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Executive Summary

Salmonella enterica is a member of the Enterobacteriaceae family and is closely related to *Escherichia coli*. *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-typhpoidal 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 US. Of concern in the US, 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 US, 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 university, 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.

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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 US and abroad. The majority of non-typhoidal 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 (18, 133, 153). 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 (50).

Current estimates indicate that exposure to *Salmonella* results in 93.76 million illnesses worldwide each year (129). In the US, it is estimated that *Salmonella* is responsible for 1.029 million illnesses, 19,000 hospitalizations, and nearly 400 deaths annually (159). Despite these figures, the Centers for Disease Control and Prevention (CDC) reported only 42,000 laboratory-confirmed clinical cases of salmonellosis (48). 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 (68). 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 U.S. Department of Agriculture Food Safety Inspection Services (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* (41).

In a recent edition of the Morbidity and Mortality Weekly Report, Crim et al. (57) reported the 2013 FoodNet findings citing 19,056 laboratory-confirmed cases of foodborne illness, which led to the hospitalization of 4,200 individuals and 80 deaths. For the 2013 FoodNet data, non-typhoidal *Salmonella* was the most commonly reported pathogen, with 7,277 cases of human illness attributed to *Salmonella* infection alone. The most commonly reported *Salmonella* serotypes were *Salmonella* serotype Enteritidis (*S*. Enteritidis: 19%), *Salmonella* serotype Typhimurium (*S*. Typhimurium: 14%), and *Salmonella* serotype Newport (*S*. Newport: 10%) (57). Furthermore, the incidence of laboratory-confirmed salmonellosis in 2013 (i.e., 15.19 cases per 100,000 individuals) was not different than that of prior FoodNet reporting years (47).

As with any surveillance system, it is important to assess possible limitations while interpreting and reporting results. A series of events must transpire in order 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) (160). 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 actually much smaller than the true population value (172). 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 (47).

In addition to the FoodNet surveillance data, the CDC also collects national *Salmonella* surveillance data from clinical diagnostic laboratories through passive surveillance (40). 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 (44). In line with the most recent FoodNet data, the annual report of the national *Salmonella* surveillance data, published in 2011, also named the top three illness-causing serotypes as *S*. Enteritidis (16.5%), *S*. Typhimurium (13.4%), and *S*. Newport (11.4%).

These surveillance systems are maintained by the CDC in order to monitor the pulse of foodborne illness in the US. 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 (138). 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 (161). 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 FSIS (51).

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 describe the magnitude 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 (31). 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 (130).

When determining the total economic cost per illness, there are several factors that must be taken into account. 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* (163). When employed by Scharff (163), 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. (159) publication, estimates provided by Mead et al. (133) were used in cost-of-illness models to project the economic burden of foodborne illnesses (164). 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. (159) from the estimates previously provided by Mead et al. (133) is the result of a change in the actual burden of disease (163) 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 (163) 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 (167) 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 (167). Public health costs associated with health department investigation and laboratory testing accounted for 16% of the total cost estimated by the survey.

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 the majority of 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 (110). 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 (95). 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 (27). 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 (49).

Salmonella Typhimurium is the second most prevalent serotype isolated from food, accounting for 14% of laboratory-confirmed cases of salmonellosis (57). *Salmonella* Typhimurium is also one of the top serotypes isolated from food-producing animals and retail meats (127). 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 (49).

The majority of 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 (23). 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) (175). Paniselloa et al. (142) demonstrated the use of retrospective analysis of foodborne illness outbreak data and its value as a means to maintain and further develop Hazard Analysis and Critical Control Point (HACCP) systems by establishing critical control points along the food production chain.

2.5 Antimicrobial Resistance and *Salmonella*

Antimicrobial resistance in *Salmonella* is a pertinent public health issue that has been an oft contentious topic of discussion for several decades (52, 174). 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 (1). 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 (186). 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 (2, 3, 52). 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 tasked with tracking changes in the susceptibility of enteric bacteria to antimicrobial agents of clinical importance in human and veterinary medicine (77). This national public health surveillance system is a collaborative effort among CDC, FDA, and USDA and is in place to monitor antimicrobial resistance of enteric bacteria isolated from ill people, retail meats, and food-producing animals (83).

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 (82). 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 (3), *S*. serotype Infantis (3), *S*. serotype Mbandaka (1), *S*. serotype Montevideo (1), and *S*. serotype Litchfield (1) 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 (82). On the other hand, only 11.1% of ground beef and 28.6% of pork isolates were classified as MDR (82).

2.6 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 (68), 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 (68).

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 (68, 94). 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 to be important reservoirs for this pathogen. The attribution of human *Salmonella* infections from food-producing animals has been described previously (68, 158). 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 (94). For example, *Salmonella* serotypes commonly associated with cattle include: *S*. serotype Anatum, *S*. Montevideo, *S*. Dublin, and *S*. Infantis (68). 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 (26, 68). 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 (68). 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 (146).

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 (107). When assessing attribution data for outbreaks and number of cases, a figure in Doyle (68) 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 (68).

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 (145). 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 (22). 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.

The combined use of multiple-source attribution methods has proven useful to estimate disease attribution and to further describe the public health burden of *Salmonella*. Batz et al. (22) 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 publically available outbreak data from the CDC's Foodborne Outbreak Online Database (FOOD) and expert elicitation for food attribution estimates (22). 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 (22). 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. Of the total illnesses attributed to *Salmonella* in this study, poultry accounted for the most with 221,045 illnesses, followed by complex foods (195,655), produce (170,264), and eggs (115,003).

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*.

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 (4, 36, 60, 93, 121, 139). 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 southern portions of the US. Fecal *Salmonella* shedding among cattle can persist for extended durations following clinical disease (34), 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 (33, 80, 117). *Salmonella* are pathogens capable of residing as transient members of the intestinal microbial population within bovine species (34). Although the prevalence of *Salmonella* within CAPFs is relatively high, especially in the southern US (25, 61, 66, 117, 188), the incidence of salmonellosis does not reflect this in mature cattle (59, 76). Young animals are frequently colonized by *Salmonella* and are most likely to experience salmonellosis within 2-4 weeks of age (103). 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 (66). Thus, reliance on overt clinical indicators of illness is not an effective indicator of *Salmonella* colonization, as infected animals may appear healthy (34).

Reasons behind the absence of clinical signs of *Salmonella* infections in cattle are currently uncertain. House et al. (103) 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 (103). However, recent data has shown that rather than acquiring this pathogen after birth, animals may be infected in utero (100). 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, as shown in **Figure 1** (100). Multiple serogroups were present with the primary serogroups consisting of C1, C2, E1, and other, 42, 30, 15, and 13%, respectively (97). 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.

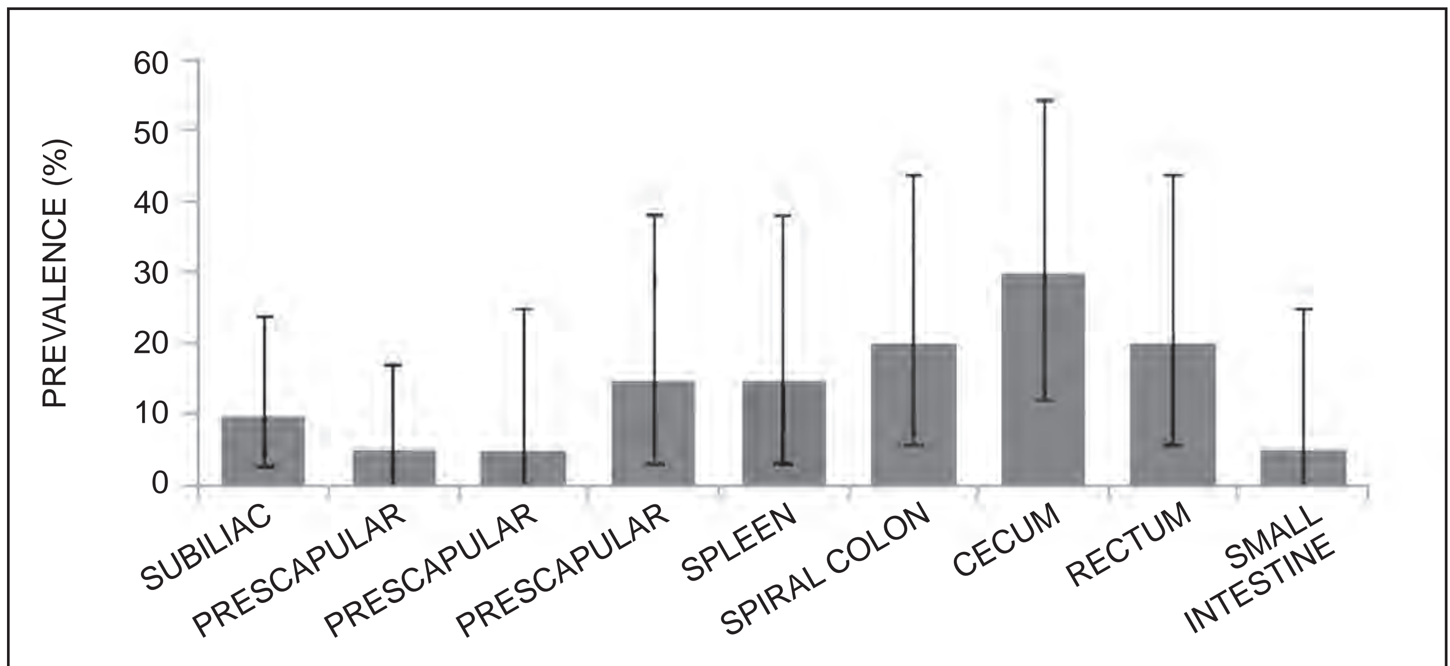

Figure 1. Prevalence of *Salmonella* by sample type collected from 20 full-term calves (97).

Empirical evidence has shown the prevalence of *Salmonella* varies significantly due to both season and region (25, 59, 61, 66, 117, 185, 188) and is apparent when evaluating the prevalence of fecal *Salmonella* in CAPFs (73, 117). Estimating the prevalence of *Salmonella* in the animals within a facility is often conducted by sampling feces and/or hides (8, 28, 37, 171). 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 (5, 65). Rather than being a function of the season, this is primarily reflective of the temperature within seasons (113), *Salmonella* typically thrives in warm weather and is suppressed in cold weather (113, 126). 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 US (76) and herd-specific in the northern regions (59, 149).

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 (25, 35, 66, 73, 117, 125, 171, 188). Edrington et al. (73) sampled 60 healthy lactating dairy cattle on each of four CAPFs in the southwest US 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. (117) 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. (188) 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 US (45% of dairies culture positive). However, Callaway et al. (37) 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 (37) compared to fecal prevalence previously reported from the southern region (66, 117), it is, however, substantially greater than other studies conducted in the northern portions of North America (149, 185).

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** (98).

The aforementioned studies exemplify the differences in fecal prevalence of *Salmonella* across regions. (66, 149, 185). The operational paradigm of dairy and feedlot operations consists of routinely purchasing and transporting animals into operations, potentially creating a biosecurity issue (4, 101). The potentially pathogenic microorganisms these animals may be harboring is moderately dependent on the region from which they were obtained. 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 (59, 61, 66, 117, 185, 188).

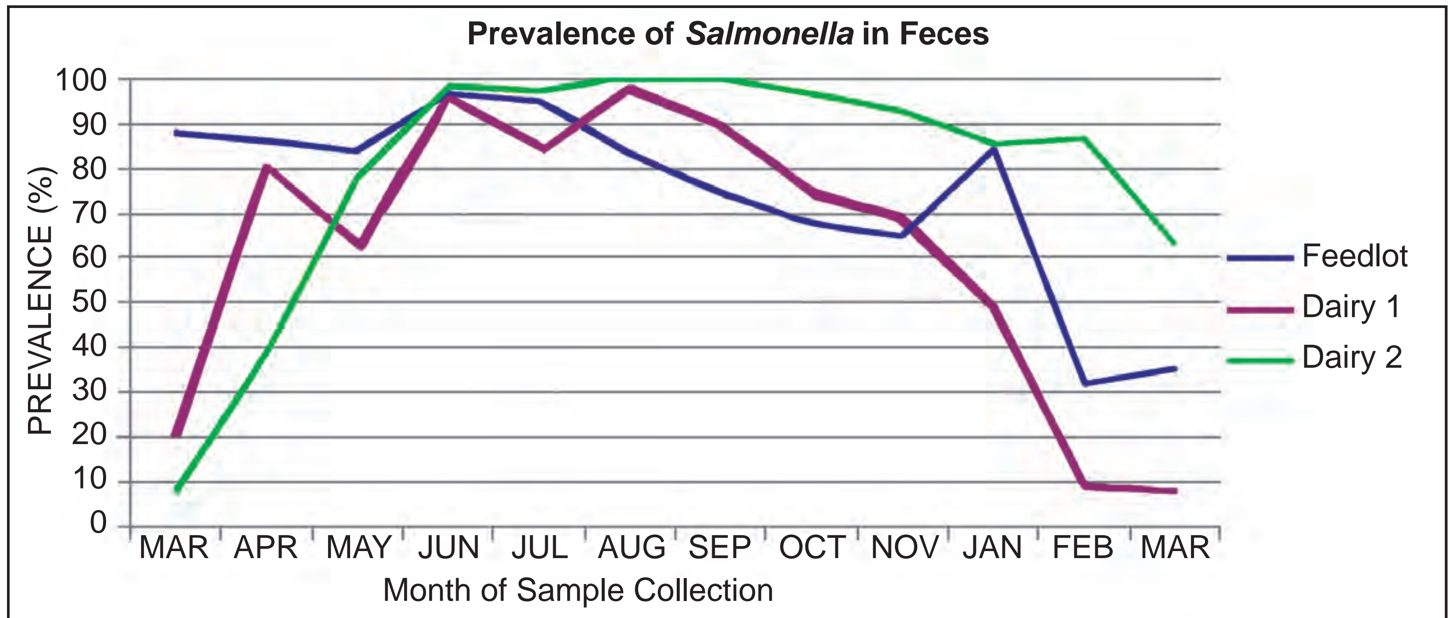

Figure 2. Prevalence of *Salmonella* in feces by commercial agricultural production facilities (CAPF) and month of sample collection post-enrichment (98).

3.3 Hide Prevalence

Pathogen prevalence on hides may reflect several sources of contamination, which accurately reflects the pathogen load of the environment (8) in addition to the load of the individual animal (151). 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 (21). Despite management practices employed by facilities, cattle activity and frequent movement throughout the day result in pulverization and subsequent aerosolization of pen floor material (131). Facilities in close proximity to one another have the capability to share bacteria due to fugitive dust generated within pens (187). 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 (131). Due to the intermittent grooming of cattle by themselves and each other, hide contamination often serves as an additional vehicle for a *Salmonella* infection (7). In an attempt to track the source of *Salmonella* contamination in ground beef, Koohmaraie et al. (115) 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. (28, 29), where the mean prevalence of hide contamination was 89.6% (n=3,040) across six different abattoirs in four geographically distinct regions of the US. 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 (150, 190).

3.4 *Salmonella* in Water

It has been shown that water troughs in CAPFs are reservoirs for *Salmonella* (76, 121). Recent studies have reported high concentrations of *Salmonella* in water troughs with seasonal fluctuations in the prevalence of

Salmonella (76). This is a significant concern due to the increased exposure when considering the number of cattle that consume water from the same trough (120). 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 (76). 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** (76, 98). The varying level of pathogens in the water could either be a reflection of 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 period of time between cleanings of the troughs (121, 141, 166).

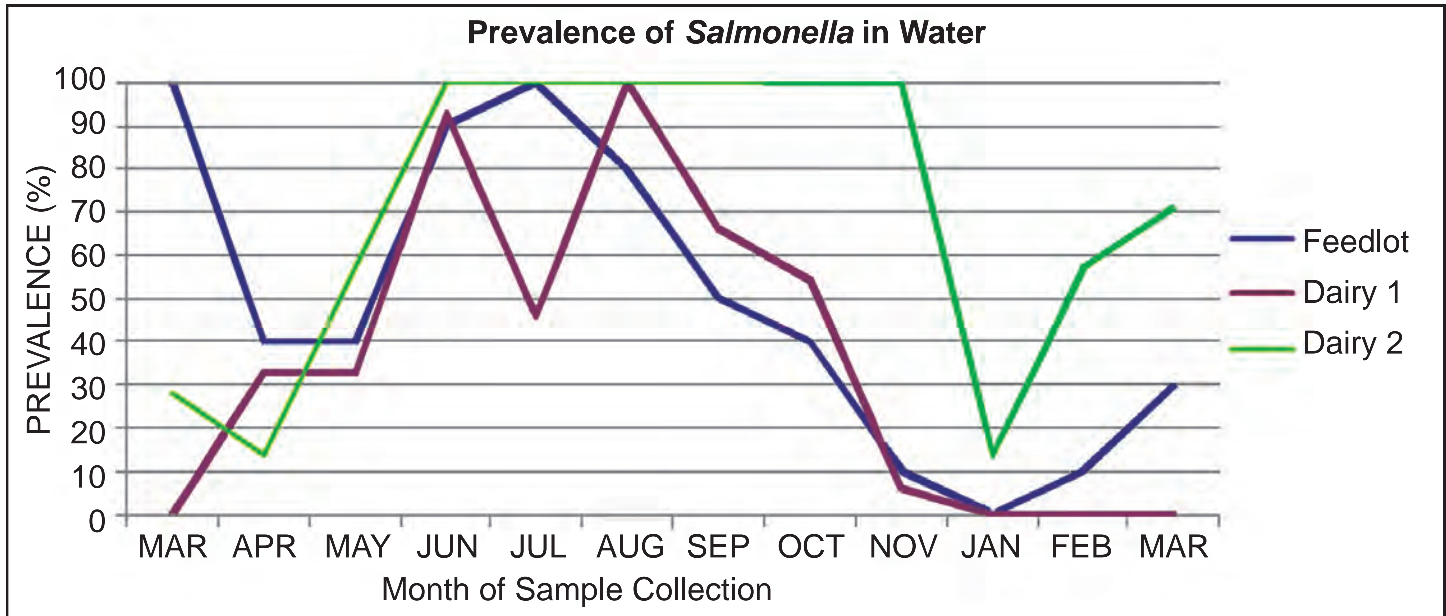

Figure 3. Prevalence of *Salmonella* in water by commercial agricultural production facilities (CAPF) and month of sample collection post-enrichment (98).

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 (121), or non-cattle-related possibilities such as birds (143). 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 (121, 171). Direct contamination from the animal's oral cavity is also a plausible route of infection; Stephens et al. (171) recovered *Salmonella* from oral swabs in 94% (n=50) of animals enrolled in a study in west Texas.

Another potential source of contamination could be non-mammalian vectors such as flies, pigeons, and additional avian species (36, 139, 143). All of the aforementioned vectors are routinely found in and around CAPFs and have been shown to carry pathogenic bacteria (139, 143). Avian species, such as Rock Dove (feral pigeons) and Eurasian-Collered Dove, inhabit CAPFs across all seasons (79, 143, 173), whereas Mourning Dove and flies are more seasonal (55, 139). In addition to consuming water from these troughs, these animals often defecate in and around troughs, and birds often use this water source to bathe themselves (38).

It is likely a combination of the aforementioned scenarios that lead to the contamination of water troughs. Water troughs have the potential to infect a large number of animals with *Salmonella* within a herd (76).

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 (109). The role of contaminated feeds in the non-clinical presence of *Salmonella* in animals is largely unknown (62). 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 (102). It is unclear if feed ingredients become contaminated with fecal bacteria prior to delivery to the feedlot and/or after arrival (62). Contamination of feeds could occur while growing, in storage, during transport, or during handling for processing into mixed feeds (62, 63, 126).

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 (102). 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 (69). In a 2011 study by Toth et al. (176) 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 (62, 102, 116). 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* (36, 38, 54, 134, 143). Avian species typically congregate in large roosting groups and exploit abundant and highly palatable food sources (79, 119). 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 (38). In addition, rodents commonly inhabit CAPFs, similarly exploiting food sources and transmitting pathogens to feedstuffs (134).

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 (131). As mentioned above, pulverized feces is easily dispersed by the frequent wind in the southwest US. Sprinklers are often utilized to mitigate the occurrence of blowing dust on CAPFs; however, data are mixed in regards to this technique's efficacy in reducing the incidence of fecal shedding of pathogenic bacteria (71, 137).

Additionally, in a recent study, Ge et al. (90) 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 (162).

In an attempt to investigate the seasonality of salmonellosis in dairy cattle, Edrington et al. (76) collected total mixed ration (TMR) samples monthly over a nine-month period directly from the feed bunk on a dairy located in the southwest US. The average prevalence of *Salmonella* in feed was 76% across the sampling period with a range of 4 to 100% (76). 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. (62). 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 (36, 139). Flies are found seasonally as are some avian species, however Rock Dove, Eurasian-Collered Dove, and European Starlings are considered peridomestic (79, 143, 173). Livestock facilities are attractive to avian species because of the availability of large quantities of feed and water (79, 108). 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 (184, 189). Wild birds have been implicated in the transmission of pathogens when the same subtypes were identified from feedlots approximately 50 to 100 km apart (184, 189).

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 (79, 108). Ingestion of feed contaminated with bird feces has been identified as a possible route of infection for cattle (60). Recent efforts have been focused on evaluating potential environmental sources within CAPFs and determining the burden of their presence (38, 70, 119). Edrington et al. (70) 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 (70).

Flies are common on CAPFs in the summer and fall months, with populations varying greatly across facilities (173). A large proportion of facilities employ management practices to mitigate the fly population (179). In addition to defecating and regurgitating in the environment, flies have the capability to infect cattle via penetrating the hide while blood feeding (139). Animal hides and manure pats are sources for flies to acquire the *Salmonella* and mechanically transmit them to an animal while feeding (35, 139). Edrington et al. (70) reported the predominate serogroups harbored by flies collected on multiple CAPFs were C1, C2, E1, and K.

3.7 Pre-harvest Interventions

The various interconnected vehicles that may potentially transmit *Salmonella* on cattle operations discussed throughout this section make control of *Salmonella* extremely complex. Each vehicle has been documented as independently impacting the transmission of *Salmonella* to cattle (38, 58, 76, 90, 121, 139, 171, 176, 177). The large proportion of the cattle located in the southern US infected with *Salmonella* creates not only a food

safety concern (88), but also a potentially detrimental 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*.

3.8 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 (6). 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 (42); 2) environmental persistence within CAPFs provides an accumulative reservoir for zoonotic pathogens (88, 89, 154); 3) vaccinated cattle are not adequately protected against the emergence of strain variants that may be more virulent (100); 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 (6, 89, 99, 103, 105).

Vaccination aims to stimulate the development of naturally acquired immunity by inoculation of non-pathogenic, but still immunogenic, components of the pathogen in question (135). 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 (124). 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 (99, 128). It has been reported that less than 1% of beef cattle operations utilize any type of commercially available *Salmonella* vaccine on their cattle (181), and less than 6% of animals fed in feedlots receive a *Salmonella* vaccine (182). 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 (180).

Although *Salmonella* vaccines are not widely adopted, evidence exists suggesting that vaccinating animals may be beneficial. Loneragan et al. (125) 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. (165) 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 (99). Conventional, commercially available vaccines are currently only capable of stimulating antibodies of serogroups B, C, or D (103). 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) (101). These SRP proteins are essential for bacterial survival as they allow iron acquisition from the environment (65). The vaccine restricts the ability of the bacteria

to gain iron from the environment via stimulating antibodies to bind to the SRP proteins (165). 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 (65, 101, 165).

3.9 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 (170). 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 (32). 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 has proven advantageous for multiple reasons: 1) DFMs have shown to effectively mitigate the shedding of *Salmonella* in feedlot cattle (170) and 2) producers often observe increased performance characteristics (e.g., weight gain and feed-to-gain ratio) in animals fed DFMs (170). The inclusion of DFMs are more widely adopted than vaccination regimens (165, 180-182); Ison (106) estimated 45.7% of feedlot-finished cattle harvested in 2012 were administered *L. acidophilus* NP51 at some point prior to harvest.

3.10 Animal Washes

Prior to entering the abattoir, the hides of cattle are often contaminated with excrement, dust, and/or mud that frequently contain pathogenic bacteria (15, 28, 112). 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 (64, 131, 150). 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 (136).

4 *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 (24, 29). Cross-contamination during fabrication is another potential hazard. The prevalence of *Salmonella* on beef carcasses, albeit low, remains cause for concern in regards to public health and prevention of *Salmonella*-related illnesses. Many interventions have been employed throughout the harvesting and fabrication processes as a means to lower, if not eliminate, pathogen contamination of beef carcasses.

4.1 Prevalence of *Salmonella* on Beef Carcasses and Ground Beef

In a study conducted by Rivera-Betancourt et al. (152), 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 located in the southern US and Plant B was located in the

northern US. 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 of the 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 (152).

In a similar prevalence study, Barkocy-Gallagher et al. (20) 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 US. 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 particular 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 (20).

A study conducted by Bacon et al. (16) aimed to determine the prevalence of *Salmonella* on the hides of beef cattle and also 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 US. 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 (16).

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). Individually, plants 1-8 showed prevalence on hides as being 47.5 (n=40), 10 (n=40), 0 (n=40), 23 (n=39), 0 (n=40), 10 (n=40), 17.5 (n=40), and 15% (n=40), respectively. Only two plants produced Salmonella-positive carcass swabs. *Salmonella* was recovered from 7.5% (n=40) of samples from Plant 1 (3 positive samples) and 2.5% (n=40) of samples from Plant 8 (1 positive sample). These results indicate the decontamination treatments used at these plants are effective at reducing *Salmonella* contamination (16).

Contamination can occur at different anatomical locations on the carcass. Sofos et al. (169) 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 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 (169).

The results of this study demonstrate the effectiveness of interventions applied to the carcass at these facilities. Overall, the percentage of *Salmonella*-positive samples collected following the final carcass wash (0.6, n=360 and 0.8%, n=360 in the wet and dry seasons, respectively, at steer-heifer plants and 3.0, n=270 and 1.8%, n=270 in cow-bull plants) was lower than that of pre-evisceration samples (2.5, n=360 and 3.6%, n=360 in wet and dry seasons, respectively, in steer-heifer plants and 8.5, n=270 and 5.2%, n=270 in cow-bull plants) (169).

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 were 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 (169).

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%

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 particular 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.

These studies and others show that while current intervention strategies are effective at significantly reducing *Salmonella* contamination of beef carcasses to very low numbers, some *Salmonella* contamination on carcasses that can lead to contaminated whole muscle cuts or ground beef products still remains.

Contamination on the surface of the beef carcass can lead to *Salmonella* contamination of the ground beef product. Zhoa et al. (191) conducted a surveillance study to determine the occurrence of *Salmonella* in retail ground beef. Samples were collected from seven cities in geographically different areas of the US. The investigators reported a prevalence of *Salmonella* in ground beef as 3.5% (n=404). In another study, Samadpour et al. (155) collected ground beef samples from retail stores in Seattle, Washington over a 12-month period. *Salmonella* was recovered from 67 out of 1750 samples (3.8%). This is similar to results of others (26).

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 (24, 104). 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 (10). 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.

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5 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.

5.1 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.(147) 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 (147). 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 (190). 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 (67). 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 FSIS as a substitute for knife trimming to remove visible contamination (190).

5.2 Acid Antimicrobials

Many acid antimicrobials are used in commercial beef plants as a means 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 (17). 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 (30, 56).

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 (84); 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 (19). 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 (190).

Lactic acid is one of the most widely used organic acids in the meat industry due to a combination of effectiveness and cost (190). It has been reported that use of lactic acid reduces aerobic plate counts by 1.5 $\log_{10}$ CFU/cm^2 (104). 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 (118). In a study that compared several decontamination treatments, lactic acid reduced *Salmonella* from by 1.80 log CFU/cm^2 (11).

5.3 Oxidizer Antimicrobials

Another category of post-harvest interventions is oxidizer antimicrobials. These can include peroxyacetic acid, electrolyzed oxidized (EO) water, or acidified sodium chlorite (ASC). Peroxyacetic acid is approved by the FSIS for use in commercial beef plants at a maximum of 1800ppm (78), although it is generally used at 200ppm. King et al. (114) 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 similar to that of ozone (13, 190). Arthur et al. (11) 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 (183). 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 (190). 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 (39). 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 (148).

Hypobromous acid is an antimicrobial agent that has been used in processing water for specific food products for a long time and is now approved for use on poultry and beef carcasses. In the beef industry, it is commonly used at 300ppm for carcass surface decontamination (190). 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 (111). Use of hypobromous acid was common in beef processing up until 2013 when it was removed from the Pathogen Reducing Technologies listed in FSIS Export Library for Japan (87).

5.4 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 (190). 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 (190). Many studies have been conducted that investigated the use of water at temperatures ranging from 74°C up to 95°C. Spraying with hot water raises the temperature of the carcass surface. The FSIS acknowledges that water greater than 74°C will produce a sanitizing effect (104). Arthur et al. (11) 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 (104).

A similar treatment to hot water washes is steam pasteurization. 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 more quickly (190). Steam pasteurization markedly reduced *S*. Typhimurium on the surface of beef. A count reduction of 3.7 log CFU/cm^2 was reported by Phebus et al. (144). In another study, steam treatment for 6 seconds reduced *Salmonella* counts by approximately 3 log CFU/cm^2 (190). Steam pasteurization cabinets are often used as a final carcass intervention in U.S. beef processing plants.

5.5 Non-thermal Interventions

Thermal treatments are highly effective at reducing contamination on beef carcasses, but heat treatments can lead to physical and chemical changes in the product resulting in loss of quality. Non-thermal technologies are either in use or are being investigated as alternative 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 (53, 190). The effective wavelength for bactericidal activity is at 253.7 nm (190). The UV light works by causing damage to DNA leading to cell death (53). The use of UV-C (wavelength of 220-300 nm with 90% of emission at 253.7 nm) has been approved by FDA for use on food products to control microorganisms (53, 81). 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. (53) observed a 1.19 log CFU/cm^2 reduction of *S*. Typhimurium on chicken breasts. Sensory aspects were also evaluated in this study, and no differences were 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 (12). 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 (12). The effect of E-beam irradiation on *Salmonella* was studied using poultry products. The investigators reported that 40% of the control chicken breast samples were positive for *Salmonella*, while none of the samples of chicken breasts exposed to electron beam irradiation yielded a positive result (122).

5.6 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. Studies have indicated greater efficacy when using a combination of decontamination strategies (149). Using a multi-hurdle approach with interventions used in sequence may result in synergistic or additive effects (190).

Understanding of 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. However, contamination of product still remains a concern for public health. With recent studies implicating lymph nodes of cattle as a mode of *Salmonella* contamination of ground beef product (10, 91, 92, 96, 115, 156, 157), it is imperative to continue researching interventions that can be implemented during further processing to reduce or eliminate *Salmonella* in beef products.

6 *Salmonella* in Bovine Lymph Nodes

6.1 Introduction to *Salmonella* in Bovine Lymph Nodes

A number of outbreaks and recalls have been reported as a result of *Salmonella*-contaminated ground beef products (45, 46, 132). Research has suggested that the carriage of such pathogens by cattle may contribute to the overall prevalence of contaminated ground beef products (26). 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. Data indicate that these interventions have resulted in tremendous

reductions in surface decontamination for both *Salmonella* and *E. coli* (43, 86). Despite the apparent success of surface decontamination intervention efforts, surveillance measures have estimated that the prevalence of *Salmonella* in ground beef products may range between 2.0 and 4.2% (26, 82). 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 (9, 91, 96, 115). Consequently, 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 (9, 91, 92, 96, 115, 157).

Lymph nodes, which are located 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. Because the *Salmonella* is encapsulated within the PLN, the 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.

6.2 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 (92, 115). 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 (92). It has been hypothesized that *Salmonella* may enter into the lymphatic system through individual, independent events (92), 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 (9, 156, 157, 168); 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 (9). 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 (123). 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 regards to food safety, including the popliteal, pre-scapular (chuck), and subiliac (flank), some of which are illustrated in **Figure 4** (9, 91, 157). 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% (9, 91, 92, 96, 115). It should be noted, however, that prevalence of *Salmonella* in small PLNs is uncommon; this is

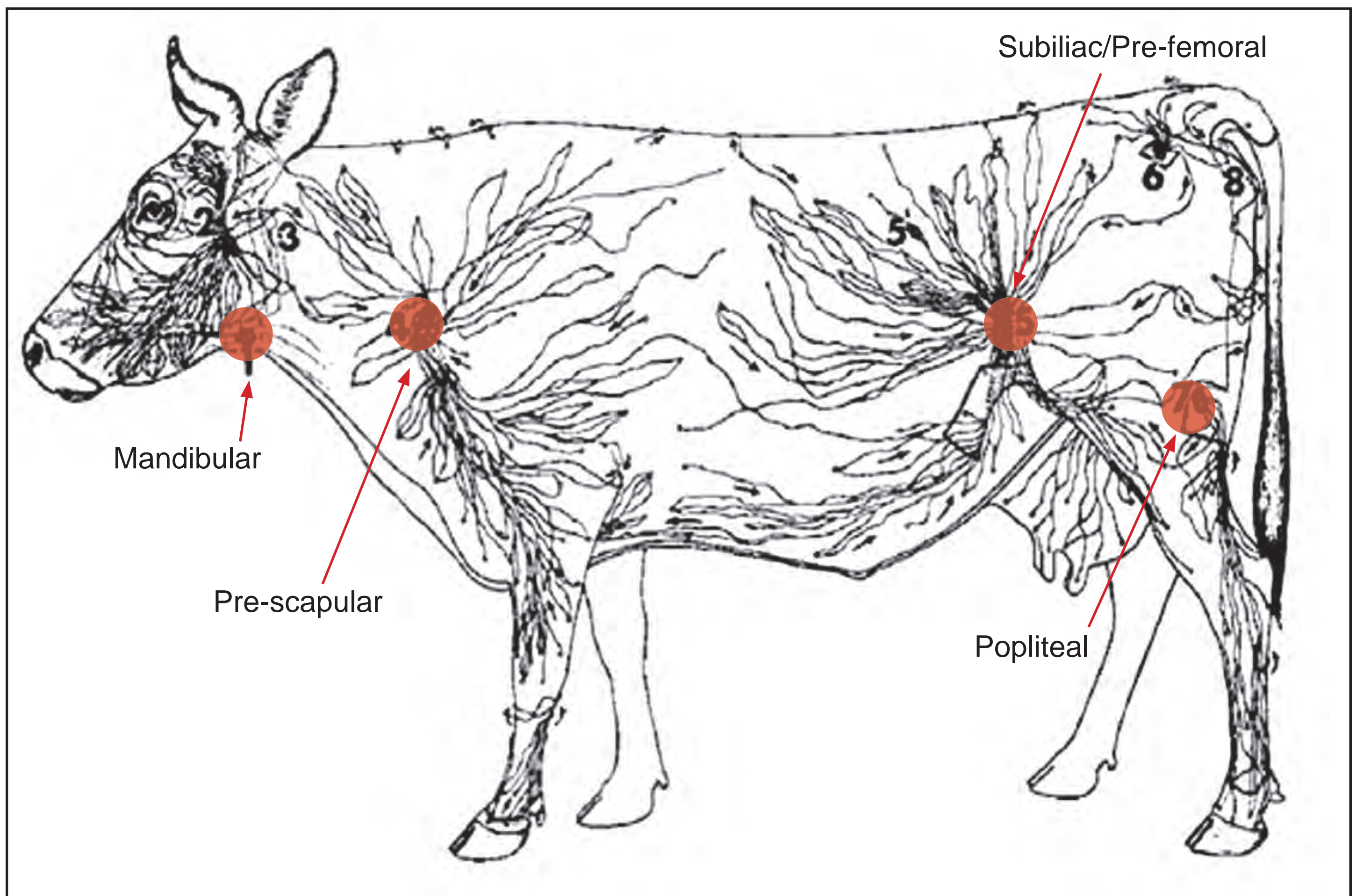

Figure 4. Mandibular, pre-scapular, subiliac/pre-femoral, and popliteal location identified on the superficial lymph flow diagram of a cow as presented by Saar and Getty in Anatomy of Domestic Animals.

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).

In an exploratory study by Gragg et al. (91), 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 (91); 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 (91). *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 US. 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 (92). As previously discussed, similar trends have been observed in fecal, hide, environmental, and food sample data (20, 73). Alternative influential variables have been hypothesized including animal temperament, animal stress levels, management styles, feeding regiments, animal origins, and environmental factors (91, 96). In addition to the seasonal and regional

trends, Haneklaus et al. (96) demonstrated that *Salmonella* prevalence in PLNs may also vary among feedlot facilities within the same geographic region. In this study, 307 pre-scapular and subiliac PLNs were collected from seven different feedlot facilities over a three-month span. Importantly, it was noted that during each sample collection, all PLNs from one facility tested negative for *Salmonella*, while PLNs from another facility tested positive for *Salmonella* with prevalence ranging from 76 to 100% (96). Despite a relatively small sample size, 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.

In addition to the potential seasonal, regional, and animal type variation reported by Gragg et al. (91), the authors also reported data regarding concentrations of *Salmonella* in PLNs. Concentrations were reported to range between 1.9 and >3.8 $\log_{10}$ CFU/ gram of lymph node (91). 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, as potential risks associated with product contaminated by particular *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 (91). While some diversity has been observed, a majority of the isolates reported by Gragg et al. (91) 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 (6, 85). *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 (91). While, overall, 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 (91). 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.

6.3 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 (74, 75, 91). 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 (72, 75, 91). 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. (75) 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 (75).

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 (74, 91, 157). 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 (74). 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 (74, 75). 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 (74).

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 (140). 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. (140).

6.4 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 (178).

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 (75). 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 (75). To date, studies assessing the efficacy of particular feeding approaches are underway, though none have reached publication.

7 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 (9, 91, 92, 96, 115). 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 (74, 75). 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*.

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