic bacteria (Edrington et al., 2009; Morrow et al., 2005).

Additionally, Ge et al. (2013) discovered 22.9% (n=201) of animal and plant by-products collected at rendering and oilseed plants were contaminated with *Salmonella* post-processing, but not prior to delivery. Although CAPFs routinely ship feedstuffs to commercial labs for testing, screening for zoonotic pathogens is not commonly practiced (Schalla et al., 2012).

To investigate the seasonality of salmonellosis in dairy cattle, Edrington et al. (2008) collected total mixed ration (TMR) samples monthly over a nine-month period directly from the feed bunk on a dairy located in the southwest U.S. The average prevalence of *Salmonella* in feed was 76% across the sampling period with a range of 4 to 100% (Edrington et al., 2008). Ten different serogroups of *Salmonella* were recovered from the TMR throughout the study with the predominate serogroups being C1, E1, and E4, which agrees with the findings of Dargatz et al. (2005). As these samples were collected from the feed bunk, it is possible that the feedstuffs may have become contaminated during the mixing process, or while in the bunk by cattle, birds, and insects as discussed above.

3.6 *Non-mammalian Vectors*

In addition to cattle, CAPFs are regularly inhabited by non-mammalian vectors (NMV), including multiple avian and biting insect species (Callaway et al., 2014; Olafson et al., 2014). Flies are found seasonally as are some avian species, however Rock Dove, Eurasian-Collered Dove, and European Starlings are considered peridomestic (Feare et al., 1992; Pedersen et al., 2006; Taylor et al., 2012). Livestock facilities are attractive to avian species because of the availability of large quantities of feed and water (Feare et al., 1992; Johnson and Glahn, 1994). Unlike cattle, these vectors are not confined to one pen as they have the capability to infect multiple locations within a facility and across multiple facilities (Van Donkersgoed et al., 2001; Wetzel and LeJeune, 2006). Wild birds have been implicated in the transmission of pathogens when the same subtypes were identified from feedlots approximately 50 to 100 km apart (Van Donkersgoed et al., 2001; Wetzel and LeJeune, 2006). Non-mammalian vectors such as wild birds and flies have also been shown to potentially harbor antimicrobial-resistant bacteria which can be introduced to the environment (Carlson et al., 2015; Xu et al., 2017).

In addition to consuming large amounts of feed, birds often contaminate feed and the farm environment (primarily the milking parlor, commodity area and shades within pens) with droppings that may spread zoonotic pathogens (Feare et al., 1992; Johnson and Glahn, 1994). Ingestion of feed contaminated with bird feces has been identified as a possible route of infection for cattle (Daniels et al., 2003). Recent efforts have been focused on evaluating potential environmental sources within CAPFs and determining the burden of their presence (Carlson et al., 2011; Edrington et al., unpublished; LeJeune et al., 2008). Edrington et al. (unpublished) sampled the internal organs (excluding the heart) of avian species from multiple
CAPFs in both the fall and winter. Prevalence of *Salmonella* in the internal organs of a combination of Rock, Eurasian Collared, and Mourning Dove collected in the southwest was 98 and 6% for fall and winter, respectively (Edrington et al., unpublished).

Flies are common on CAPFs in the summer and fall months, with populations varying greatly across facilities (Taylor et al., 2012; Xu et al., 2017). A large proportion of facilities employ management practices to mitigate the fly population (USDA, 2008). In addition to defecating and regurgitating in the environment, flies have the capability to infect cattle via penetrating the hide while blood feeding (Olafson et al., 2014). Animal hides and manure pats are sources for flies to acquire the *Salmonella* and then mechanically transmit them to an animal while feeding (Callaway et al., 2006; Olafson et al., 2014; Olafson et al., 2016). Research has indicated that after horn flies acquire *Salmonella* from hides, it can persist and survive for at least 5 days. With long-term feeding from horn flies, prevalence of *Salmonella* in cattle lymph nodes increased from 8% to 50% from day 5 to 11, respectively (Olafson et al., 2016). The increased prevalence can potentially be attributed to the repeated inoculations from infected flies rather than a single inoculation event. Edrington et al. (unpublished) reported the predominate serogroups harbored by flies collected on multiple CAPFs were C1, C2, E1, and K.

4   Pre-harvest Interventions

It has been established that the beef feedlot environment may function as a reservoir of *Salmonella* populations. The various interconnected vehicles that may potentially transmit *Salmonella* on cattle operations discussed throughout the previous section make control of *Salmonella* extremely complex. Each vehicle has been documented as independently impacting the transmission of *Salmonella* to cattle (Carlson et al.,2011, 2015; Cummings et al., unpublished; Edrington et al., 2008; Ge et al., 2013; LeJeune et al., 2001b; Olafson et al., 2014; Stephens et al., 2007b; Toth et al., 2011; Toth et al., 2013; Xu et al., 2017). The large proportion of the cattle located in the southern U.S. infected with *Salmonella* creates not only a food safety concern (Fossler et al., 2005a), but also a potential animal health concern. A better understanding of the ecology of these microorganisms in and around CAPFs will assist in developing interventions, which could aid in reducing the incidence and burden of *Salmonella*. While not all are widely utilized, various pre-harvest interventions currently exist which may have an effect on *Salmonella* populations in a live animal setting.

4.1 *Vaccines*

Disease outbreaks compromise animal welfare, promote antimicrobial use and subsequently lead to selection for antimicrobial resistance in zoonotic pathogens, which compromises productivity and, at times, elevates mortality rates (Anderson et al., 2001). Efforts to control *Salmonella* are often less effective than desired for the following reasons:

1) disease outbreaks are sporadic and frequently caused by certain serogroups (CDC, 2014b)
2) environmental persistence within CAPFs provides an accumulative reservoir for zoonotic pathogens (Fossler et al., 2005a, 2005b; Ruzante et al., 2010)
3) vaccinated cattle are not adequately protected against the emergence of strain variants that may be more virulent (Heithoff et al., 2012)
4) general management practices, such as failure to clean water troughs or pens, and environmental events, concurrent with heat stress or suppressing dry matter intake, may potentially increase the exposure of a pathogen and/or compromise host immunity (Anderson et al., 2001; Fossler et al., 2005b; Heithoff et al., 2015; House et al., 2001; Hughes et al., 2014).

Vaccination aims to stimulate the development of naturally acquired immunity by inoculation of nonpathogenic, but still immunogenic, components of the pathogen in question (Meeusen et al., 2007). Vaccines that induce protective immunity against colonization of pathogens may offer distinct advantages because of likely acceptance by cattle producers and ready incorporation into existing vaccination protocols (Loneragan et al., 2005). Vaccination represents a sustainable, although minimally adopted, approach for promoting animal health, animal welfare, and food safety through mitigating pathogen exposure at the onset of commercial food production (Heithoff et al., 2015; Mahan et al., 2012). It has been reported that less than 1% of beef cattle operations utilize any type of commercially available *Salmonella* vaccine on their cattle (USDA-APHIS, 2010), and less than 6% of animals fed in feedlots receive a *Salmonella* vaccine (USDA-APHIS, 2013). The latest statistic on the percentage of dairy farms that vaccinate against *Salmonella* reported by the USDA National Animal Health Monitoring System was 10% in 2007 (USDA-APHIS, 2009).

Although *Salmonella* vaccines are not widely adopted, evidence exists suggesting that vaccinating animals may be beneficial. Loneragan et al. (2012) reported that the recovery of fecal *Salmonella* was 78% less likely in animals culled from herds that practiced whole-herd vaccination than observed in animals from herds that did not practice vaccination. In addition, Smith et al. (Smith et al., 2014) reported that calves that received colostrum from dams vaccinated in the previous dry period had elevated *Salmonella* antibodies when compared to calves receiving colostrum from unvaccinated dams.

In the past, immunity obtained from being vaccinated with a conventional vaccine was restricted to a narrow range of closely related strains within a specific serogroup (Heithoff et al., 2015). Conventional, commercially available vaccines are currently only capable of stimulating antibodies of serogroups B, C, or D (House et al. 2001). However, more recently, a vaccine has become commercially (NB: conditionally licensed at the time of

writing) available that targets proteins possessed by *Salmonella* Newport but may afford some cross protection against non-Newport serotypes. This is a subunit vaccine that is composed of purified extracts of siderophore receptors and porins (SRP) (Hermesch et al., 2008). These SRP proteins are essential for bacterial survival as they allow iron acquisition from the environment (Dodd et al., 2011). The vaccine restricts the ability of the bacteria to gain iron from the environment via stimulating antibodies to bind to the SRP proteins (Smith et al., 2014). In theory, targeting a protein possessed by all *Salmonella* organisms should induce immunity to multiple serogroups; however, clinical trials have shown mixed results of the efficacy of this vaccine (Dodd et al., 2011; Hermesch et al., 2008; Smith et al., 2014).

4.2 *Direct-Fed Microbials*

Direct-fed microbials (DFM) such as *Lactobacillus acidophilus* NP51 have been effective in mitigating the shedding of *Salmonella* in feedlot cattle when administered to cattle throughout the feeding period and prior to harvest at high doses (Stephens et al., 2007a). Pre-harvest interventions, such as DFM, can be implemented in conjunction with other sanitation procedures to create a multi-hurdle approach designed to control foodborne pathogens throughout the beef production system (Callaway et al., 2002). Unlike vaccination regimens, the inclusion of DFMs is relatively easy to incorporate into CAPFs by simply including them into the TMR.

The use of DFMs is proven advantageous by effectively mitigating the shedding of *Salmonella* in feedlot cattle and producers often observe increased performance characteristics (e.g., weight gain and feed-to-gain ratio) in animals fed DFMs (Stephens et al., 2007a). The inclusion of DFMs are more widely adopted than vaccination regimens (Smith et al., 2014; USDA-APHIS, 2009; 2010; 2013). Ison (2013) estimated 45.7% of feedlot-finished cattle harvested in 2012 were administered *L. acidophilus* NP51 at some point prior to harvest. Further research has shown when fed DFMs the prevalence and concentration of *Salmonella* in subiliac lymph nodes of cattle at slaughter may be decreased (Vipham et al., 2015).

4.3 *Animal Washes*

The operational paradigm of dairy and feedlot operations consists of routinely purchasing and transporting animals into operations (Adhikari et al., 2009; Hermesch et al., 2008). Prior to entering the abattoir, the hides of cattle are often contaminated with excrement, dust, and/or mud that frequently contain pathogenic bacteria (Bacon et al., 2000; Bricha-Harhay et al., 2011; Kalchayanand et al., 2009a). This could be due to wind or muddy conditions at the time of shipping, the close confinement during transportation, the length of transport, and/or the facilities used for lairage (Dewell et al., 2008; McEachran et al., 2015; Reicks et al., 2007). Carcass pathogen intervention systems have been widely studied; however, minimal research efforts have been directed toward the effects of intervention systems applied to animals prior to entry into the abattoir (Mies et al., 2004).

4.4 *Bacteriophages*

The use of Bacteriophages could be an intervention in the live animal operations according to research by Xie et al. (2016). In this study three feedlots in south Texas were utilized and 27 samples from various locations were collected. Recovery of bacteriophages in *Salmonella*-free environments suggests that phages might play a role in suppressing populations in the feedlot environment (Xie et al., 2016).

5   *Salmonella* Contamination of Beef Carcass Surfaces and Ground Beef

Despite implementation of pre-harvest interventions, post-harvest measures are also necessary to mitigate contamination that commonly occurs during the harvesting and disassembly (aka fabrication) process. It is widely known that beef carcasses can become contaminated with microorganisms such as *Salmonella* during the harvesting process. As thoroughly discussed in the previous section, cattle are natural carriers of *Salmonella* and as such, it is often found on their hides. Hide removal as well as evisceration are harvesting processes that provide an opportunity for contamination of the carcass (Bell, 1997; Brichta-Harhay et al., 2008). Cross-contamination during fabrication is another potential hazard. The prevalence of *Salmonella* on beef carcasses, albeit low, remains cause for concern regarding public health and prevention of *Salmonella*-related illnesses. Many interventions have been employed throughout the harvesting and fabrication processes to lower, if not eliminate, pathogen contamination of beef carcasses.

5.1 *Prevalence of Salmonella on Beef Carcasses*

In a study conducted by Rivera-Betancourt et al. (2004), the prevalence of *Salmonella* on the hides of cattle and on the carcass, both pre-evisceration and post-application of interventions was investigated. These samples were collected from two facilities, Plant A was in the southern U.S. and Plant B was located in the northern U.S. Collections were conducted in April, May, July, August, and October. These facilities employed the following post-harvest interventions: steam vacuum, knife trimming, pre-evisceration carcass wash, and a post-evisceration carcass wash.

Overall prevalence of *Salmonella* was significantly higher on the hides of cattle at Plant A, and the prevalence of *Salmonella* on fence panels was also higher at Plant A. Although the prevalence of *Salmonella* on hides was high (91.8%; n=510), the prevalence of *Salmonella* on the carcass swabs taken both pre-evisceration and after application of interventions was markedly lower in Plant A. Carcass swabs taken prior to evisceration showed a 23.3% (n=511) and 26.8% (n=522) prevalence in Plants A and B, respectively. After all post-harvest interventions had been employed, swabs of the carcass were taken again. Prevalence of *Salmonella* at this point was 0% (n=499) in Plant A, and 0.8% (n=520) in Plant B. This reduction in prevalence demonstrates the efficacy of post-harvest interventions used at these two slaughter facilities (Rivera-Betancourt et al., 2004).

In a similar prevalence study, Barkocy-Gallagher et al. (2003) investigated the prevalence of *Salmonella* in feces, on the hides of cattle, and on the carcass pre-evisceration and after post-harvest interventions at three fed-beef slaughter plants located in the midwestern U.S. Animals were tracked throughout the harvesting process, and all sample types were collected from the same animal. Investigators collected samples in four

separate seasons: spring, summer, fall, and winter. Spring was defined as late April through early May, summer as August, fall as late October through mid-November, and winter as late January through mid-February. The authors reported the highest prevalence during the summer and fall time frames. As observed in pre-harvest facilities, seasonal effects on the prevalence of *Salmonella* have also been well demonstrated in harvesting facilities by many studies.

In this study, fecal samples were collected from each animal to identify the prevalence of animals shedding *Salmonella*. The authors determined fecal prevalence to be 2.1 (n=285), 9.1 (n=287), 2.8 (n=218), and 2.5% (n=197) during spring, summer, fall and winter, respectively. The prevalence of *Salmonella* in feces was much lower than what was reported on hides. *Salmonella* was recovered from 61.4 (n=306), 91.6 (n=321), 97.7 (n=219), and 27.7% (n=220) of hide samples during spring, summer, fall and winter, respectively. Pre-evisceration carcass swabs were collected immediately after hide removal and prior to the first carcass wash. Pre-evisceration carcass swabs are valuable in that they measure not only the transfer of pathogens from the hide to the carcass, but they also can be compared to post-intervention carcass swabs to determine the efficacy of the interventions applied. Prevalence of *Salmonella* on pre-evisceration carcasses was reported as 3.0 (n=305), 19.7 (n=319), 24.9 (n=217), and 4.1% (n=219) during spring, summer, fall and winter, respectively. During the summer, *Salmonella* was recovered from only 0.3% (n=301) post-intervention carcasses with an overall prevalence of 0.1% (n=1016). In this case, *Salmonella* was detected in the feces, on the hide, and on the pre-evisceration carcass of the same animal (2003).

A study conducted by Bacon et al. (2002) aimed to determine the prevalence of *Salmonella* on the hides of beef cattle and on the carcass of the same animal. Carcass swabs were obtained after application of decontamination strategies, and each carcass was swabbed at the brisket, flank, and rump using a single swab. Samples were collected from five steer-heifer facilities (labeled 1-5) and three cow-bull facilities (labeled 6-8). These facilities are commercial beef packing plants geographically dispersed throughout the U.S. At the time of the study, Plants 1-4 employed the following intervention strategies: steam vacuum, pre-evisceration carcass wash, pre-evisceration application of an organic acid solution, thermal pasteurizing, post-evisceration carcass wash, and a post-evisceration organic acid solution rinsing. Plant 5 used the same strategies except for the pre- and post- evisceration organic acid solution. Plants 6-8 applied the following interventions to carcasses: steam-vacuum, thermal pasteurizing, post-evisceration carcass wash and a post-evisceration lactic acid rinse (Bacon et al., 2002). Overall, the prevalence of *Salmonella* on the hides of cattle sampled at these facilities was 15.4% (n=319), and the prevalence on carcasses was 1.3% (n=319). Results indicate the decontamination treatments used at these plants are effective at reducing *Salmonella* contamination (Bacon et al., 2002).

Contamination can occur at different anatomical locations on the carcass. Sofos et al. (1999b) collected baseline contamination data at seven slaughter plants: four steer-heifer and three cow-bull facilities. Collections were made during a dry season defined as November to January and a wet season defined as May to June. Samples were excised from three anatomically distinct sites on each carcass (brisket, flank, and

rump). These sites are used by the USDA-FSIS to test for contamination. Swabs were taken at each of the following points in the harvesting process: pre-evisceration, after the final carcass wash just before carcass chilling, and following the 24-hour chilling period (Sofos et al., 1999b).

Presented in **Table 1** are the percentage of *Salmonella*-positive samples from the brisket, flank, and rump taken at the same three points in the slaughtering process. For all sample types, 100 cm^2 of surface tissue was excised. Brisket samples were taken anterior to the navel along the ventral midline; flank samples were taken posterior to the navel on the ventral midline; and rump samples were obtained from the cushion of the round. After the 24-hour chilling period, the average prevalence of *Salmonella* in steer-heifer plants at the brisket, flank, and rump was 0.8 (n=120), 0 (n=120), and 2.5% (n=120), respectively, during the wet season and 0.8 (n=120), 0 (n=120) and 0% (n=120), respectively, in the dry season. At cow-bull facilities, the average prevalence of *Salmonella* after the 24-hour chilling at the same three locations was 4.4 (n=90), 2.2 (n=90), and 1.1% (n=90), respectively, in the wet season, and 2.2 (90), 1.1 (90) and 0% (90), respectively, during the dry season (Sofos et al., 1999b). The results of this study demonstrate the effectiveness of interventions applied to the carcass at these facilities.

Table 1. Percentage of Salmonella-positive samples collected from three locations on the beef carcass, at three points in the harvesting process.

Fed Cattle		Wet	Dry
Pre-evisceration	Brisket	3.9%	4.2%
	Flank	0.8%	1.7%
	Rump	3.3%	5.0%
Post-carcass Wash	Brisket	0%	0.8%
	Flank	0%	0.8%
	Rump	1.7%	0.8%
Post 24-hour Chilling	Brisket	0.8%	0.8%
	Flank	0%	0%
	Rump	2.5%	0%
Cow-Bull			
Cow-Bull Pre-evisceration	Brisket	15.5%	5.5%
	Flank	5.5%	2.1%
	Rump	2.5%	0%
Post-carcass Wash	Brisket	6.7%	3.3%
	Flank	1.1%	1.1%
	Rump	1.1%	1.1%
Post 24-hour Chilling	Brisket	4.4%	2.2%
	Flank	2.2%	1.1%
	Rump	1.1%	0%

A study by Villarreal-Silva et al. (2016) was intended to simulate pathogen cross-contamination that would occur in a post-harvest environment. At three different abattoirs, brisket areas of 13 hide-on carcasses were inoculated with a slurry containing a cocktail of fluorescent *Escherichia coli* biotype I, which is an approved surrogate for *Salmonella*. Samples were taken from carcasses after hide opening, prior to and after evisceration, after splitting, and after final intervention. Additionally, samples were taken from the environment (floor, walls, air), equipment (knives, meat hooks, hide pullers, and splitting saws), and personal garment (gloves, boots, aprons). Results showed cross-contamination occurred between the inoculated hide and the carcass as well as transfer to the adjacent, noninoculated carcasses (Villarreal-Silva et al., 2016). Cross-contamination also occurred from carcasses to environment, personal garments, and equipment. Counts of surrogate bacteria were highest on equipment (15%), then environmental samples (10%), then with personal garments having the lowest contamination (7%). Surrogates were undetected (<1.4 log CFU)/300cm^2) in all abattoirs after the final intervention stage (Villarreal-Silva et al., 2016).

These studies and others show that current intervention strategies are effective at significantly reducing *Salmonella* contamination of beef carcasses to very low numbers. Understanding the potential contamination of the carcass at different locations is essential to employing effective interventions. Certain intervention strategies may be more effective on a portion of the carcass as opposed to other areas. Knowing where contamination is likely to occur and at what stage in the harvesting and dressing process that part of the carcass is most vulnerable is imperative to an effective multi-hurdle, post-harvest decontamination strategy.

5.2 *Prevalence of Salmonella in Ground Beef*

Even with the previously discussed reduction, *Salmonella* contamination on carcasses can still lead to contaminated whole muscle cuts or ground beef products. From 1973-2011, of the 1965 outbreaks associated with food, 96 were attributed to beef. There was a shift in this time period from the type of beef implicated, from whole muscle roasts to ground beef. Ground beef emerged as a vehicle of transmission in the 2000s and was implicated in 17 of the 38 beef-attributed outbreaks reported during 2002-2011 (Laufer et al., 2015).

Several studies have been conducted throughout the U.S. to determine the occurrence of *Salmonella* in retail ground beef. On average prevalence has ranged from 3.5-4.2% of samples testing positive. These studies were conducted in different cities and at different times throughout the year to gain an accurate understanding of prevalence in retail (Bosilevac et al., 2009; Samadpour et al., 2005; Zhoa et al., 2002).

In the past, it was thought that surface contamination of the carcass was the most common cause of contamination in ground beef, with cross-contamination via food contact surfaces also playing a role (Bell, 1997; Huffman, 2002). However, it is important to note that recent studies have revealed *Salmonella* is harbored within several cattle lymph nodes commonly incorporated into ground beef via trim (Arthur et al., 2008a; Li et al., 2015). These lymph nodes will be further discussed in section 6. This information is important when considering application and mode of action of currently used post-harvest interventions.

.

6 Post-harvest Interventions

Several studies including those aforementioned, have shown that a multi-hurdle approach of post-harvest interventions markedly reduces prevalence of *Salmonella* on the surface of beef carcasses. These interventions fall into several categories including physical decontamination of the carcass, the use of acid antimicrobials and oxidizer antimicrobials, thermal interventions and non-thermal interventions. Each of these mediations works in a unique fashion to reduce or eliminate pathogenic bacterial contamination, including *Salmonella*, of the beef carcass.

6.1 *Physical Interventions*

Physical decontamination refers to removal of visible contamination on the carcass. This is accomplished using several methods including knife trimming, the use of ambient temperature water for rinsing the carcass, and steam-vacuuming. Knife trimming has been shown to be an effective method to remove visible contamination such as hair, fecal material, or ingesta. Prasai et al. (1995) excised samples of the surface of beef carcass sides in a commercial slaughter plant. Samples were collected from carcasses classified as the following: not trimmed and not washed (NTNW), trimmed but not washed (TNW), trimmed and washed (TW) or not trimmed but washed (NTW). The mean aerobic plate counts (APC) were reported. When compared to the NTNW carcasses, the TNW carcasses saw a 3.0 $\log_{10}$ colony forming units (CFU) per cm^2 reduction in total APC. The TW saw a 0.9 $\log_{10}$ CFU/ cm^2 reduction, and the NTW carcasses showed a 0.3 $\log_{10}$ CFU/cm^2 reduction. These results indicate that trimming is an effective means of decontamination. Since, carcasses that had been trimmed and washed showed APC counts that were 2 $\log_{10}$ CFU/cm^2 higher than those that were only trimmed, a possible conclusion is that washing with ambient temperature water (i.e. not using hot water or an antimicrobial wash) could potentially spread contamination to adjacent areas of the carcass (Prasai et al., 1995). While knife trimming is an acceptable corrective action for visible contamination, it is not sufficient in itself to remove all contamination, as microbial contamination is not visible.

Steam vacuuming is another method to remove visible contamination, especially along the lines of the hide removal pattern or small spots on the carcass (Wheeler et al., 2014). The steam vacuum is a handheld device and removes bacterial and visible contaminants by applying steam and/or hot water, typically 88-94°C, while simultaneously vacuuming the area. Steam vacuuming has been shown to reduce contamination as effectively as knife trimming, reducing aerobic plate counts as much as 3 $\log_{10}$ CFU per cm^2 (Dorsa, 1996). Steam vacuuming is not effective on the entirety of the carcass as it is difficult to use along the awkward angles and curves of a beef carcass. Steam vacuuming is approved by the USDA-FSIS as a substitute for knife trimming to remove visible contamination (Wheeler et al., 2014).

6.2 *Acid Antimicrobials*

Many acid antimicrobials are used in commercial beef plants to reduce contamination. Organic acids are the more commonly used and studied agents. These include acetic, citric, and lactic acids. There are many factors that influence the effectiveness of these acids including concentration, pH and pKa (Baird-Parker,

1980). It is thought that these acids interfere with the transmembrane proton gradient of microbial cells and with structures of the cell surface, which disrupt nutrient transport and microbial growth (Brown and Booth, 1991; Corlett and Brown, 1980).

To date, most organic acids are permitted for use at 1.5 to 2.5% of the solution for carcass washing in commercial beef plants (USDA-FSIS, 1996); however, some can be used at levels up to 5% concentration. Organic acids are applied as a rinse to the surface of the carcass. This rinse is most commonly applied immediately prior to entering the cooler; however, it can be and is used at other points in the slaughter process, such as prior to evisceration and/or after hide removal. These organic acid treatments have been shown to be more effective when applied as a warm (i.e. 50 to 55°C) carcass rinse (Barkate et al., 1993). Several factors can influence the efficacy of the acid treatment such as whether the bacteria are protected on the carcass surface (by a crevasse in the fat) such that the organic acid does not reach the bacteria (Wheeler et al., 2014).

Lactic acid is one of the most widely used organic acids in the meat industry due to a combination of effectiveness and cost (Wheeler et al., 2014). Many research studies have shown the efficacy of using lactic acid as an intervention to decrease *Salmonella* and other bacterial populations (Arthur et al., 2008c; Katawal et al., 2018; Laury et al., 2009; Pokharel et al., 2016; Yang, 2017;). It has been reported that use of lactic acid reduces aerobic plate counts by 1.5 log$_{10}$ CFU/cm^2 (Huffman, 2002). The combination of various organic acids has proven to be effective at reducing bacterial contamination as well. It has been shown that spraying for 20 seconds with a commercially available product that consists of a blend of lactic and citric acids reduced the population of *Salmonella* by 1.1 log CFU/100cm^2 on inoculated fresh beef (Laury et al., 2009). In a study that compared several decontamination treatments, lactic acid reduced *Salmonella* from by 1.80 log CFU/cm^2 (Arthur et al., 2008c). Another study examined the relationship of heated (52℃) and unheated (21℃) lactic acid on the reduction of *Salmonella* populations on beef carcass surface tissue. Lactic acid reduced *Salmonella* populations by 2.4 log CFU/cm^2 for both heated and unheated. Regardless of temperature lactic acid showed a significant reduction (0.3 log CFU/cm^2) and showed to be an effective intervention for bacterial populations on beef carcass surface tissue (Yang et al, 2017).

6.3 *Oxidizer Antimicrobials*

Another category of a post-harvest intervention is oxidizer antimicrobials. These can include peroxyacetic acid, electrolyzed oxidized (EO) water, or acidified sodium chlorite (ASC). Peroxyacetic acid is approved by the USDA-FSIS for use in commercial beef plants at a maximum of 1800ppm (FDA, 2015), although it is generally used at 200ppm. King et al. (King et al., 2005) reported that use of peroxyacetic acid prior to chilling reduced *Salmonella* by 0.7 log$_{10}$ CFU/cm^2 on the carcass surface.

Recently, electrolyzed oxidized water emerged as an intervention in the food industry. Electrolyzed oxidized water (EO) is made by passing a current of electricity through a diluted saltwater solution. A product of the reaction is sodium hydroxide (NaOH), and the other is hypochlorous acid, which has a low pH, contains active

chlorine, and has a strong oxidation reduction potential like that of ozone (Ayebah and Hung, 2005; Wheeler et al., 2014). Arthur et al. (2008c) tested the use of EO water on *Salmonella* contamination of beef carcasses and reported a reduction ranging from 0.57 to 0.75 $\log_{10}$ CFU/cm^2.

Acidified sodium chlorite is approved for use between 500 and 1200 ppm in the commercial beef industry (UDSA-FSIS, 2013). Acidified sodium chlorite works through the oxidative effect of chlorous acid, which is derived from the conversion of chlorite ions into its acid form under acidic conditions such as mixing with citric acid or phosphoric acid (Wheeler et al., 2014). Acidified sodium chlorite has been proven to successfully reduce *Salmonella* contamination on beef carcasses as demonstrated by the results of a study that compared the effectiveness of a water wash to both phosphoric acid-activated acidified sodium chlorite and citric acid-activated acidified sodium chlorite on *S*. Typhimurium contamination (Castillo et al., 1999). The investigators reported a reduction of 2.3 log CFU/cm^2 when using the water wash. With the use of phosphoric acid-activated ASC a reduction of 3.9 log CFU/cm^2 was observed and, with the citric acid-activated ASC, a 4.6 log CFU/cm^2 reduction was seen. In other studies, a reduction of 1.9 to 2.3 log CFU/cm^2 in both *Salmonella* and *E. coli* O157:H7 has been reported when using a spray wash of sodium chlorite activated with citric acid (Ransom et al., 2003).

Hypobromous acid is an antimicrobial agent that has been used in processing water for specific food products. In the beef industry, it is used at 300ppm for carcass surface decontamination (Wheeler et al., 2014). Hypobromous acid reduced *Salmonella* on fresh beef by 0.7 log CFU/cm^2. The same study showed a reduction in aerobic plate count and Enterobacteriaceae by 2.8 to 3.6 log CFU/cm^2, respectively (Kalchayanand et al., 2009b). Use of hypobromous acid was common in beef processing up until 2013 when it was removed from the list of Pathogen Reducing Technologies approved in the USDA-FSIS export requirements for Japan (USDA-FSIS, 2014).

6.4 *Thermal Interventions*

Heat treatment is used as an intervention in many food processing environments including beef production. Steam vacuuming, which was mentioned earlier is a combination of physical and thermal treatments, as it uses hot water and the vacuum to remove contamination (Wheeler et al., 2014). Hot water is also used as an intervention step on its own. Hot water wash cabinets are common in beef processing plants as pre-evisceration and final carcass interventions (Wheeler et al., 2014). Many studies have been conducted that investigated the use of water at temperatures ranging from 74°C up to 95°C. The USDA-FSIS acknowledges that water greater than 74°C will produce a sanitizing effect (Huffman, 2002). Arthur et al. (2008c) reported that the use of a hot water (i.e. 74°C) wash for 20 seconds reduced *Salmonella* contamination on the carcass by 1.04 to 2.10 log CFU/cm^2. Other studies have shown reductions up to 3 $\log_{10}$ cycles using hot water washes at temperatures as high as 95°C (Huffman, 2002).

A similar treatment to hot water washes is steam pasteurization. Steam pasteurization cabinets are often used as a final carcass intervention in U.S. beef processing plants. Since steam at 100°C has a higher heat

capacity than water at the same temperature, the steam can raise the surface temperature of the carcass much quicker (Wheeler et al., 2014). Phebus et al. (1997) reported that steam pasteurization markedly reduced *S.* Typhimurium on the surface of beef by a count reduction of 3.7 log CFU/cm^2. In another study, steam treatment for 6 seconds reduced *Salmonella* counts by approximately 3 log CFU/cm^2 (Wheeler et al., 2014).

6.5 *Non-thermal Interventions*

As previously discussed, thermal treatments are highly effective at reducing contamination on beef carcasses. One drawback of thermal interventions is the physical and chemical changes that are attributed to these treatments. Non-thermal technologies are either in use or are being investigated to provide alternate interventions to reduce quality loss associated with thermal interventions.

Ultraviolet (UV) light irradiation is often used for decontamination of surfaces and water in hospitals and laboratories. UV treatment has been used in water purification for years and research into application of UV to foods is ongoing (Chun et al., 2010; Wheeler et al., 2014). The UV light works by causing damage to DNA leading to cell death (Chun et al., 2010). The use of UV-C (wavelength of 220-300 nm with 90% of emission at 253.7 nm, which has been proven as an effective wavelength for bactericidal activity) has been approved by FDA for use on food products to control microorganisms (Chun et al., 2010; FDA, 2007; Wheeler et al., 2014). Using UV-C is not expensive and does not require the use of chemicals or heat. The effectiveness of UV-C light treatment against *Salmonella* has been reported on poultry. Chun et al. (2010) observed a 1.19 log CFU/cm^2 reduction of *S.* Typhimurium on chicken breasts. Sensory aspects were evaluated in this study as well, with no differences observed.

Electron beam (E-beam) irradiation technology has recently evolved to a point where low-dose, low-penetration E-beam irradiation can be used to effectively treat large, non-uniform surface areas such as an entire carcass side after chilling (Arthur et al., 2005). The E-beam only has about 15mm of penetration, so the surface of a carcass can be treated without adverse effects on the quality of products. It has been demonstrated that E-beam radiation of chilled beef primals reduced *E. coli* O157:H7 by 4 log CFU/cm^2, with no adverse effects on quality attributes (Arthur et al., 2005). The effect of E-beam irradiation on *Salmonella* has been studied using poultry products as well. The investigators reported that 40% of the control chicken breast samples were positive for *Salmonella*, while none of the of chicken breast samples exposed to electron beam irradiation yielded a positive result (Lewis et al., 2002).

Research has also been conducted on the use of bacteriophages in red meats and poultry and their effect on *Salmonella* populations in the final ground product (Yeh et al., 2017). In this study bacteriophages were added during tumbling of ground meat. The meat trim was inoculated to result in a contamination level of 7.0 log CFU/g. Following tumbling for ground beef *Salmonella* populations were reduced by 1.1 log CFU/g. This study shows the potential use of bacteriophage application for control in ground products (Yeh et al., 2017).

6.6 *Multiple Hurdle Strategy*

It is widely understood that no single intervention is 100% effective. This is due to the variation in pathogen susceptibility to interventions, and in part to the non-uniform beef carcass surface, which provides opportunities for pathogens to avoid contact with interventions. Using a multi-hurdle approach with interventions used in sequence may result in synergistic or additive effects and provide greater efficacy of decontamination strategies (Rao et al., 149; Wheeler et al., 2014; Yeh et al., 2017).

Understanding of the best carcass dressing practices has greatly improved over the years, and the implementation of post-harvest interventions has markedly improved the safety of beef. The combination of physical decontamination methods and use of antimicrobial compounds such as organic acids has contributed to this improvement.

7 *Salmonella* in Bovine Lymph Nodes

Contamination of beef products remain a concern for public health. Recent studies have implicated lymph nodes of cattle as a mode of *Salmonella* contamination of ground beef products (Arthur et al., 2008a; Gragg et al., 2013a, 2013b; Haneklaus et al., 2012; Koohmaraie et al., 2012; Samuel et al., 1981; Samuel et al., 1979). With lymph nodes being a cause of contamination, it is imperative to continue researching to understand the epidemiology, route of entry, and potential interventions that can be implemented to reduce occurrence of *Salmonella* in the lymph nodes. Further understanding will aid in reducing or eliminating *Salmonella* in beef products.

7.1 *Introduction to Salmonella in Bovine Lymph Nodes*

As previously discussed, interventions have been developed and implemented in the production process in order to mitigate risks associated with surface contamination at critical control points within the production process. Many of these intervention strategies are based on our understanding that pathogens commonly enter ground beef products by way of surface contamination on beef trim. Previous discussion of interventions has shown tremendous reductions in surface decontamination for *Salmonella*. As a result of this ongoing food safety concern, the beef industry is investigating alternative routes of contamination with the anticipation of developing a strategy to mitigate the burden of *Salmonella* in ground beef (Arthur et al., 2008a; Gragg et al., 2013; Haneklaus et al., 2012; Koohmaraie et al., 2012). Recent publications have provided evidence that pathogen contamination of ground beef products may also occur via the animal's lymphatic system, specifically through the inclusion of PLNs in ground beef products (Arthur et al., 2008a; Gragg et al., 2013a, 2013b; Haneklaus et al., 2012; Koohmaraie et al., 2012; Li et al., 2015; Samuel et al., 1979).

Lymph nodes, which are in the adipose tissues of the animal, act as a filtration system to sequester and destroy invaders such as bacteria and viruses within the body The presence of *Salmonella* in PLNs is problematic, as PLNs are a common component of beef trimmings incorporated into ground beef products in usual proportions; e.g., inclusion is a result of proximity to the beef trimmings utilized. . Li et al. (2015)

reported that in a 2000 lb production lot, on average over 90% of the *Salmonella* CFU load originated from lymph nodes. Because *Salmonella* is encapsulated within PLNs, in-plant surface decontamination interventions cannot make contact with the pathogen, thus rendering the control measures insufficient; consequently, implementation of current pathogen reduction and HACCP plans may not be the appropriate methodology necessary to address this specific food safety hazard.

7.2 *Epidemiological trends of Salmonella in Peripheral Lymph Nodes*

Importantly, it has been noted that *Salmonella* can be recovered from various PLNs that are distributed throughout the carcass (Gragg et al., 2013a; Koohmaraie et al., 2012). While *Salmonella* has been recovered from lymph nodes of differing anatomical origins, widespread dissemination of *Salmonella* throughout the lymphatic system does not appear to be common (Gragg et al., 2013a). It has been hypothesized that *Salmonella* may enter into the lymphatic system through individual, independent events (Gragg et al., 2013a), though discussion regarding hypothesized routes of entry will be further discussed in the next section.

Many early lymph node studies focused their efforts on investigating *Salmonella* in the mesenteric lymph nodes (Arthur et al., 2008a; Samuel et al., 1981; Samuel et al., 1979; Sofos et al., 1999a); however, it is noteworthy that gastrointestinal tract (GIT)-associated lymph nodes, such as these, are discarded during the evisceration process and, thus, do not pose a direct food safety hazard. In contrast, PLNs that reside in the adipose tissues are associated with important muscle cuts; it is these PLNs that should be used to determine the magnitude of the food safety hazard posed by harborage of *Salmonella* in the PLNs of healthy cattle presented for harvest, as they have greater potential to be incorporated into ground beef products (Arthur et al., 2008). Indeed, a recent risk assessment – albeit limited by available data and parameter estimates – indicated that the contribution of *Salmonella* in ground beef is largely from PLNs compared to *Salmonella* from the carcass surface (Li et al., 2015). Due to the complexity in removing certain lymph nodes at harvest, many recent studies have focused on examining PLNs that are more accessible during harvest and may be important in regard to food safety, including the popliteal, pre-scapular (chuck), and subiliac (flank), some of which are illustrated in **Figure 4** (Arthur et al., 2008a; Gragg et al., 2013b; Samuel et al., 1979). As exploration of *Salmonella* in the PLNs is in the early stages, publications and data are relatively scarce regarding epidemiological trends associated with harborage. Preliminary research suggests that prevalence of *Salmonella* in PLNs of healthy cattle presented for harvest can range between 1.6 and 88% (Arthur et al., 2008a; Gragg et al., 2013a, 2013b; Haneklaus et al., 2012; Koohmaraie et al., 2012). It should be noted, however, that prevalence of *Salmonella* in small PLNs is uncommon; this is true even in regions and cattle types in which *Salmonella* has been commonly recovered from large PLNs (such as the subiliac lymph node; Loneragan, data unpublished).
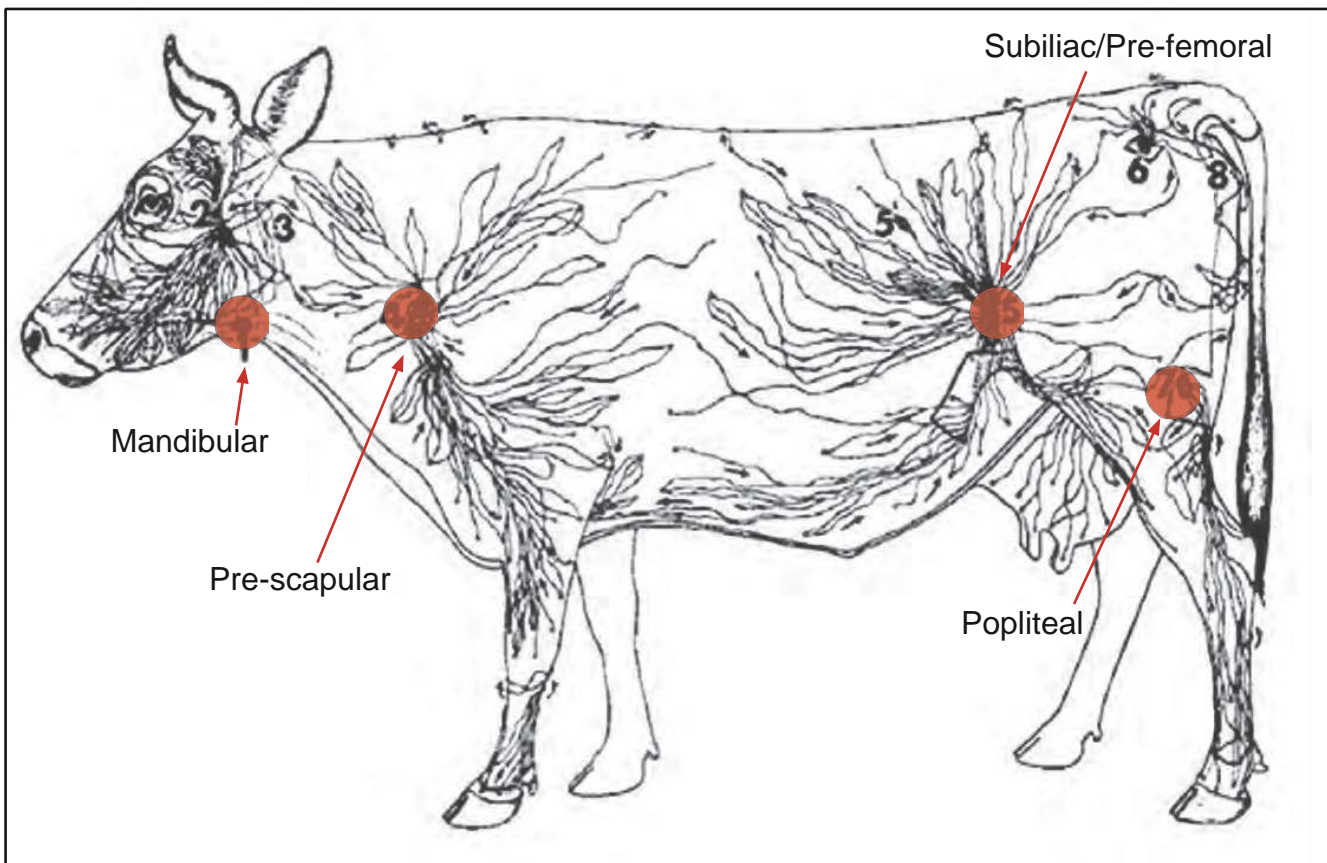
Figure 4. Mandibular, pre-scapular, subiliac/pre-femoral, and popliteal location identified on the superficial lymph flow diagram of a cow as presented by Saar and Getty in Anatomy of Domestic Animals.

In an exploratory study by Gragg et al. (2013b), an overall mean prevalence of 7.5% was observed in 3,327 subiliac lymph nodes. Importantly, the authors reported trends suggesting that harborage of *Salmonella* may be affected by factors such as animal-type (i.e. feedlot and cull animals), season, and region (Gragg et al., 2013b); moreover, the authors reported that the overall mean prevalence may have been skewed by these variables. Upon stratification of the data, it was observed that the prevalence of *Salmonella* was greater in the feedlot cattle populations relative to cull cattle populations (Gragg et al., 2013b). *Salmonella* prevalence in the cull cattle populations remained consistently low (0.65%) and did not appear to be affected by region or season. Alternatively, *Salmonella* prevalence in the feedlot cattle populations appeared to be low in the cooler season yet peaked in the warmer season, particularly in the southwest region of the U.S. (Nickelson et al., 2018; Webb et al., 2017). An additional study evaluating *Salmonella* prevalence in lymph nodes collected from cattle presented for harvest in Mexico supports the findings of a seasonal and regional trend (Gragg et al., 2013b). As previously discussed, similar trends have been observed in fecal, hide, environmental, and food sample data (Barkocy-Gallagher et al., 2003; Edrington et al., 2004). Alternative influential variables have been hypothesized including animal temperament, animal stress levels, management styles, feeding regiments, animal origins, and environmental factors (Gragg et al., 2013b; Haneklaus et al., 2012; Nickelson et al., 2018). In addition to the seasonal and regional trends, *Salmonella* prevalence in PLNs may also vary among feedlot facilities within the same geographic region (Haneklaus et al., 2012; Belk et al., 2018). These findings may illustrate that variables at a feedlot level, such as animal husbandry practices, animal origin, and environmental factors, may greatly influence *Salmonella* harborage in the PLNs. Further research will need to

be conducted to understand further differences such as age, immune function, and other factors not yet identified and their role in prevalence of *Salmonella* in cattle (Brown et al., 2015).

In addition to potential seasonal, regional, and animal type variation (Gragg et al.,2013b; Nickelson et al., 2018; Webb et al., 2017), data regarding concentrations of *Salmonella* in PLNs has been established (Gragg et al., 2013b; Webb et al., 2017). Concentrations were reported to range between 1.6 to 4.9 $\log_{10}$ CFU/PLN (Webb et al., 2017). While many of the PLNs that tested positive for *Salmonella* were below the limit of quantification (LOQ), 33% were found to harbor *Salmonella* at concentrations above the LOQ with the methods deployed in the study. Although the grinding process associated with the production of ground beef may dilute any *Salmonella* encapsulated in the PLNs, higher concentrations of *Salmonella* represent an important public health problem that may be contributing to the overall detection of *Salmonella* in ground beef products.

Also noteworthy is the characterization of the *Salmonella* isolates collected from PLNs. These isolates can present potential risks associated with product contaminated by Salmonella serotypes harboring virulence factors or antimicrobial resistance exist. Various *Salmonella* serotypes have been recovered from PLNs, with some publications reporting as many as 24 different serotypes (Gragg et al., 2013b). While some diversity has been observed, majority of the isolates reported were *S*. Montevideo and *S*. Anatum (44 and 25%, respectively), which have also been reported as the two most commonly isolated serotypes in ground beef products (Anderson et al., 2001; USDA-FSIS, 2011; Gragg et al., 2013b). Webb et al. (2017) reported that the most common serotypes isolated from PLNs were Montevideo (26.9%), Lille (14.9%), Cerro (13.0%), Anatum (12.8%), and Dublin (6.9%). *S*. Typhimurium and *S*. Newport, which are commonly associated with human illness, have been recovered from PLNs; while the prevalence of these serotypes was reported to be relatively low, it is noteworthy that these were largely isolated from cull animal populations (Gragg et al., 2013b). In 2013, antimicrobial susceptibility testing revealed that a majority (86%) of *Salmonella* isolates collected from PLNs were pansusceptible, 8.3% were MDR, which (as opposed to the discussion above) was defined as resistance to two or more antimicrobial classes (Gragg et al., 2013b). More recent data has shown that majority of isolates were still pansusceptible (80.6%), however 10.7% of isolates were MDR (Webb et al., 2017). Notably, MDR phenotypes were also more commonly isolated from cull cattle PLNs relative to feedlot cattle PLNs. This may indicate that while *Salmonella* prevalence in cull animal populations remains relatively low throughout all regions and seasons, the presence of medically important serotypes and MDR strains within this population may warrant further investigation to decrease the potential risk imposed.

7.3 *Route of Entry*

The growing recognition of *Salmonella* in PLNs has also generated questions regarding the route by which *Salmonella* infects the PLNs and the duration of infection (Edrington et al., 2013a; 2013b; Gragg et al., 2013b). Upon entry into the body, an intruder, such as a virus or bacteria, is recognized and engulfed by the cells of the immune system to be transported to the lymph node for destruction. The lymphatic system works in branches, with various parts of the body draining to specific lymph nodes within relatively close anatomical

regions (**Figure 4**). The subiliac lymph node, for example, is responsible for the filtration of lymph draining from the skin of the abdominal wall, pelvis region, and hind limbs (Edrington et al., 2013a, 2013c; Gragg et al., 2013b). Experimental models have been performed, in which cattle were challenged with *Salmonella* via hypothesized routes of infection, namely oral, subcutaneous injections, and intradermal injections, with the objective of achieving PLNs that are predictably *Salmonella*-positive. As *Salmonella* is typically associated with the GIT, initial hypotheses proposed that *Salmonella* in the PLNs might originate from the GIT. Exploratory challenge models conducted by Edrington et al. (2013a) demonstrated that *Salmonella* can reach the PLNs via oral exposure. While natural oral inoculation is not an impractical route of infection, the observations suggested that the concentrations of oral exposure necessary to achieve PLNs that are predictably *Salmonella*-positive at a detectable level are substantial and may not be typical of naturally occurring environmental settings (Edrington et al., 2013a).

Consequently, alternative hypothesized routes of infection have been investigated in which *Salmonella* infection of the PLNs occurs via transdermal routes, namely insect bites or abrasions on the hide, and is then drained to the regional PLNs (Edrington et al., 2013b; Gragg et al., 2013b; Samuel et al., 1979). Gastrointestinal colonization typically occurs via *Salmonella* founder cells, but migration of bacteria from intradermal delivery sites are less known. Transmission of bacteria from the gastrointestinal tract to the lymphatic system is frequently observed but, there is little occurrence of founder *Salmonella* cells found in lymph nodes (Porwollik et al., 2018). The transdermally delivered isolates were present in the animal's gut environment but was a more rare occurrence. Bacteria is known to drain from the transdermal injection sites into the PLN while subiliac/prefemoral nodes harbored bacteria that had been delivered to the rear legs. Peripheral lymph nodes also harbored orally delivered bacterial clones (McClelland and Edrington, unpublished; Porwollik et al., 2018). One experimental inoculation method involved a tuberculin syringe to administer *Salmonella* to the leg of the animal at an intradermal depth. While the authors reported that this method produced predictably positive PLNs, it also resulted in swelling and lameness in the treated animals, presumably due to difficulties in governing the penetration depth of the needle (Edrington et al., 2013b). As a result, a transdermal challenge model was developed in which various *Salmonella* serotypes were applied to the skin of the animal using a multi-prong inoculator allergy skin-testing device that allowed for greater control of penetration depth during application (Edrington et al., 2013a; 2013b). This method was also able to yield predictably positive PLNs in the corresponding region of the animal that was inoculated with *Salmonella* without resulting in swelling and lameness. While it was reported that this device produces PLNs with *Salmonella* concentrations above the limit of detection for at least eight days post-inoculation, a salient limitation of this approach is that these concentrations were below the LOQ (Edrington et al., 2013b).

As previously discussed, insects that commonly inhabit CAPF can be persistent carriers of bacteria associated with foodborne illness, including, but not limited to, *Salmonella*. A recent publication identified biting flies, such as horned flies (*Haemetobia irritans*), as an opportune route of entry for *Salmonella* to breach the skin barrier, thus resulting in drainage into the regional lymph node as part of the animal's immune response (Olafson et al., 2014). It has been illustrated that *Salmonella* harborage can occur in the fly's mouthparts and

digestive tracts, a contamination that may transpire through grooming practices or while pursuing fresh fecal pats for egg deposition. As previously discussed, it is through such events that flies, when feeding, may mechanically transmit the bacteria from the animal's hide or environment into lesions created in the skin barrier. (Olafson et al., 2014). Following a single inoculation, *Salmonella* has been shown to persist in cattle for approximately 28 days, yet in production settings a repeated inoculation will typically occur which can allow for a continuous route of infection (Edrington et al., 2016; Olafson et al., 2016).

7.4 *Potential Interventions*

Harborage of *Salmonella* in lymph nodes is an important food safety hazard and development of effective and practical solutions is necessary to mitigate the risk. Removal of all lymph nodes from the bovine carcass during fabrication is not a practical solution. Hundreds of lymph nodes are present in varying sizes, thus complete removal would be tedious, time consuming, as well as expensive due to the quantity and distribution of PLNs throughout the carcass. While removal of all lymph nodes may be an impractical control measure, it may be practical to remove large, easily accessible PLNs during fabrication. Targeting particular lymph nodes for removal would require a greater understanding of the distribution of *Salmonella* throughout the lymphatic system in order to determine which PLNs are associated with the greatest risk. Currently, the Federal Purchase Ground Beef Program, directed by the USDA Agricultural Marketing Services (AMS), requires removal of the three superior lymph nodes, namely the subiliac/pre-femoral, popliteal, and pre-scapular (**Figure 4**), as a within-plant *Salmonella* control effort for the National School Lunch Program (USDA-AMS, 2013).

Moreover, it may be appropriate to also approach this risk at the pre-harvest level with implementation of interventions within the feedlot setting. Specifically, commercial siderophore receptors and porins (SRP) *Salmonella* vaccines or the utilization of particular feeding approaches may provide alternative strategies for the reduction of *Salmonella* in the PLNs of cattle populations presented for harvest. In an exploratory study published in 2013, two cohorts of animals, one of which was vaccinated and the other served as an unvaccinated control group, were challenged with *S.* Newport and *S.* Montevideo by the oral and intradermal models previously described (Edrington et al., 2013a). The authors reported a vaccine effect in animals inoculated with *S.* Newport, although the same effect was not reported in animals inoculated with *S.* Montevideo. These findings may suggest that some serotypes may have an increased rate of clearance or a reduced duration of infection within the PLN, eluding to the hypothesis that vaccine impact should be investigated on a serotype basis (Edrington et al., 2013a).

The utilization of antimicrobials has also been established to decrease risk of *Salmonella* concentrations in cattle. Cattle that were treated for salmonellosis showed a decrease in hide prevalence of around 38%. The same cattle at harvest also showed only one positive out of 227 lymph nodes tested. This data presents the idea that following salmonellosis, there is not persistent *Salmonella* contamination of PLNs (Arthur and Harhay, unpublished). When a DFM is utilized in a production setting, prevalence of *Salmonella* concentrations has been shown to decrease in lymph nodes. Vipham et al. (2015) reported that with use of a

DFM, *Salmonella* concentration varied among animals but was seen to decrease with a reduction in *Salmonella* concentration in lymph nodes ranged from 31-82%.

Research has been conducted into methodology that would allow for testing the efficacy of pre-harvest intervention methods on *Salmonella* concentrations in PLNs. McClelland and Edrington (unpublished) utilized individually barcoded clones which allowed for estimates of *Salmonella* populations to be obtained in cattle. A real-time PCR (qPCR) assay has been developed that would allow for detection and quantification of *Salmonella* in cattle lymph nodes. The qPCR was seen to be more accurate in detecting concentration in lymph nodes than a standard culture method, with more qPCR samples testing positive than by culture method (Bai et al., 2018).

8   Conclusions

In summary, *Salmonella* can be recovered from the lymph nodes of healthy cattle presented for harvest, which may be contributing to the burden of *Salmonella* in ground beef products (Arthur et al., 2008a; Gragg et al., 2013a, 2013b; Haneklaus et al., 2012; Koohmaraie et al., 2012). The lymph node may act as a protective capsule allowing the *Salmonella* to evade chemical and thermal antimicrobial interventions implemented in the harvesting facility. As little is known about how *Salmonella* reaches and persists in the lymphatic system of cattle, more research is necessary to better understand the potential factors responsible for differences observed in seasons of the year, regions of the country, and animal types.

The recovery of *Salmonella* from a multitude of large PLNs in a carcass, from both cattle presented at harvest and from animals enrolled in challenge models, suggests that PLN infection may originate from multiple routes of entry. Moreover, initial findings have indicated that when challenged with *Salmonella*, bovine PLNs may have the ability to clear a *Salmonella* infection quickly, suggesting that frequency of exposure/ inoculation may play an important role in persistence (Edrington et al., 2013a; 2013b). Evidence also suggests that duration of infection might be serotype specific, thus representing an important knowledge gap to be investigated in the future. Through the modeling of potential routes of PLN infection, as well as the duration of infection, more information may be gained to assess efficacy of potential intervention strategies in the future.

Salmonella remains an important pathogen in terms of human public health due to the burden of illness associated with contaminated food. We have illustrated that many factors can contribute to the persistence of *Salmonella* in seemingly healthy cattle populations allowing it to evade current post-harvest interventions. Therefore, investing in both pre- and post-harvest intervention technologies for beef and beef products is not only necessary, but is also critical to mitigate the public health risk imposed by *Salmonella*.

References

Aarestrup, F. M. Antimicrobial Resistance in Bacteria of Animal Origin. ASM Press, 2006.

Aarestrup, F. M., and S. M. Pires. 2009. Comment on: Causal regulations vs. political will: Why human zoonotic infections increase despite precautionary bans on animal antibiotics. *Environment International*. 35:760-761.

Acar, J. F., and G. Moulin. 2006. Antimicrobial resistance at farm level. *Scientific and Technical Review of the Office International des Epizooties*. 25:775-792.

Adhikari, B., T. E. Besser, J. M. Gay, L. K. Fox, M. A. Davis, R. N. Cobbold, A. C. Berge, and D. D. Hancock. 2009. The role of animal movement, including off-farm rearing of heifers, in the interherd transmission of multidrugresistant *Salmonella*. *J Dairy Sci*. 92:4229-38.

Alam, M. J., D. G. Renter, S. E. Ives, D. U. Thomson, M. W. Sanderson, L. C. Hollis, and T. G. Nagaraja. 2009. Potential associations between fecal shedding of *Salmonella* in feedlot cattle treated for apparent respiratory disease and subsequent adverse health outcomes. *Vet Res*. 40:2.

Anderson, R. J., J. K. House, B. P. Smith, H. Kinde, R. L. Walker, B. J. Vande Steeg, and R. E. Breitmeyer. 2001. Epidemiologic and biological characteristics of salmonellosis in three dairy herds. *American Veterinary Medical Association*. 219:310-322.

Arave, C. W., and J. L. Albright. 1981. Cattle Behavior. *J Dairy Sci*. 64:1318-1329.

Arthur, T. M., and D. Harhay. Unpublished. Examination of the Impact of Pre-harvest Events and Practices on *Salmonella* Contamination of Peripheral Lymph Nodes. (Seen in project summary at http://www.beefresearch.org)

Arthur, T. M., D. M. Brichta-Harhay, J. M. Bosilevac, M. N. Guerini, N. Kalchayanand, J. E. Wells, S. D. Shackelford, T. L. Wheeler, and M. Koohmaraie. 2008a. Prevalence and Characterization of *Salmonella* in Bovine Lymph Nodes Potentially Destined for Use in Ground Beef. *J Food Prot*. 71:1685-1688.

Arthur, T. M., J. M. Bosilevac, D. M. Brichta-Harhay, N. Kalchayanand, D. A. King, S. D. Shackelford, T. L. Wheeler, and M. Koohmaraie. 2008b. Source tracking of *Escherichia coli* O157:H7 and *Salmonella* contmaination in the lairage environment at commercial U.S. beef processing plants and identification of an effective intervention. *J Food Prot*. 71:1752-1760.

Arthur, T. M., N. Kalchayanand, J. M. Bosilevac, D. M. Brichta-Harhay, S. D. Shackelford, J. L. Bono, T. L. Wheeler, and B. Koohmaraie. 2008c. Comparison of effects of antimicrobial interventions on multidrug-resistant *Salmonella*, susceptible *Salmonella*, and *Escherichia coli* O157:H7. *J. Food Prot*. 71:2177-2181.

Arthur, T. M., T. L. Wheeler, S. D. Shackelford, J. M. Bosilevac, X. Nou, and B. Koohmaraie. 2005. Effects of lowdose, low-penetration electron beam irradiation of chilled beef carcass surface cuts on *Escherichia coli* O157-H7 and meat quality. *Journal of Food Protection*. 68:666-672.

Ayebah, B., and Y. Hung. 2005. Electrolyzed water and its corrosiveness on various surface materials commonly found in food processing facilities. *Food Process Engineering*. 28:247-264.

Bacon, R. T., J. N. Sofos, K. E. Belk, D. R. Hyatt, and G. C. Smith. 2002. Prevalence and Antibiotic Susceptibility of *Salmonella* Isolated from Beef Animal Hides and Carcasses. *J Food Prot*. 65:284-290.

Bacon, R. T., K. E. Belk, J. N. Sofos, R. P. Clayton, J. O. Reagan, and G. C. Smith. 2000. Microbial Populations on Animal Hides and Beef Carcasses at Different Stages of Slaughter in Plants Employing Multiple-Sequential Interventions for Decontamination. *J Food Prot*. 63:1080-1086.

Bai, J., V. Trinetta, X. Shi, L. W. Noll, G. Magossi, W. Zheng, E. P. porter, N. Cernicchiaro.,
D. G. Renter, and T. G. Nagaraja. 2018. A Multiplex Real-Time PCR Assay, Based on *inv*A and *pag*C Genes, for theDetection and Quantification of *Salmonella enterica* from Cattle Lymph Nodes. *J Microbiol Methods.* 148: 110-116.

Baird-Parker, A. C. 1980. Organic Acids. p. 126-135. In R.P. Elliot, et al. (ed.), Microbial Ecology of Foods. International Commission on Microbiological Specifications for Foods., vol. 1. Academic Press, New York.

Baird-Parker, A. C. 1990. Foodborne salmonellosis. *The Lancet*. 336:1231-1235.

Barkate, M. L., G. R. Acuff, L. M. Lucia, and D. S. Hale. 1993. Hot water decontamination of beef carcasses for reduction of intitial bacterial numbers. *Meat Science*. 35:397-401.

Barkocy-Gallagher, G. A., T. M. Arthur, M. Rivera-Betancourt, X. Nou, S. D. Shackelford, T. L. Wheeler, and M. Koohmaraie. 2003. Seasonal Prevalence of Shiga Toxin-Producing *Escherichia coli*, Including O157:H7 and Non-O157 Serotypes, and *Salmonella* in Commercial Beef Processing Plants. *J Food Prot*. 66:1978-1986.

Batz, M. B., S. Hoffmann, and J. G. Morris, Jr. 2012. Ranking the disease burden of 14 pathogens in food sources in the United States using attribution data from outbreak investigations and expert elicitation. *Journal of Food Protection*. 75:1278-91.

Bean, N. H., and P. M. Griffin. 1990. Foodborne disease outbreaks in the United States, 1973-1987: Pathogens, vehicles, and trends. *Journal of Food Protection*. 53:804-817.

Belk, A. D., A. N. Arnold, J. E. Sawyer, D. B. Griffin, T. M. Taylor, J. W. Savell, and K. B. Gehring. 2018. Comparison of *Salmonella* Prevalence Rates in Bovine Lymph Nodes across Feeding Stages. *J Food Prot.* 81(4):549-553.

Bell, R. G. 1997. Distribution and sources of microbial contamination on beef carcasses. *Appl. Microbiol*. 82:292300.

Blau, D. M., B. J. McCluskey, S. R. Ladely, D. A. Dargatz, P. J. Fedorka-Cray, K. Ferris, and M. L. Headrich. 2005. *Salmonella* in Dairy Operations in the United States: Prevalence and Antimicrobial Drug Susceptibility. *J Food Prot*. 68:696-702.

Bosilevac, J. M., M. N. Guerini, N. Kalchayanand, and M. Koohmaraie. 2009. Prevalence and characterization of *Salmonella* in commercial ground beef in the United States. *Appl Environ Microbiol*. 75:1892-1900.

Braden, C. R. 2006. *Salmonella enterica* serotype Enteritidis and eggs: A national epidemic in the United States. *Clinical Infectious Diseases*. 43:512-517.

Brichta-Harhay, D. M., M. N. Guerini, T. M. Arthur, J. M. Bosilevac, N. Kalchayanand, S. D. Shackelford, T. L. Wheeler, and M. Koohmaraie. 2008. *Salmonella* and *Escherichia coli* O157:H7 contamination on hides and carcasses of cull cattle

presented for slaughter in the United States: an evaluation of prevalence and bacterial loads by immunomagnetic separation and direct plating methods. *Appl Environ Microbiol*. 74:6289-97.

Brichta-Harhay, D. M., T. M. Arthur, J. M. Bosilevac, N. Kalchayanand, S. D. Shackelford, T. L. Wheeler, and M. Koohmaraie. 2011. Diversity of multidrug-resistant *Salmonella enterica* strains associated with cattle at harvest in the United States. *Appl Environ Microbiol*. 77:1783-96.

Brown, M. H., and I. R. Booth. 1991. Acidulants and Low pH. p. 22. In N.J. Russell, and G.W. Gould (ed.), Food Preservations Van Nostand Reinhold, New York.

Brown, T. R., E. S. Edrington, G. H. Loneragan, D. L. Hanson, K. Malin, J. J. Ison, and D. J. Nisbet. 2015. Investigation into possible differences in Salmonella Prevalence in the Peripheral Lymph Nodes of cattle Derived from Distinct Production Systems and of Different Breed Types. *J Food Prot.* 78(11):2081-2084.

Buzby, J. C., and T. Roberts. 2009. The Economics of enteric infections: Human foodborne disease costs. *Gastroenterology*. 136:1851-1862.

Callaway, T. R., J. E. Keen, T. S. Edrington, L. H. Baumgard, L. Spicer, E. S. Fonda, K. E. Griswold, T. R. Overton, M. E. VanAmburgh, R. C. Anderson, K. J. Genovese, T. L. Poole, R. B. Harvey, and D. J. Nisbet. 2005. Fecal Prevalence and Diversity of *Salmonella* Species in Lactating Dairy Cattle in Four States. J Dairy Sci. 88:3603-3608.

Callaway, T. R., R. C. Anderson, K. J. Genovese, T. L. Polle, T. J. Anderson, J. A. Byrd, L. F. Kubena, and D. J. Nisbet. 2002. Sodium Chlorate Supplementation Reduces *E. coli* O157:H7 Populations in Cattle. *J Anim Sci*. 80:1683-1689.

Callaway, T. R., S. E. Dowd, T. S. Edrington, R. C. Anderson, N. Krueger, N. Bauer, P. J. Kononoff, and D. J. Nisbet. 2010. Evaluation of bacterial diversity in the rumen and feces of cattle fed different levels of dried distillers grains plus solubles using bacterial tag-encoded FLX amplicon pyrosequencing. *J Anim Sci*. 88:3977-83.

Callaway, T. R., T. S. Edrington, A. D. Brabban, J. E. Keen, R. C. Anderson, M. L. Rossman, M. J. Engler, K. J. Genovese, B. L. Gwartney, J. O. Reagan, T. L. Poole, R. B. Harvey, E. M. Kutter, and D. J. Nisbet. 2006. Fecal prevalence of E. coli O157, *Salmonella*, *Listeria*, and Bacteriophage Infecting *E. coli* O157:H7 in Feedlot Cattle, in the Southern Plains Region of the United States. *Foodborne Pathog Dis*. 3:234-244.

Callaway, T. R., T. S. Edrington, and D. J. Nisbet. 2014. Isolation of *Escherichia coli* O157:H7 and *Salmonella* from migratory brown-headed cowbirds (Molothrus ater), common Grackles (Quiscalus quiscula), and cattle egrets (Bubulcus ibis). *Foodborne Pathog Dis*. 11:791-4.

Callaway, T. R., T. S. Edrington, R. C. Anderson, J. A. Byrd, and D. J. Nisbet. 2008. Gastrointestinal microbial ecology and the safety of our food supply as related to *Salmonella*. *J Anim Sci*. 86:E163-72.

Carlson, J. C., A. B. Franklin, D. R. Hyatt, S. E. Pettit, and G. M. Linz. 2011. The role of starlings in the spread of *Salmonella* within concentrated animal feeding operations. Journal of Applied Ecology. 48:479-486.

Carlson, J. C., D. R. Hyatt, J. W. Ellis, D. R. Pipkin, A. M. Mangan, M. Russell, D. S. Bolte, R. M. Engeman, T. J. DeLiberto, and G. M. Linz. 2015. Mechanisms of antimicrobial resistant *Salmonella enterica* transmission associated with starling-livestock interactions. *Vet Microbiol.* 179(1-2): 60-68.

Castillo, A., L. M. Lucia, G. K. Kemp, and G. R. Acuff. 1999. Reduction of *Escherichia coli* O157:H7 and *Salmonella* Typhimurium on Beef Carcass Surfaces Using Acidified Sodium Chlorite. *J Food Prot*. 62:580-584.

CDC. 2011. National *Salmonella* Surveillance Overview. Atlanta, Georgia: US Department of Health and Human Services, CDC.

CDC. 2014. Foodborne Diseases Active Surveillance Network (FoodNet): FoodNet Surveillance Report for 2012 (Final Report). Atlanta, Georgia: U.S. Department of Health and Human Services, CDC.

CDC. 2014. Reports of selected *Salmonella* outbreak investigations. In, Atlanta, Georgia: CDC.

CDC. April 5, 2012. What is Salmonellosis? Available at: http://www.cdc.gov/Salmonella/general/index.html. Accessed.

CDC. August 8, 2014. Foodborne Outbreak Tracking and Reporting. Available at: http://www.cdc.gov/foodsafety/fdoss/. Accessed.

CDC. Date, February 28, 2018. National Enteric Disease Surveillance: *Salmonella* Annual Report, 2016. Available at https://www.cdc.gov/nationalsurveillance/salmonella-surveillance.html. Accessed.

CDC. Date, November 5 2013, Foodborne Diseases Active Surveillance Network (FoodNet). Available at: http://www.cdc.gov/foodnet/index.html. Accessed.

CDC. December 18, 2014. *Salmonella*. Available at: http://www.cdc.gov/Salmonella/. Accessed.

Cherubin, C. E. 1981. Antibiotic-Resistance of *Salmonella* in Europe and the United States. *Reviews of Infectious Diseases*. 3:1105-1126.

Chun, H. H., J. Y. Kim, B. D. Lee, D. J. Yu, and K. B. Song. 2010. Effect of UV-C irradiation on the inactivation of inoculated pathogens and quality of chicken breasts during storage. *Food Control*. 21:276-280.

Clark, L., and R. G. McLean. 2003. A Review of Pathogens of Agricultural and Human Health Interest Found in Blackbirds. p. 103-108. In G.M. Linz (ed.), Symposium on Management of North American Blackbirds, Bismarck, ND.

Corlett, D. A., and M. H. Brown. 1980. pH and Acidity. p. 92. In J.H. Silliker, et al. (ed.), Microbial Ecology of Foods. International Commission on Microbiological Specifications for Foods., vol. 1. Academic Press, New York.

Cummings, K. J., L. D. Warnick, K. A. Alexander, C. J. Cripps, Y. T. Grohn, P. L. McDonough, D. V. Nydam, and K. E. Reed. 2009. The incidence of salmonellosis among dairy herds in the northeastern United States. *J Dairy Sci*. 92:3766-74.

Cummings, K. J., T. S. Edrington, D. L. Hanson, G. H. Loneragan, and et al. CRISPR Diversity and Antimicrobial Susceptibility of *Salmonella* Isolates from Dairy Farm Environments in Texas. *Unpublished*.

Daniels, M. J., M. R. Hutchings, and A. Greig. 2003. The risk of disease transmission to livestock posed by contamination of farm stored feedby wildlife excreta. *Epedemiol Infect*. 130:561-568.

Dargatz, D. A., C. A. Kopral, M. M. Erdman, and P. J. Fedorka-Cray. 2016. Prevalence and Antimicrobial Resistance of Salmonella Isolated from Cattle Feces in Unites States Feedlots in 2011. *Foodborne Pathog Dis.* 13(9): 483-489.

Dargatz, D. A., P. J. Fedorka-Cray, S. R. Ladely, C. A. Kopral, K. E. Ferris, and M. L. Headrick. 2003. Prevalence and antimicrobial susceptibility of *Salmonella* spp. isolates from US cattle in feedlots in 1999 and 2000. Journal of *Applied Microbiology*. 95:753-761.

Dargatz, D. A., R. A. Strohmeyer, P. S. Morley, D. R. Hyatt, and M. D. Salman. 2005. Characterization of *Escherichia coli* and *Salmonella enterica* from Cattle Feed Ingredients. *Foodborne Pathog Dis*. 2:341-347.

Davies, R. H., and C. Wray. 1997. Distribution of *Salmonella* contamination in ten animal feedmills. *Veterinary Microbiology*. 51:159-169.

Dewell, G. A., C. A. Simpson, R. D. Dewell, D. R. Hyatt, K. E. Belk, J. A. Scanga, P. S. Morley, T. Grandin, G. C. Smith, D. A. Dargatz, B. A. Wagner, and M. D. Salman. 2008. Risk Associated with Transportation and Lairage on Hide Contamination with *Salmonella enterica* in Finished Beef Cattle at Slaughter. *J Food Prot*. 71:2228-2232.

Dodd, C. C., D. G. Renter, D. U. Thomson, and T. G. Nagaraja. 2011. Evaluation of the effects of a commercially available *Salmonella* Newport siderophore receptor and porin protein vaccine on fecal shedding of *Salmonella* bacteria and health and performance of feedlot cattle. *American Journal of Veterinary Research*. 72:239-247.

Dodd, C. C., D. R. Renter, X. Shi, M. J. Alam, T. G. Nagraja, and M. W. Sanderson. 2011. Prevalence and Persistence of *Salmonella* in Cohorts of Feedlot Cattle. *Foodborne Pathog Dis*. 8:781-789.

Dorsa, W. J. 1996. Decontamination of Beef Carcasses by Commercial Steam-Vacuum. *Recip. Meat Conf. Proc*. 49:114-120.

Doyle, M. E. 2013. White paper on human illness caused by *Salmonella* from all food and non-food vectors, Update 2013. *FRI Food Safety Reviews*.

Doyle, M. P., and M. C. Erickson. 2008. Summer meeting 2007 - the problems with fresh produce: an overview. *J Appl Microbiol*. 105:317-30.

Edrington, T. S., B. H. Carter, T. H. Friend, G. R. Hagevoort, T. L. Poole, T. R. Callaway, R. C. Anderson, and D. J. Nisbet. 2009. Influence of sprinklers, used to alleviate heat stress, on faecal shedding of *E. coli* O157:H7 and *Salmonella* and antimicrobial susceptibility of *Salmonella* and *Enterococcus* in lactating dairy cattle. *Lett Appl Microbiol*. 48:738-43.

Edrington, T. S., G. H. Loneragan, J. Hill, K. J. Genovese, D. M. Brichta-Harhay, R. L. Farrow, N. A. Krueger, T. R. Callaway, R. C. Anderson, and D. J. Nisbet. 2013a. Development of challenge models to evaulate the efficacy of a vaccine to reduce carriage of *Salmonella* in peripheral lymph nodes of cattle. *J. Food Prot*. 76:1259-1263.

Edrington, T. S., G. H. Loneragan, K. J. Genovese, D. L. Hanson, D. J. Nisbet. 2016. *Salmonella* Persistence within the Peripheral Lymph Nodes of Cattle following Experimental Inoculation. *J Food Prot.* 79(6):1032-1035.

Edrington, T. S., G. H. Loneragan, K. J. Genovese, H. He, T. R. Callaway, R. C. Anderson, D. M. Brichta-Harhay, and D. J. Nisbet. 2013b. Development of a transdermal *Salmonella* challenge model in calves. *Journal of Food Protection*. 76:1255-1258.

Edrington, T. S., M. E. Hume, M. L. Looper, C. L. Schultz, A. C. Fitzgerald, T. R. Callaway, K. J. Genovese, K. M. Bischoff, J. L. McReynolds, R. C. Anderson, and D. J. Nisbet. 2004. Variation in the faecal shedding of *Salmonella* and *E. coli* O157: H7 in

lactating dairy cattle and examination of *Salmonella* genotypes using pulsed-field gel electrophoresis. *Letters in Applied Microbiology*. 38:366-372.

Edrington, T. S., R. L. Farrow, M. E. Hume, P. N. Anderson, G. R. Hagevoort, D. J. Caldwell, T. R. Callaway, R. C. Anderson, and D. J. Nisbet. 2013c. Evaluation of the Potential Antimicrobial Resistance Transfer from a Multi-Drug Resistant *Escherichia coli* to *Salmonella* in Dairy Calves. *Current Microbiology*. 66:132-137.

Edrington, T. S., T. R. Brown, D. L. Hanson, and G. H. Loneragan. Unpublished. *Salmonella* Diversity in Non-Mammalian Vectors from Agricultural Operations in the Southern High Plains.

Edrington, T. S., T. T. Ross, T. R. Callaway, C. H. Martinez, M. E. Hume, K. J. Genovese, T. L. Poole, R. C. Anderson, and D. J. Nisbet. 2008. Investigation into the seasonal salmonellosis in lactating dairy cattle. *Epidemiol Infect*. 136:381-90.

FDA. 2011. National Antimicrobial Resistance Monitoring System- Enteric Bacteria (NARMS): 2011 Retail Meat Report. In U.S. Department of Health and Human Services, FDA, Rockville, MD.

FDA. 2012. National Antimicrobial Resistance Monitoring System- Enteric Bacteria (NARMS): 2010 Executive Report. In U.S. Department of Health and Human Services, FDA, Rockville, MD.

FDA. Date, 2007, Irradiation in the production, processing and handling of food, 21 CFR, part 179.39. Available at: http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch. Cfm?CFRPart=179. Accessed 21DEC, 2014.

FDA. Date, 2015, Inventory of effective food contact substance (FCS) notifications. FCN no. 1490. Available at: http://www.accessdata.fda.gov/scripts/fdcc/?set=fcn&id=1490. Accessed August 7, 2015.

Feare, C. J., P. Douville de Franssu, and S. J. Peris. 1992. The Starling in Europe: Multiple Approaches to a Problem Species. P. 83-88. In, Vertebrate Pest Conference, vol. 15. University of Calif., Davis.

Fedorka-Cray, P. J., D. A. Dargatz, L. A. Thomas, and J. T. Gray. 1998. Survey of *Salmonella* Serotypes in Feedlot Cattle. *J Food Prot*. 61:525-530.

Fossler, C. P., S. J. Wells, J. B. Kaneene, P. L. Ruegg, L. D. Warnick, J. B. Bender, L. E. Eberly, S. M. Godden, and L. W. Halbert. 2005a. Herd-level factors associated with isolation of *Salmonella* in a multi-state study of conventional and organic dairy farms I. *Salmonella* shedding in cows. *Prev Vet Med*. 70:257-77.

Fossler, C. P., S. J. Wells, J. B. Kaneene, P. L. Ruegg, L. D. Warnick, J. B. Bender, L. E. Eberly, S. M. Godden, and L. W. Halbert. 2005b. Herd-level factors associated with isolation of *Salmonella* in a multi-state study of conventional and organic dairy farms II. *Salmonella* shedding in calves. *Prev Vet Med*. 70:279-91.

Ge, B., P. C. LaFon, P. J. Carter, S. D. McDermott, J. Abbott, A. Glenn, S. L. Ayers, S. L. Friedman, J. C. Paige, D. D. Wagner, S. Zhao, P. F. McDermott, and M. A. Rasmussen. 2013. Retrospective analysis of *Salmonella*, *Campylobacter*, *Escherichia coli*, and *Enterococcus* in animal feed ingredients. *Foodborne Pathog Dis*. 10:684-91.

Gragg, S. E., G. H. Loneragan, K. K. Nightingale, D. M. Brichta-Harhay, H. Ruiz, J. R. ELder, L. G. Garcia, M. F. Miller, A. Echeverry, R. G. Ramirez Porras, and M. M. Brashears. 2013a. Substantial within-animal diversity of *Salmonella* isolates from lymph nodes, feces, and hides of cattle at slaughter. *Applied and Environmental Microbiology*. 79:4744-4750.

Gragg, S. E., G. H. Loneragan, M. M. Brashears, T. M. Arthur, J. M. Bosilevac, N. Kalchayanand, R. Wang, J. W. Schmidt, J. C. Brooks, S. D. Shackelford, T. L. Wheeler, T. R. Brown, T. S. Edrington, and D. M. Brichta-Harhay. 2013b. Cross-sectional study examining *Salmonella* enterica carriage in subiliac lymph nodes of cull and feedlot cattle at harvest. *Foodborne Pathog. Dis*. 10:368-74.

Greig, J. D., and A. Ravel. 2009. Analysis of foodborne outbreak data reported internationally for source attribution. *International Journal of Food Microbiology*. 130:77-87.

Greig, J., A. Rajic, I. Young, M. Mascarenhas, L. Waddell, and J. LeJeune. 2014. A Scoping Review of the Role of Wildlife in the Transmission of Bacterial Pathogens and Antimicrobial Resistance to the Food Chain. *Zoonoses Public Health*.

Guard-Petter, J. 2001. The chicken, the egg and *Salmonella* Enteritidis. *Environmental Microbiology*. 3:421-430.

Haneklaus, A. N., K. B. Harris, D. B. Griffin, T. S. Edrington, L. M. Lucia, and J. W. Savell. 2012. *Salmonella* prevalence in bovine lymph nodes differs among feedyards. *Journal of Food Protection*. 75:1131-1133.

Hanson, D. L., G. H. Loneragan, T. R. Brown, D. J. Nisbet, M. E. Hume, and T. S. Edrington. 2016. Evidence supporting vertical transmission of *Salmonella* in dairy cattle. *Epidemiol Infect.* 144(5):962-967.

Heithoff, D. M., J. K. House, P. C. Thomson, and M. J. Mahan. 2015. Development of a *Salmonella* cross-protective vaccine for food animal production systems. *Vaccine*. 33:100-7.

Heithoff, D. M., W. R. Shimp, J. K. House, Y. Xie, B. C. Weimer, R. L. Sinsheimer, and M. J. Mahan. 2012. Intraspecies variation in the emergence of hyperinfectious bacterial strains in nature. *PLoS Pathog*. 8:e1002647.

Helke, K. L., M. A. McCrackin, A. M. Galloway, A. Z. Poole, C. D. Salgado, and B. P. Marriott. 2017. Effects of antimicrobial use in agricultural animals on drug-resistant foodborne salmonellosis in humans: A systematic literature review. *Crit Rev Food Sci Nutr.* 56(13):2115-2132.

Hermesch, D. R., D. U. Thomson, G. H. Loneragan, D. R. Renter, and B. J. White. 2008. Effects of commercially available vaccine against *Salmonella enterica* serotype Newport on milking production, somatic cell count, and shedding of *Salmonella* organisms in female dairy cattle with no clinical signs of salmonellosis. *American Journal of Veterinary Research*. 69:1229-1234.

Hinton, M. H. 2000. Infections and intoxications associated with animal feed and forage which may present a hazard to human health. *Vet J*. 159:124-38.

House, J. K., M. M. Ontiveros, N. M. Blackmer, E. L. Dueger, J. B. Fitchhorn, G. R. McArthur, and B. P. Smith. 2001. Evaluation of an autogenous *Salmonella* bacterin and a modified live *Salmonella* serotype Choleraesuis vaccine on a commercial dairy farm. *American Journal of Veterinary Research*. 62:1897-1902.

Huffman, R. D. 2002. Current and future technologies for the decontamination of carcasses and fresh meat. *Meat Sci*. 62:285-294.

Hughes, H. D., J. A. Carroll, N. C. Burdick Sanchez, and J. T. Richeson. 2014. Natural variations in the stress and acute phase responses of cattle. *Innate Immun*. 20:888-96.

Ison, J. J. 2013. A meta-analysis of the association of *Lactobacilus acidophilus* NP51 administration with *Escherichia coli* O157 in feces and on hides of feedlot cattle. In, Department of Animal and Food Science, vol. Master of Science. Texas Tech University, Lubbock, Texas, USA.

Jackson, B. R., P. M. Griffin, D. Cole, K. A. Walsh, and S. J. Chai. 2013. Outbreak-associated *Salmonella enterica* serotypes and food commodities, United States, 1998-2008. *Emerging Infectious Diseases*. 19:1239-1244.

Johnson, R. J., and J. F. Glahn. 1994. European Starlings. p. 109-120. In S.E. Hygenstrom, R.M. Timm, and G.E. Larson (ed.), The Handbook: Prevention and Control of Wildlife Damages., University of Nebraska-Lincoln.

Jones, P. W., P. Collins, G. T. H. Brown, and M. Aitken. 1982. Transmission of *Salmonella* mbandaka to cattle from contaminated feed. *Journal of Hygiene*. 88:255-263.

Jones, T. F., L. A. Ingram, P. R. Cieslak, D. J. Vugia, M. Tobin-D'Angelo, S. Hurd, C. Medus, A. Cronquist, and F. J. Angulo. 2008. Salmonellosis outcomes differ substantially by serotype. *Journal of Infectious Disease*. 198:109114.

Kalchayanand, N., D. M. Brichta-Harhay, T. M. Arthur, J. M. Bosilevac, M. N. Guerini, T. L. Wheeler, S. D. Shackelford, and M. Koohmaraie. 2009a. Prevalence rates of *Escherichia coli* O157:H7 and *Salmonella* at different sampling sites on cattle hides at a feedlot and processing plant. *J Food Prot*. 72:1267-1271.

Kalchayanand, N., T. M. Arthur, J. M. Bosilevac, D. M. Brichta-Harhay, M. N. Guerini, S. D. Shackelford, T. L. Wheeler, and M. Koohmaraie. 2009b. Effectiveness of 1,3-Dibromo-5,5 Dimethylhydantoin on reduction of *Esherichia coli* O157:H7- and *Salmonella*-inoculated fresh meat. *Journal of Food Protection*. 72:151-156.

Karp, B. E., H. Tate, J. R. Plumblee, U. Dessai, J. M. Whichard, E. L. Thacker, K. R. Hale, W. Wilson, C. R. Friedman, P. M. Griffin, and P. F. McDermott. 2017. National Antimicrobial Resistance Monitoring System: Two Decades of Advancing Public Health Trhough Integrated Surveillance of Antimicrobial Resistance. *Foodborne Pathog Dis.* 14(10):545-557.

Katawal, I., M. M. Brashears, A. Echeverry, A. Calle, and M. F. Miller. 2018. Validation of Interventions on Raw Ground Beef Components; Cheek Meat and Weasand. Meat and Muscle Biology 2:141-141.

Kendrovski, V., Z. Karadzovski, and M. Spasenovska. 2011. Ambient maximum temperature as a function of *Salmonella* food poisoning cases in the Republic of Macedonia. *N Am J Med Sci*. 3:264-7.

King, D. A., L. M. Lucia, A. Castillo, G. R. Acuff, K. B. Harris, and J. W. Savell. 2005. Evaluation of peroxyacetic acid as a post-chilling intervention for control of *Escherichia coli* O157:H7 and *Salmonella* Typhimurium on beef carcass surfaces. *Meat Sci*. 69:401-7.

Koohmaraie, M., J. A. Scanga, M. De La Zerda, B. Koohmaraie, L. Tapay, V. Beskhlebnaya, T. Mai, K. Greeson, and M. Samadpour. 2012. Tracking the sources of *Salmonella* in ground beef produced from nonfed cattle. *Journal of Food Protection*. 75:1464-1468.

Krytenburg, D. S., D. D. Hancock, D. H. Rice, T. E. Besser, C. C. Gay, and J. M. Gay. 1998. A pilot survey of *Salmonella enterica* contamination of cattle feeds in the Pacific northwestern USA. *Animal Feed Science and Technology*. 75:75-79.

Kunze, D. J., G. H. Loneragan, T. M. Platt, M. F. Miller, T. E. Besser, M. Koohmaraie, T. Stephens, and M. M. Brashears. 2008. *Salmonella enterica* burden in harvest-ready cattle populations from the southern high plains of the United States. *Appl Environ Microbiol*. 74:345-51.

Laufer, A. S., J. Grass, K. Holt, J. M. Whichard, P. M. Griffin, and L. H. Gould. 2015. Outbreaks of *Salmonella* infections attributed to beef – Unites States, 1973-2011. *Epidemiol Infect.* 143(9): 2003-2013.

Laury, A. M., M. V. Alvarado, G. Nace, C. Z. Alvarado, J. C. Brooks, A. Echeverry, and M. M. Brashears. 2009. Validation of a lactic acid- and citric acid-based antimicrobial product for the reduction of *Escherichia coli* O157:H7 and *Salmonella* on beef tips and whole chicken carcasses. *Journal of Food Protection*. 72:2208-2211.

LeJeune, J. T., T. E. Besser, and D. D. Hancock. 2001. Cattle water troughs as reservoirs of *Escherichia coli* O157. *Appl Environ Microbiol*. 67:3053-7.

LeJeune, J. T., T. E. Besser, N. L. Merrill, D. H. Rice, and D. D. Hancock. 2001. Livestock Drinking Water Microbiology and the Factors Influencing the Quality of Drinking Water Offered to Cattle. *J Dairy Sci*. 84:1856-1862.

LeJeune, J., J. Homan, G. Linz, and D. L. Pearl. 2008. Role of the European Starling in the Transmission of *E. coli* O157 on Dairy Farms. p. 31-34. In R.M. Timm, and M.B. Madon (ed.), Vertebr. Pest Conf., vol. 23. Univ. of Calif., Davis.

Lewis, S. J., A. Velasquez, S. L. Cuppet, and S. R. McKee. 2002. Effect of electron beam irradiation on poultry meat safety and quality. *Poultry Science*. 81:896-903.

Li, M., S. Malladi, H. S. Hurd, T. J. Goldsmith, D. M. Brichta-Harhay, and G. H. Loneragan. 2015. *Salmonella* ssp. In Lymph Nodes of Fed and Cull Cattle: Relative Assessment of Risk to Ground Beef. *Food Control.* 50- 423-434.

Likavec, T., A. F. Pires, and J. A. Funk. 2016. Association between thermal environment and *Salmonella* in fecal samples from dairy cattle in midwestern United States. *Can J Vet Res.* 80(3): 183-188.

Loneragan, G. H., and M. M. Brashears. 2005. Pre-harvest interventions to reduce carriage of *E. coli* O157 by harvest-ready feedlot cattle. *Meat Sci*. 71:72-8.

Loneragan, G. H., D. U. Thomson, R. M. McCarthy, H. E. Webb, A. E. Daniels, T. S. Edrington, D. J. Nisbet, S. J. Trojan, S. C. Rankin, and M. M. Brashears. 2012. *Salmonella* diversity and burden in cows on and culled from dairy farms in the Texas High Plains. *Foodborne Pathog Dis*. 9:549-55.

Losinger, W. C., L. P. Garber, M. A. Smith, H. Scott Hurd, L. G. Biehl, P. J. Fedorka-Cray, L. A. Thomas, and K. Ferris. 1997. Management and nutritional factors associated with the detection of *Salmonella* sp. from cattle fecal specimens from feedlot operations in the United States. *Prev Vet Med*. 31:231-244.

Louden, B. C., D. Haarmann, J. Han, S. L. Foley, and A. M. Lynne. 2012. Characterization of antimicrobial resistance in *Salmonella enterica* serovar Typhimurium isolates from food animals in the U.S. *Food Research International*. 45:968-972.

Mahan, M. J., D. M. Heithoff, and J. K. House. 2012. *Salmonella* cross-protective vaccines: fast-forward to the next generation of food safety. *Future Microbiology*. 7:805-808.

Majowicz, S. E., J. Musto, E. Scallan, F. J. Angulo, M. Kirk, S. J. O'Brien, T. F. Jones, A. Fazil, R. M. Hoekstra, and S. International Collaboration on Enteric Disease 'Burden of Illness. 2010. The global burden of nontyphoidal *Salmonella* gastroenteritis. *Clinical Infectious Diseases*. 50:882-889.

Mangen, M. J., M. B. Batz, A. Kasbohrer, T. Hald, J. G. Morris, Jr., M. Taylor, and A. H. Havelaar. 2010. Integrated approaches for the public health prioritization of foodborne and zoonotic pathogens. *Risk Analysis*. 30:782-797.

McClelland, M., and T. S. Edrington. 2017. Unpublished. Collaborator I – Investigation into the Origin of *Salmonella* in the Peripheral Lymph Nodes of Fed Beef Cattle at Slaughter in the Southwestern United States. (seen in project summary at http://www.beefreserach.org)

McEachran, A. D., B. R. Blackwell, J. D. Hanson, K. J. Wooten, G. D. Mayer, S. B. Cox, and P. N. Smith. 2015. Antibiotics, Bacteria, and Antibiotic Resistance Genes: Aerial Transport from Cattle Feed Yards via Particulate Matter. *Environ Health Perspect*. 123:337-43.

Mead, P. S., L. Slutsker, V. Dietz, L. F. McCaig, J. S. Bresee, C. Shapiro, P. M. Griffin, and R. V. Tauxe. 1999. Foodrelated illness and death in the United States. Emerging Infectious Diseases. 5:607-625.

Meerburg, B. G., and A. Kijlstra. 2007. Role of rodents in transmission of Salmonella and Campylobacter. Journal of the Science of Food and Agriculture. 87:2774-2781.

Meeusen, E. N., J. Walker, A. Peters, P. P. Pastoret, and G. Jungersen. 2007. Current status of veterinary vaccines. Clin Microbiol Rev. 20:489-510, table of contents.

Mies, P. D., B. R. Covington, K. B. Harris, L. M. Lucia, G. R. Acuff, and J. W. Savell. 2004. Decontamination of Cattle Hides Prior to Slaughter Using Washes with and without Antimicrobial Agents. J Food Prot. 67:579-582.

Morrow, J. L., F. M. Mitloehner, A. K. Johnson, M. L. Galyean, J. W. Dailey, T. S. Edrington, R. C. Anderson, K. J. Genovese, T. L. Poole, S. E. Duke, and T. R. Callaway. 2005. Effect of water sprinkling on incidence of zoonotic pathogens in feedlot cattle. *Journal of Animal Science*. 83:1959-1966.

Nickelson, K. J., T. M. Taylor, D. B. Griffin, J. W. Savell, K. B. Gehring, and A. N. Arnold. 2018. *Salmonella* Prevalence in Lymph Nodes of U. S. and Mexican Cattle Presented for Slaughter during 2 Seasons in Texas. *Meat and Muscle Biol.* 2(2):145-146.

Office of Disease Prevention and Health Promotion (ODPHP). Date, 2014, Evidence-Based Resource Summary. Available at: http://www.healthypeople.gov/2020/tools-resources/evidence-based-resource/foodborne-illnessacquired-united-states%E2%80%94major. Accessed.

Olafson, P. U., K. H. Lohmeyer, T. S. Edrington, and G. H. Loneragan. 2014. Survival and Fate of *Salmonella enterica* serovar Montevideo in Adult Horn Flies (Diptera: Muscidae). *Journal of Medical Entomology*. 51:993-1001.

Olafson, P. U., T. R. Brown, K. H. Lohmeyer, R. B. Harvey, D. J. Nisbet, G. H. Loneragan, and T. S. Edrington. 2016. Assessing Transmission of *Salmonella* to Bovine Peripheral Lymph Nodes upon Horn Fly Feeding. *J Food Prot*. 79(7): 1135-1142.

Pachepsky, Y. A., R. A. Blaustein, G. Whelan, and D. R. Shelton. 2014. Comparing temperature effects on *Escherichia coli*, *Salmonella*, and *Enterococcus* survival in surface waters. *Lett Appl Microbiol*. 59:278-83.

Paniselloa, P. J., R. Rooney, P. C. Quanticka, and R. Stanwell-Smith. 2000. Application of foodborne disease outbreak data in the development and maintenance of HACCP systems. *International Journal of Food Microbiology*. 59:221-234.

Pedersen, K., L. Clark, W. F. Andelt, and M. D. Salman. 2006. Prevalence of Shiga Toxin-Producing *Escherichia coli* and *Salmonella enterica* in Rock Pigeons Captured in Fort Collins, Colorado. *Journal of Wildlife Disease*s. 42:46-55.

Phebus, R. K., A. L. Nutsch, D. E. Schafer, R. C. Wilson, M. J. Riemann, J. D. Leising, C. L. Kastner, J. R. Wolf, and R. K. Prasai. 1997. Comparison of steam pasteurization and other methods for reduction of pathogens on surfaces of freshly slaughtered beef. *Journal of Food Protection*. 60:476-484.

Pires, S. M. 2013. Assessing the applicability of currently available methods for attributing foodborne disease to sources, including food and food commodities. *Foodborne Pathogens and Disease*. 10:206-213.

Pires, S. M., E. G. Evers, W. Van Pelt, T. Ayers, E. Scallan, F. J. Angulo, A. Havelaar, T. Haid, and t. M.-V.-N. W. W. Group. 2009. Attributing the Human Disease Burden of Foodborne Infections to Specific Sources. *Foodborne Pathogens and Disease*. 6:417-424.

Porwollik, S., K. Genovese, W. Chu, G. H. Loneragan, T. S. Edrington, and M. McClelland. 2018. Neutral barcoding of genomes reveals the dynamics of *Salmonella* colonization in cattle and their peripheral lymph nodes. *Vet Microbiol.* 220: 97-106.

Prasai, R. K., R. K. Phebus, C. M. Garcia Zepeda, C. L. Kastner, A. E. Boyle, and D. Y. C. Fung. 1995. Effectiveness of trimming and/or washing on microbiological quality of beef carcasses. *Journal of Food Protection*. 58:1114-1117.

Queiroz, O. C. M., I. M. Ogunade, Z. Weinberg, and A. T. Adesogan. 2018. Silage review: Foodborne pathogens in silage and their mitigation by silage additives. *J Dairy Sci.* 101(5): 4132-4142.

Ransom, J. R., K. E. Belk, J. N. Sofos, J. D. Stopforth, J. A. Scanga, and G. C. Smith. 2003. Comparison of intervention technologies for reducing *Escherichia coli* O157:H7 on beef cuts and trimmings. *Food Protection Trends*. 23:24-34.

Rao, S., J. Van Donkersgoed, V. Bohaychuk, T. Besser, X. Song, B. Wagner, D. Hancock, D. R. Renter, D. A. Dargatz, and P. S. Morley. 2010. Antimicrobial Drug Use and Antimicrobial Resistance in Enteric Bacteria Among Cattle from Alberta Feedlots. *Foodborne Pathog Dis*. 7:449-457.

Reicks, A. L., M. M. Brashears, K. D. Adams, J. C. Brooks, J. R. Blanton, and M. F. Miller. 2007. Impact of Transportation of Feedlot Cattle to the Harvest Facility on the Prevalence of E*scherichia coli* O157-H7, *Salmonella*, and Total Aerobic Microoorganisms on Hides. *J Food Prot*. 70:17-21.

Renter, D. G, T. G. Nagaraja, N. Cernicchiaro, T. S. Edrington, G H. Loneragan, and D. L. Hanson. 2014 Unpublished. *Salmonella* in the Peripheral Lymph Nodes of Cattle: Host, Agent and Environmental Factors. (seen in project summary at http://www.beefresearch.org).

Rhoades, J. R., G. Duffy, and K. Koutsoumanis. 2009. Prevalence and concentration of verocytotoxigenic *Escherichia coli*, *Salmonella enterica* and *Listeria monocytogenes* in the beef production chain: A review. *Food Microbiology*. 26:357-376.

Rivera-Betancourt, M., S. D. Shackelford, T. M. Arthur, K. E. Westmoreland, G. Bellinger, M. L. Rossman, J. O. Reagan, and M. Koohmaraie. 2004. Prevalence of *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* in two geographically distant commercial beef processing plants in the United States. *Journal of Food Protection*. 67:295-302.

Rodrique, D. C., R. V. Tauxe, and B. Rowe. 1990. International increase in *Salmonella* enteritidis: A new pandemic? *Epidemiology and Infection*. 105:21-27.

Ruzante, J. M., J. E. Lombard, B. Wagner, C. P. Fossler, J. S. Karns, J. S. Van Kessel, and I. A. Gardner. 2010. Factors Associated with *Salmonella* Presence in Environmental Samples and Bulk Tank Milk from US Dairies. *Zoonoses and Public Health*. 57:e217-e225.

Samadpour, M., M. W. Barbour, T. Nguyen, T. M. Cao, F. Buck, G. A. Depavia, E. Mazengia, P. Yang, D. Alfi, M. Lopes, and J. D. Stopforth. 2005. Incidence of enterohemorrhagic *Escherichia coli*, *Escherichia coli* O157, *Salmonella*, and *Listeria monocytogenes* in retail fresh ground beef, sprouts, and mushrooms. *Journal of Food Protection*. 69:441-443.

Samuel, J. L., D. A. Oboyle, W. J. Mathers, and A. J. Frost. 1979. Isolation of *Salmonella* from mesenteric lymph nodes of healthy cattle at slaughter. *Research in Veterinary Science*. 28:238-241.

Samuel, J. L., J. A. Eccles, and J. Francis. 1981. *Salmonella* in the intestinal-tract and associated lymph nodes of sheep and cattle. *Journal of Hygiene*. 87:225-232.

Scallan, E., and B. E. Mahon. 2012. Foodborne Diseases Active Surveillance Network (FoodNet) in 2012: a foundation for food safety in the United States. *Clinical Infectious Diseases*. 54:S381-S384.

Scallan, E., R. M. Hoekstra, B. E. Mahon, T. F. Jones, and P. M. Griffin. 2015. An assessment of the human health impact of seven leading foodborne pathogens in the United States using disability adjusted life years. *Epidemiol Infect.* 143:2795-2804.

Scallan, E., R. M. Hoekstra, F. J. Angulo, R. V. Tauxe, M.-A. Widdowson, S. L. Roy, J. L. Jones, and P. M. Griffin. 2011. Foodborne illness acquired in the United States—Major pathogens. *Emerging Infectious Diseases*. 17:7-15.

Scallan, E., T. F. Jones, A. Cronquist, S. Thomas, P. Frenzen, D. Hoefer, C. Medus, F. J. Angulo, and F. W. Group. 2006. Factors associated with seeking medical care and submitting a stool sample in estimating the burden of foodborne illness. *Foodborne Pathogens and Disease*. 3:432-438.

Schalla, A., L. Meyer, Z. Meyer, S. Onetti, A. Schultz, and J. Goeser. 2012. Hot topic: apparent total-tract nutrient digestibilities measured commercially using 120-hour in vitro indigestible neutral detergent fiber as a marker are related to commercial dairy cattle performance. *J Dairy Sci*. 95:5109-14.

Scharff, R. L. 2012. Economic burden from health losses due to foodborne illness in the United States. *Journal of Food Protection*. 75:123-131.

Scharff, R. L., J. McDowell, and L. Mederios. 2009. Economic cost of foodborne illness in Ohio. *Journal of Food Protection*. 72:128-136.

Schmidt, J. W., G. E. Agga, J. M. Boilevac, D. M. Brichta-Harhay, S. D. Shackelford, R. Wang, T. L. Wheeler, T. M. Arthur. 2015. Occurrence of Antimicrobial-Resistant *Escherichia coli* and *Salmonella enterica* in the Beef Cattle Production and Processing Continuum. *Appl Environ Microiol*. 81(2):71-725.

Smith, G. W., M. L. Alley, D. M. Foster, F. Smith, and B. W. Wileman. 2014. Passive immunity stimulated by vaccination of dry cows with a *Salmonella* bacterial extract. *J Vet Intern Med*. 28:1602-5.

Smith, R. P., G. A. Paiba, and J. Ellis-Iversen. 2008. Short communication: turbidity as an indicator of *Escherichia coli* presence in water troughs on cattle farms. *J Dairy Sci*. 91:2082-5.

Sockett, P. N., and J. A. Roberts. 1991. The social and economic-impact of salmonellosis - A report of a national survey in England and Wales of laboratory-confirmed *Salmonella* infections. *Epidemiology and Infection*. 107:335-347.

Sofos, J. N., S. L. Kochevar, G. R. Bellinger, D. R. Buege, D. D. Hancock, S. C. Ingham, J. B. Morgan, J. O. Reagan, and G. C. Smith. 1999a. Sources and extent of microbiological contamination of beef carcasses in seven United States slaughtering plants. *Journal of Food Protection*. 62:140-145.

Sofos, J. N., S. L. Kochevar, J. O. Reagan, and G. C. Smith. 1999b. Incidence of *Salmonella* on beef carcasses relating to the U.S. Meat and Poultry Inspection regulations. *Journal of Food Protection*. 62:467-473.

Stephens, T. P., G. H. Loneragan, E. Karunasena, and M. M. Brashears. 2007a. Reduction of *Escherichia coli* O157 and *Salmonella* in feces and on hides of feedlot cattle using various doses of a direct-fed microbial. *Journal of Food Protection*. 70:2386-2391.

Stephens, T. P., G. H. Loneragan, T. W. Thompson, A. Sridhara, L. A. Branham, S. Pitchiah, and M. M. Brashears. 2007b. Distribution of *Escherichia coli* O157 and *Salmonella* on Hide Surfaces, the Oral Cavity, and in Feces of Feedlot Cattle. *J Food Prot*. 70:1346-1349.

Tack, D. M., E. P. Marder, P. M. Griffin, P. R. Cieslak, J. Dunn, S. Hurd, E. Scallan, S. Lathrop, A. Muse, P. Ryan, K. Smith, M. Tobin-D'Angelo, D. J. Vugia, K. G. Hold, B. J. Wolpert, R. Tauxe, and A. L. Geissler. 2019. Preliminary Incidence and Trends of Infections with pathogens Trasmitted Commonly Through Food – Foodborne Diseases Active Surveillance Network, 10 U.S. Sites, 2015-2018. *MMWR Morb Mortal Wkly Rep.* 68(16):369-373.

Tauxe, R. V., M. P. Doyle, T. Kuchenmuller, J. Schlundt, and C. E. Stein. 2010. Evolving public health approaches to the global challenge of foodborne infections. *International Journal of Food Microbiology*. 139:S16-S28.

Taylor, D. B., R. D. Moon, and D. R. Mark. 2012. Economic Impact of Stable Flies (Diptera: Muscidae) on Dairy and Beef Cattle Production. *Journal of Medical Entomology*. 49:198-209.

Threlfall, E. J., L. R. Ward, J. A. Frost, and G. A. Willshaw. 2000. The emergence and spread of antibiotic resistance in food-borne bacteria. *International Journal of Food Microbiology*. 62:1-5.

Tirado, C., and K. Schmidt. 2001. WHO surveillance program for control of foodborne infections and intoxications: Preliminary results and trends across greater Europe. *Journal of Infection*. 43:80-84.

Toth, J. D., H. W. Aceto, S. C. Rankin, and Z. Dou. 2011. Survival characteristics of *Salmonella enterica* serovar Newport in the dairy farm environment. *J Dairy Sci*. 94:5238-46.

Toth, J. D., H. W. Aceto, S. C. Rankin, and Z. Dou. 2013. Short communication: Survey of animal-borne pathogens in the farm environment of 13 dairy operations. *J Dairy Sci*. 96:5756-61.

USDA. 2008. Part III:Reference of Dairy Cattle Health and Management Practices in the United States, 2007. In, Dairy 2007 USDA-APHIS-VS, CEAH, Fort Collins, CO.

USDA-AMS. Date, 2013, Technical Requirements Schedule-- GB--2013 for USDA Purchases of Ground Beef Items, Frozen. Available at: http://www.ams.usda.gov/AMSv1.0/getfile?dDocName=STELPRDC5103936. Accessed.

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

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

USDA-APHIS. Date, 2013, Vaccine Usage in U.S. Feedlots. United States Department of Agriculture, Animal and Plant Health Inspection Services. Accessed 22DEC, 2013.

USDA-ERS. Cost Estimates of Foodborne Illnesses. http://ers.usda.gov/data-products/cost-estimates-of-foodborne-illnesses.aspx.

USDA-FSIS. Date, 1996, Notice of policy change; achieving the zero-tolerance performance standard for beef carcasses by knife trimming and vacuuming with hot water or steam; use of acceptable carcass interventions for reducing carcass contamination without prior agency approval. Accessed 19DEC, 2014.

USDA-FSIS. Date, 2011, Serotypes profile of *Salmonella* isolates from meat and poultry products: January 1998 through December 2010. Available at: http://www.fsis.usda.gov/wps/portal/fsis/topics/ data-collection-and-reports/microbiology/annual-serotyping-reports. Accessed April 4, 2013.

USDA-FSIS. Date, 2013, FSIS Directive 7120.1- Safe and suitable ingredients used in the production of meat, poultry, and egg products. Available at: http://www.fsis.usda.gov/wps/portal/fsis/topics/regulations/directives/7000series/safe-suitable-ingredients-related-document. Accessed 20DEC, 2014.

USDA-FSIS. Date, 2014, Export requirements for Japan. Available at: http://www.fsis.usda.gov/wps/portal/fsis/topics/international-affairs/exporting-products/export-library-requirements-by-country/japan. Accessed 11OCT, 2014.

Van Donkersgoed, J., J. Berg, A. Potter, D. Hancock, T. Besser, D. Rice, J. LeJeune, and S. Klashinsky. 2001. Environmental sources and transmission of *Escherichia coli* O157 in feedlot cattle. *Canadian Veterinary Journal*. 42:714-720.

Van Donkersgoed, J., V. Bohaychuk, T. Besser, X. Song, B. Wagner, D. D. Hancock, D. R. Renter, and D. A. Dargatz. 2009. Occurrence of foodborne bacteria in Alberta feedlots. *Canadian Veterinary Journal*. 50:166-172.

Varma, J. K., K. Mølbak, T. J. Barrett, J. L. Beebe, T. F. Jones, T. Rabatsky-Ehr, K. E. Smith, D. J. Vugia, H. H. Chang, and F. J. Angulo. 2005. Antimicrobial-resistant non-typhoidal *Salmonella* is associated with excess bloodstream infections and hospitalizations. *Journal of Infectious Diseases*. 191:554-561.

Villarreal-Silva, M., D. P. Genho, I. Ilhak, L. M. Lucia, J. S Dickson, K. B. Gehring, J. W. Savell, and A. Castillo. 2016. Tracing Surrogates for Enteric Pathogens Inoculated on Hide through the Beef Harvesting Process. *J Food Prot.* 79(11):1860-1867.

Vipham, J. L., G. H. Loneragan, L. M. Guillen, J. C. Brooks, B. J. Johnson, A. Pond, N. Pond, and M. M. Brashears. 2015. Reduced burden of *Salmonella enterica* in bovine subiliac lymph nodes associated with administration of a direct-fed microbial. *Zoonoses Public Hlth.* 62(8): 599-608.

Von Essen, S. G., and B. W. Auvermann. 2005. Health effects from breathing air near CAFOs for feeder cattle or hogs. *J Agromedicine*. 10:55-64.

Webb, H. E., D. M. Brichata-Harhay, M. M. Brashears, K. K. Nightinglae, T. M. Arthur, J. M. Bosilevac, N. Kalchayanand, J. W. Schmidt, R. Wang, S. A. Granier, T. R. Brown, T. S. Edrington, S. D. Shackelford, T. L. Wheeler, and G. H. Loneragan. 2017. *Salmonella* in Peripheral Lymph Nodes of Healthy Cattle at Slaughter. *Front Microbiol.* 8:2214.

Wells, S. J., P. J. Fedorka-Cray, D. A. Dargatz, K. Ferris, and A. Green. 2001. Fecal Shedding of *Salmonella* spp by dairy cows on farm and at cull cow markets. *J Food Prot*. 64:3-11.

Wetzel, A. N., and J. T. LeJeune. 2006. Clonal dissemination of *Escherichia coli* O157:H7 subtypes among dairy farms in northeast Ohio. *Appl Environ Microbiol*. 72:2621-6.

Wheeler, T. L., N. Kalchayanand, and J. M. Bosilevac. 2014. Pre- and post-harvest interventions to reduce pathogen contamination in the U.S. beef industry. *Meat Sci*. 98:372-82.

Xie, Y., J. W. Savell, A. N. Arnold, K. B. Gehring, J. L. Gill, and T. M. Taylor. 2016. Prevalence and Characterization of *Salmonella enterica* and *Salmonella* Bacteriophages Recovered from Beef Cattle Feedlots in South Texas. *J Food Prot.* 8:1304-1459*.*

Xu, Y., S. Tao, N. Hinkle, M. Harrison, and J. Chen. 2017. *Salmonella*, including Antibiotic-Resistant *Salmonella*, from Flies Captured from Cattle Farms in Georgia, U.S.A. *Sci Total Environ.* 10:324.

Yang, X., B. R. Bullard, I. Geornaras, S. Hu, D. R. Woerner, R. J. Delmore, J. B. Morgan, and K. E. Belk. 2017. Comparison of the Efficacy of a Sulfuric Acid-Sodium Sulfate Blend and Lactic Acid for the Reduction of *Salmonella* on Prerigor Beef Carcass Surface Tissue. *J Food Prot.* 3:809-813.

Yeh, Y., F. de Moura, K. Van Den Broek, M. Fonseca, and A. S. De Mello. 2017. Effects of Ultraviolet Light, Organic Acids, and Bacteriophage Interventions on *Salmonella* Populations in Ground Beef. *Meat Sci.* 139: 44-48.

Zhoa, T., M. P. Doyle, P. J. Fedorka-Cray, P. Zhoa, and S. Ladely. 2002. Occurence of *Salmonella enterica* Serotype Typhimurium DT104A in retail ground beef. *Journal of Food Protection*. 65:403-407.