THESIS

SALMONELLA CONTAMINATION IN POULTRY—ARE WE MISSING A POTENTIAL RESERVOIR?

Submitted by

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ABSTRACT

SALMONELLA CONTAMINATION IN POULTRY—ARE WE MISSING A POTENTIAL RESERVOIR?

The objective of this study was to assess presence and characteristics of *Salmonella enterica* found in synovial fluid of broiler carcasses. Synovial fluid of three individual joints from 500 broiler carcasses was individually sampled (1,500 total samples) from five broiler processing facilities located in the Southeast and Western U.S. The external surface of broiler carcass was decontaminated prior to sampling of the shoulder, coxofemoral, and tibiofemoral joints. Individual samples were enriched, composited, and subjected to rapid PCR-based detection of *Salmonella*. Individual samples from any positive composites were also enriched before determination of *Salmonella* presence in the same manner. Positive individual samples were subjected to secondary enrichment before plating onto selective agar for isolation of *Salmonella. Salmonella* isolates were serotyped before determination of antimicrobial susceptibility. Overall, 1.00% (5 out of the 500 broiler carcasses) of composite samples, and 0.47% (7 out of 1,500 samples) of individual samples were positive for *Salmonella.* Five of the seven isolates were susceptible to all drugs and determined to be *Salmonella* Enteritidis. The remaining two isolates, identified as *Salmonella* Typhimurium, were resistant to streptomycin. To our knowledge, no previous assessments of *Salmonella* in synovial fluid of broilers has been reported; however, results of the present study suggested that *Salmonella* may be present in synovial fluid of broilers. Although low prevalence, this information provides valuable insight into potential poultry contamination pathways and warrants further exploration.

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"Destiny is no matter of chance. It is a matter of choice. It is not a thing to be waited for, it is a thing to be achieved." - William Jennings Bryan

"Do not go where the path may lead, go instead where there is no path and leave a trail." - Ralph Waldo Emerson

For me, these have been quotes that have helped define who I am; to go out and achieve my own future and my dreams. However, there is no way I could reach this point without the help of others. First and foremost, I have to thank my family as they have been my absolute rock and foundation, there to help in any way possible through thick and thin. To all of my advisors, teachers, and mentors, I thank you all for the guidance and leadership along this long winding path called life. You all have helped me realize I can accomplish far more than I ever thought I could and I will always be indebted to you for that. It would be wrong to write this and not thank my fellow grad students. We've spent countless hours together in packing plants and in microbiology labs. You all are absolutely the best, I look forward to working with everyone in the future once we all have "real jobs". And finally, I would not be where I am today without my best friend, you've kept me sane during some of the toughest times and been there to help celebrate the best, I'll never be able to fully thank you enough. Thank you all, I can't wait to see what the future will hold!

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1.1 Introduction

While advancements have been made in securing safe food for the global population,

one of the biggest area of concern is the reduction of pathogenic *Salmonella* enterica*.* Divided

into two distinct serovar groups, Typhoidal and non-Typhoidal, based on the form of infection,

non-Typhoidal *Salmonella* is one of the leading causes of foodborne illnesses worldwide with an

estimated 93.8 million cases and 155,000 deaths each year due to complications from

gastrointestinal infections (*46*). While normally resulting in diarrhea, vomiting, nausea, fever

and abdominal pain, non-typhoidal *Salmonella* can also lead to reactive arthritis, post-infectious

irritable bowel syndrome (PI-IBS) and bacteremia (*35*, *64*). Painter et al. (*58*) discovered

between 1998 and 2008, among bacterial etiologic agents, *Salmonella* was responsible for the

highest number of reported outbreaks, outbreak-associated illnesses, estimated illnesses,

estimated hospitalizations, and estimated deaths. These findings were later supported by

Scallan et al. (*65*) who estimated over 1 million illnesses, 19,000 hospitalizations, and 400

deaths have been contributed to *Salmonella* infections with Hoffmann et al. (*34*) estimating

annual costs of $3.7 million (in 2013 U.S. dollars) when accounting for productivity lost, medical

expenses and expenditures on disease prevention.

Gastrointestinal infections stem from exposure to food and water that have been

contaminated, with fecal matter from an infected individual or animal (*20*). While the risk for

Salmonella spp. induced gastroenteritis is seen worldwide, higher risk occurs in places with

reduced sanitation and access to clean water. Today, *Salmonella* infection are found in almost

all foodstuffs with the most common sources within the U.S. being poultry, eggs, and fresh

produce (*20*, *24*). Between 1998 and 2012, the Interagency Food Safety Analytics Collaboration

(*37*) determined that poultry meat products accounted for 17% (chicken products 10%; turkey

products 7%) of all *Salmonella* outbreaks, the highest of any meat protein source. This is

alarming considering that within the United States, domestic consumers eat more poultry than

any other meat protein with an estimated 49 kg of total poultry products consumed as

compared to 48 kg of total red meat products (*20*, *54*). An example of the risk associated with

Salmonella contaminated poultry was an outbreak of multidrug resistant *Salmonella* Heidelberg

from Foster Farms chicken which resulted in over 600 reported illnesses and 240

hospitalizations (*74*). Fortunately, antimicrobial resistance phenotypes expressed in this

outbreak did not include any antibiotics used to commonly treat salmonellosis such as

fluoroquinolones or third generation cephalosporins.

In an effort to control the prevalence of foodborne pathogens, the United States

Department of Agriculture Food Safety Inspection Service (USDA-FSIS; *94*) established the

Salmonella verification program in 1996 as a part of the Pathogen Reduction; Hazard Analysis

and Critical Control Point (PR/HACCP) Systems Final Rule. The initial standards, set in 1996,

provided benchmarks used to measure process control in meat and poultry slaughter. Today,

adjusting for changes in processing and production of poultry products, maximum acceptable

Salmonella and *Campylobacter* positive performance standards have been set for 5 poultry

product categories (*94*). The current performance standards used by USDA-FSIS for *Salmonella*

prevalence are as follows: broiler carcasses (7.5%), turkey carcasses (1.7%), comminuted

chicken (25.0%), comminuted turkey (13.5%), and chicken parts (15.4%; *94*). As of June of 2017,

based on 52-week moving window data between April 2016 and June 2017, 82.45% of broiler

establishments met the standards, however only 67.20% of establishments met the standards

for chicken parts (*95*). This may be associated with sampling location as whole bird carcass

rinses are often performed immediately post chilling and, until recently, did not include a

neutralizing buffer to deactivate the antimicrobials within the chilling water.

While not the conventional means for *Salmonella* contamination in poultry, several

studies (*9*, *15*, *32*, *51*, *53*, *99*) have associated *Salmonella* presence with the skeletal system.

Higgins et al. (*32*), noted in 1944 presence of *Salmonella* in association with joint inflammation

in turkeys. While it's role isn't fully understood, *Salmonella* has also been associated as either

the sole bacterium or as part of a cocktail of bacteria present in cases of bacterial

chondronecrosis with osteomyelitis (BCO), commonly known as femoral head necrosis (*9*, *51*).

Butterworth et al. (*9*) suggested BCO is a common cause of lameness in broilers during the final

weeks of production and could be exacerbated by poor conformation, rapid growth curves, and

heavy weights. The idea that these are protagonists towards broiler lameness have resulted in a

push from sectors of the broiler industry to produce slower growing broilers in an effort to

reduce lameness therefore improve broiler welfare. The association of *Salmonella* in the

skeletal system of animals has additionally been documented in cattle (*26*), horses (*4*) and pigs

(*8*). In all three cases, the detection of *Salmonella* from the skeletal system was not the

objective of the study, but rather a research finding.

Aside from zoonotic cases, *Salmonella* has been identified as contributor to joint health

problems in humans. Scallan et al. (*64*), estimated that 8% of all cases of non-typhoidal

Salmonella gastroenteritis cases result in a condition known as reactive arthritis. While not

appearing to have a large impact on public health, 8% of an estimated 1 million illnesses becomes an area of public health interest. Shirtliff and Mader (*67*) describe reactive arthritis as the result of a microbial infection at a distant site, such as the gastrointestinal system, that result in joint inflammation and infection without traditional evidence of sepsis. Published literature (*35*, *47*) suggests that reactive arthritis normally lasts between a few weeks and a few months, but can result in severe, prolonged polyarticular reactive joint disease which has been shown to not be altered by long-term antibiotic therapy.

With published literature highlighting the ability of *Salmonella* to be harbored within the skeletal system of humans and animals, it's important that further research be done to understand the mechanisms behind its survival and mitigation as they apply to food safety. The objective of this thesis project was to investigate the likelihood of *Salmonella* prevalence in synovial fluid as an alternative reservoir for contamination during fabrication of poultry products, especially poultry parts. In an effort accurately represent the domestic poultry industry, broilers were sourced from two different production systems (antibiotic-free and conventional) located in two distinctly different production regions (Southeast and West) of the U.S.

1.2 Meat Safety

Meat science encompasses activities of packers, processors and purveyors; it is the segment of the industry that converts live animals into food products and then distributes such products to merchandisers (*1*). Since the earliest recorded history, people have recognized the importance of wholesome sources and proper processing of their meat supply (*1*). In the 1880's due to technology advancements such as refrigerated rail cars and electricity, the possibility of

year-round meat packing became a reality (*93*). Processors then had the ability to refrigerate

their products and ship to other markets, outside of their neighborhood, without fear of

product spoilage due to temperature abuse from heat exposure. At the same time, however,

the press focused public attention on the problem of quality and purity of food products that

were being sold to the public, primarily meat products coming from the Chicago packers (*1*).

The meat inspection program that developed during the turn of the century used

organoleptic methods, based on sight, touch, and smell to evaluate meat quality and safety as

opposed to microbiological methods used today (*36*). The major public health concerns of the

time were the potential for transmission of diseases from sick animals to humans and the lack

of sanitary conditions for animal slaughter and production of processed products (*36*). In 1890,

in response to the growing demand for inspection of meat products, President Benjamin

Harrison signed the first law requiring inspection of meat products destined for export

however, this law did nothing to help with domestic unrest and uncertainty around the meat

industry (*1, 36*, *93*). It would later be amended in 1891 and 1895 to establish the antemortem

inspection for all cattle, pigs and sheep destined for interstate commerce only, not intrastate,

and to prevent the transportation of condemned carcasses of said livestock (*1*).

In 1906, much needed change was brought to the meat packing industry with the

passing of the Federal Meat Inspection Act by President Theodore Roosevelt (*1, 36, 93*) driven

by consumer outcry from the 1905 novel *The Jungle* written by Upton Sinclair (*1, 36*). The novel

took aim at the poor working conditions of the Chicago Meat Packing districts and described in

dramatic detail the threat it posed to consumer safety (*93*). The primary goal of the Federal

Meat Inspection Act was to prohibit sale of adulterated or misbranded meat and meat products

for food, and ensure that meat and meat products were slaughtered and processed under sanitary conditions (*93*). The law not only required a carcass-by-carcass inspection within harvesting facilities, but also provided continuous United States Department of Agriculture (USDA) inspection at processing operations (*36*). At that time poultry was not considered important enough, deemed "a minor meat product," to be a part of the Federal Meat Inspection Act as it was thought of as a Sunday dinner specialty (*1*, *55*).

In 1926, the Federal Poultry Inspection Service (FPIS) was established to voluntarily assist local producers with their inspection programs as well as inspect live poultry at railroad terminals and poultry markets in and around New York City (*55*). Before 1926, most poultry was purchased, and inspected for wholesomeness, by consumers as either live birds or "New York dressed" carcasses having only the blood and feathers removed (*1*, *55*). Then in 1957, the Poultry Products Inspection Act was signed into law with two main purposes: (1) to ensure the wholesomeness of the poultry products through continual inspection and (2) to promote sales by enabling processors to transport their product into jurisdictions that required certification (*1*, *55*, *93*).

The Meat Inspection Act of 1906 and the Poultry Products Inspection Act of 1957 only applied to animals harvested and products processed for interstate and foreign commerce, not intrastate commerce (*1*). In order to change the void, the Wholesome Meat Act and Whole Poultry Products Act were enacted by Congress in 1967 and 1968 respectively (*1*, *55*, *93*). These acts established federal-state cooperative programs in order to ensure the inspection of all meat products sold to consumers, providing for federal financial, technical, and laboratory

assistance in setting up state meat and poultry inspection programs equivalent to federal standards (*1*, *55*).

During most of the 20th century, the previously mentioned acts were thought to be fairly effective at controlling pathogens and maintaining food safety. Then, starting in 1980, studies and reports by the National Academy of Science, the Government Accounting Office and the USDA addressing adequacy of the meat inspection system for ensuring safe, wholesome meat and poultry products began to recommend the implementation of more science-based inspection procedures (*1*, *93*). The call for change was heeded after a 1993 outbreak of *Escherichia coli* O157:H7 in the Pacific-Northwest sickened 700 people and killed four children (*11*, *36*, *93*).

Then in July of 1996, the USDA Food Safety and Inspection Service (FSIS) issued its landmark, sweeping new regulations, the Pathogen Reduction and Hazard Analysis Critical Control Point (HACCP) system (*1*, *36*, *93*). The Federal regulators believed the best way to achieve the goal of improving meat and poultry safety was to require that all meat and poultry establishments adopt PR/HACCP systems (*36*). Designed by the Pillsbury Company as a means of assuring the safety of food supplied to the U.S. space program, this program was established to reduce occurrence and numbers of pathogenic microorganisms on meat and poultry products, reduce the incidence of foodborne illnesses caused by consumption of these products, and to modernize the meat and poultry inspection system (*1*, *36*). The HACCP system is comprised of 7 main principles; identification of all hazards associated with a product, identification of all points where hazards can be controlled or prevented, establishment of critical limits for each control point, establishment of monitoring system for each control point,

establishment of corrective actions to be taken when there is a deviation from the critical limit, effective record keeping, and procedures to verify the HACCP system is in fact working (*36*). Even with establishment of world-class food safety, there is continuing research being done to improve upon the methods and techniques used today.

1.3 Modern Poultry Production

What started as a humble beginning for Mrs. Wilmer Steele of Sussex County, Delaware, with the first commercial operation of 500 chicks, has now become the largest source of animal protein in the United States, and the largest poultry industry in the world (*69*, *96*). Today the poultry industry in the United States is a $38.7 billion industry with 83% ($32.1 billion) attributed to the sale of 8.78 billion broilers and 244 million young turkeys (*97*). In 2010 chicken overtook beef as the number one choice of meat protein for consumers within the United States with an estimated 41.3 kg of chicken products consumed compared to only 24.6 kg of beef products in 2016 (*20*, *54*). This trend is further carried into total red meat consumption versus total poultry consumption, U.S. consumers consumed and estimated 49.3 kg of total poultry products compared to 48.0 kg of total red meat products (*54*). The success of the United States broiler and turkey industries can be attributed to four key components: high integration, production contracts, growers specialized in specific weight ranges and relatively small and specialized farms (*45*).

In the U.S., over 70% of broilers are produced in the Southeast, while over 70% of all young turkeys are produced in the upper Midwest and Eastern U.S. (*97*). Most of the poultry industry is centered around adequate land and water, favorable weather conditions, and access to major components of poultry diets including corn and soybeans (*45*). According to a 2011

Agricultural Resource Management Survey partially conducted by USDA Economic Research

Service (*45*), 99% of all contract grow-out broiler farms were family farms that raise broilers for

an integrator, who in return is responsible for providing chicks, feed, vaccines, and

veterinary/technical assistance (*45*). Generally, these farms are very specialized with little

diversification into other agricultural commodities.

Because of the substantial amount of control that the integrators have on the

production of broilers, the poultry industry is largely vertically integrated. The primary goal is to

reduce variation among all steps of the production system, therefore allowing integrators to

control the size of broilers coming into the processing facilities (*45*). In 2012, 20 integrators

accounted for 96% of all broilers produced in the U.S. with the top three accounting for 50% of

all production (*45*).

Many processing facilities use equipment designed to handle a certain weight range of

birds for the given production period therefore run most efficiently when at full capacity with

birds of the same size; hence, growers are contracted out to raise all of the birds within one

barn to the same finish weight to help meet the preferred weight (*45*). The USDA Agricultural

Marketing Service (AMS; *90*) classifies broilers into four classes according to their final market

weight; 1.93 kg or less, 1.93 – 2.84 kg, 2.84 – 3.52 kg, and 3.52 kg and higher. For the week

ending May 27th of 2017 (*90*), the U.S. processed 167,360,000 broilers with an average weight

of 2.84 kg.

Most U.S. poultry processing facilities follow a fairly typical sequence for processing

birds, both broilers and turkeys (*3*). Once birds have been unloaded, stunned, and

exsanguinated the remainder of the harvesting process, including scalding, defeathering, and

evisceration, is highly automated with little manual labor (*3*). At the conclusion of the

harvesting process, the birds enter into a chilling system, usually either immersion chilling or air

chilling in order to maintain carcass quality and inhibit pathogen growth (*3*). In the case of

immersion chilling, antimicrobials (i.e. peracetic acid or free chlorine) are normally added to the

chilled water to aid with pathogen reduction (*3*).

Once chilled, appropriate disassembly and packaging will be determined by weight and

grade of the poultry carcass. Carcass grades are assigned through a voluntary USDA program,

developed in 2002, that evaluates poultry carcasses for the following attributes: conformation,

fleshing, fat covering, defeathering, exposed flesh, disjointed and broken bones and missing

parts, discolorations, backs and freezing defects (*91*). Much like previous steps in poultry

processing, portioning and packaging can be highly automated using technologies such as a

water jet to produce consistently sized products every time to meet the growing demand from

consumers for consistently sized, convenient products (*3*).

In the 1960's and 70's, it was common (54-83%) for broilers to be processed and then

sold as whole carcass birds (*68*). However, due to changes in lifestyle and, therefore, buying

preferences of consumers, the industry has moved to further process close to 50% of broiler

meat into products such as breaded chicken nuggets or tenders (*68*). Moreover, another 40% of

broilers are marketed as chicken parts such as boneless chicken breasts, thighs, drumsticks or

wings (*68*). The USDA-AMS (*90*) reports that, while the smaller broilers are normally marketed

bone-in into the fast food and food service sectors, larger sized broilers are normally deboned

and then further processed.

1.4 *Salmonella* spp.

Theobald Smith discovered the genus *Salmonella* in 1885 while working under the supervision of Daniel E. Salmon (*20*, *66*). Smith and Salmon worked for the U.S. Bureau of Animal Industry on industry issues such as hog cholera, bovine pleuropneumonia, Texas cattle fever, turkey blackhead and bovine tuberculosis (*66*). While attempting to isolate the source of hog cholera, Smith actually isolated, what was then named, *Salmonella choleraesuis,* now known as *Salmonella enterica* (*20*, *66*). It was later determined that hog cholera is a viral infection, but *Salmonella* is a common secondary infection (*66*).

Salmonella is defined as a facultative anaerobic, gram negative, motile, non-sporeforming, rod shaped bacterium classified as a member of the *Enterobacteriaceae* family (*89*). Using taxonomy developed by Le Minor and Popoff in 1987, *Salmonella* has species *enterica* and *bongori* further divided into six subspecies (*20*, *89*). Previously, the genus *Salmonella* was broken down into several more species, because it was initially thought that each unique serotype was its own species (*25*). As more advanced genetic techniques have become available such as DNA sequencing and hybridization, it was determined that many of the serotypes shared a high degree of genetic similarity (*25*). While *Salmonella bongori* contains only the subspecies V, *Salmonella enterica* was divided into six subspecies; *enterica* (subspecies I), *salamae* (subspecies II), *arizonae* (subspecies IIIa), *diarizonae* (subspecies IIIb), *houtenae* (subspecies IV), and *indica* (subspecies VI; *20*, *89*).

In 1934, there were only 44 different serotypes of *Salmonella* identified; today, there are over 2,500 different serotypes (*24*, *29*, *40*, *89*). Given the vast number of serotypes and the capability to infect different hosts, an intricate naming system for *Salmonella* serotypes is used.

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The initial *Salmonella* serotypes were named according to the associated disease, the animals from which they were isolated, the person who isolated them or the place where they were initially isolated (*20*). The officially recognized system used today was first developed in the 1930's by P.B. White and F. Kauffmann using the lipopolysaccharide (O antigen) and flagella (H antigens; *20*). Due to the extensive number of isolated serovars discovered during the mid 1900's, the previously mentioned division of subspecies was used to assist with the identification of serotypes in scientific literature (*20*). Today, the Centers for Disease Control and Prevention (CDC; *7*) recognizes names for serotypes in subspecies I and uses antigenic formulas for unnamed serotypes described after 1966 in subspecies II, IV, and VI and in *Salmonella bongori* under recommendation from the World Health Organization (WHO).

Within *Salmonella enterica* subspecies *enterica*, there are a diversity of *Salmonella* serotypes that have the potential to invade different animal hosts, these account for more than 60% of serotypes identified and 99% of diseases in warm-blooded animals (*12*). Foley et al. (*25*) suggest the ability of different *Salmonella* serotypes to survive and thrive in different host environments involves several interconnected factors, including differences in host environments (pH, temperature, and sites of attachment, etc.), strength of host immune system and its response to different serotypes, any commensal organisms present, and genetic makeup of the pathogen itself. These serotypes are divided into typhoidal (affects only humans) and non-typhoidal (affects humans and animals) *Salmonella* (*89*). *Salmonella enterica* infections are normally acquired from contaminated food or water that has come in contact with fecal matter (*20*, *24*, *30*, *89*). Published research (*2*, *8*, *28*) has also suggested within-animal reservoirs such as the lymphatic system may result in contamination of ground products, especially

ground beef and pork, as lymph nodes are commonly included in the grinding process. While

Salmonella exposure does not always lead to infection; infections are usually seen in the

elderly, immune-challenged or young. These infections can result in one of the following: a

systemic infection leading to enteric fever or an intestinal infection resulting in gastroenteritis

(*20*).

Typhoidal *Salmonella* infections, caused by serotypes *Salmonella* Typhi and *Salmonella*

Paratyphi A, commonly result in enteric fever known as typhoid and paratyphoid fevers (*27*,

89). Typhoid fever is found only in humans and is a much more serious life-threatening,

systemic disease with an estimated annual global effect of 27 million illnesses and over 216,000

deaths (*16*, *20*, *27*). Enteric fever is endemic in the developing world in regions that lack clean

water and adequate sanitation, therefore facilitating spread of these pathogens by the fecal-

oral route (*27*). In the late 1870's *Salmonella* Typhi was first identified as the cause of enteric

fever that was transmitted via water and milk (*20*).

With *Salmonella* Typhi and *Salmonella* Paratyphi A, infective doses can be less than

1,000 cells, with normal onset in one to three weeks post ingestion, but it may take as long as

two months for the onset of symptoms (*89*). Typical symptoms include a fever of 103° to 104°F,

lethargy, gastrointestinal symptoms such as abdominal pains and diarrhea, headache, achiness,

loss of appetite and occasionally a rash of flat, rose-colored spots (*89*). While the infection itself

may only last a couple of weeks, lasting effects such as septicemia and the colonization of

others tissues and organs such as endocarditis may occur. Septic arthritis may also occur; this is

when the infection directly affects skeletal joints and can be difficult to treat. Additionally,

chronic infection of the gallbladder may also occur, resulting in the infected person becoming a

chronic, asymptomatic carrier, which continues to excrete 10^6-10^{10} *Salmonella* Typhi bacteria per gram of feces for more than 12 months (*27*, *89*).

While typhoidal *Salmonella* is caused by only two serovars, non-typhoidal *Salmonella* (NTS) infections can be the result of all other serovars and is unique in the sense that it can take as little as one cell to make an individual sick, depending on their age, health and strain of bacteria (*27*, *89*). Non-typhoidal *Salmonella* is most commonly associated with foodborne illnesses, is generally self-limiting in the ileum and colon, and causes diarrhea, vomiting, nausea, fever and abdominal pain with onset in 6 to 72 hours following exposure (*27*, *46*, *57*, *89*). In addition to normal gastroenteritis symptoms, NTS gastroenteritis has also been shown to lead to reactive arthritis and post-infectious irritable bowel syndrome (PI-IBS) and bacteremia (*35*, *64*). Symptoms normally last four to seven days, but may last longer depending on host factors, dose ingested, and strain characteristics (*89*).

Both domestically and abroad, independent of source, NTS has been the costliest foodborne pathogen for several years (*6*, *33*, *34*, *64*, *65*). Globally, NTS is the leading bacterial cause of acute gastroenteritis and is estimated to account for 93.8 million cases with 155,000 deaths annually when under-reporting and under-diagnosis are taken into account (*17*, *46*, *57*). Within the U.S., NTS is estimated to be responsible for over one million illnesses, 19,000 hospitalizations and close to 400 deaths per year (*65*). A spring 2017 report from the CDC (*49*) suggested that 7,554 cases with 2,163 hospitalizations and 39 deaths occurred that were associated with NTS from 10 U.S. sites in 2016. Hoffman et al. (*34*) estimated NTS infections to have an annual economic burden of $3.7 million U.S. dollars with an estimate of $3,568 per case (in 2013 U.S. dollars).

For *Salmonella* infection, common penetration and passage start with the organism

surviving the lactoperoxidases in saliva and low-pH, acidic environment of the stomach (*18*, *24*,

25). Once so, it is able to proceed to the gastrointestinal tract (GIT) and colonize in multiple

sites including, but not limited to, the small intestine, colon and cecum (*25*). While epithelial

and immune cells lining the GIT provide the initial protective barrier, intestinal adhesion is

facilitated by flagella and fimbriae present on the bacteria cell surface (*24*, *25*).

Once attached to the intestinal epithelium, *Salmonella* bacteria express a type III

secretion system (T3SS), which is a multiprotein complex that facilitates endothelial uptake and

invasion (*24*, *25*, *48*). The highly regulated T3SS is a complex of proteins that allow for the

transfer of toxins and other effector proteins into intestinal cells under specific environmental

conditions and is associated with structural and regulatory proteins involved with cellular

invasion (*25*, *48*). The virulent genes that encode for *Salmonella* pathogenicity island 1 (SPI-1)

and *Salmonella* pathogenicity island 2 (SPI-2) both illicit T3SS responses and are thought to be

an insertion of a large region of DNA in the bacteria chromosome (*48*). The T3SS is not unique

however only to *Salmonella,* other pathogens such as *Yersinia* spp*., Escherichia Coli,* and

Shigella spp. have been shown to utilize similar systems (*48*). The SPI-1 complex is visually

referred to pedestal-like and aids with the adhesion of the bacteria to the cell membrane (*24*,

48). Within the SPI-1 is a needle-like structure which is used to inject effector proteins into the

cell cytosol that then interact with the actin cytoskeleton causing the targeted cell membrane

to extend outwards, resulting in what is commonly known as membrane ruffling (*24*, *25*). In

addition to ruffling the cytoskeleton membrane, effector proteins can cause the activation of

secretory pathways, facilitate inflammation and alter ion balances within cells, therefore

leading to secretion of fluids in the GIT and consequent diarrhea in humans and animals (*25*).

Ruffling of the cell membrane facilitates the immersion of *Salmonella* cells into the host

cell membrane, therefore, internalized in a membrane-bound *Salmonella*-containing vacuole

(SCV; *24*, *25*). As the SCV migrates from the luminal border to the basal membrane, it avoids

destruction by the host cell by excreting proteins that mask the vacuole making appear to

belong within the cell (*24*, *25*). Once internalized, *Salmonella* cells express SPI-2, this is only

activated within the SCV causing systemic infections resulting in intracellular pathogenesis (*24*,

25). While in the SCV, the SPI-2 secrete effector proteins that interact with cytoskeleton and

motor proteins, forming *Salmonella*-induced filaments (SIFs) that protrude out of the SCV

facilitating the merger of SCVs with other vesicles in the cell and may assist with replication (*24*,

25). In the case that the *Salmonella* cell is consumed by macrophage cells through phagocytosis,

a SCV will be also be produced by using the SPI-2 T3SS (*48*) which has been shown to allow for

the growth of *Salmonella* cells within macrophages therefore reducing the immune system's

ability to remove the infection (*25*, *48*). Upon replication, either within the intestinal cells or

macrophages, the infection can quickly become systemic spreading from the intestine to the

mesenteric lymph nodes, liver, spleen, bone marrow, and gallbladder (*27*).

In an effort to enhance the laboratory-based surveillance of *Salmonella* infections and to

minimize the burden of this pathogen on a global scale, the World Health Organization (WHO)

developed the WHO Global *Salmonella* Surveillance program in January of 2000, now known as

the WHO Global Foodborne Infections Network (GFN; *29*, *30*). Traditional serotyping is done by

mixing a suspension of *Salmonella* with a series of antisera specific for a variety of O and H

surface antigens (*24*). Following incubation, mixtures are observed for agglutination; the specific agglutination profile is used to determine serotype of the isolate in question (*24*). Among all *Salmonella* serovars responsible for causing foodborne illnesses, the two most common serotypes are *Salmonella* Enteritidis and Typhimurium (*20*, *23*, *27*, *30*). Other serotypes commonly isolated from humans include *Salmonella* serovars Agona, Hadar, Heidelberg, Infantis, Javiana, Montevideo, Muenchen, Newport, Saintpaul, and Virchow (*27*, *30*, *87*). Of the 7,554 confirmed *Salmonella* cases in the U.S. between 2013 and 2016 from 10 U.S. sites, 87% had serotype data available with Enteritidis (17%), Newport (11%), and Typhimurium (9%) as the most common serotypes (*49*).

Non-typhoidal *Salmonella* is an important foodborne disease, now commonly associated with almost all foodstuffs with the most common sources within the U.S. being poultry, eggs, and fresh produce (*20*, *24*). First linked to beef in the late 1880's by Gärtner, *Salmonella* was associated with contaminated fishmeal formulated for pig and poultry feeds in the 1960's (*Salmonella* Agona), and then connected to chickens and turkeys in the mid 1970's (*Salmonella* Hadar; *20*, *57*, *63*). Interestingly, within species, the common serotype isolated can vary depending on when and where the sample is collected. For instance, in 2014, the most commonly isolated U.S. serovars from retail chicken product were, in order of prevalence, Typhimurium, Kentucky, and Enteritidis (*87*). However, among isolates derived from regulatory testing, the most common isolated serovars, in order of prevalence, were Kentucky, Enteritidis, and Heidelberg (*87*). The same trend can be seen in *Salmonella* isolates from cattle sources. The top isolates from retail ground beef were Dublin, Typhimurium and Montevideo, while top isolates from, regulatory testing were Montevideo, Dublin and Cerro (*87*).

As *Salmonella* can easily adapt to its host environment, it's expected to see a wide range

of outbreaks related to *Salmonella*. Already in 2017, according to the U.S. CDC, there are

multiple ongoing outbreaks connected to live poultry in backyard flocks (*71*). As of May 25,

2017, 372 people were infected with multiple different serotypes of *Salmonella* from 47 states

with 71 people being hospitalized (*71*). Of those who became ill, 83% stated that they had had

contact with live poultry within the week prior to becoming ill; this is not surprising as poultry

are known to be asymptomatic carriers of *Salmonella* (*71*).

In 2016, there were 14 reported outbreaks of *Salmonella* infections resulting in a total of

1,045 reported illnesses, 247 reported hospitalizations, and three reported deaths (*82*). The

largest contributing factors to the number of illnesses in 2016 were eight multistate outbreaks

of salmonellosis connected to the handling of live poultry in backyard flocks. These accounted

for 895 reported illnesses, 209 hospitalizations and three deaths stemming from seven different

serovars of *Salmonella* (*Salmonella* Enteritidis, Muenster, Hadar, Indiana, Mbandaka, Infantis,

and Braenderup; *71*). The other outbreaks from 2016, involving nine different *Salmonella*

serotypes, only amassed 150 reported illnesses and 38 hospitalizations linked to alfalfa sprouts,

shell eggs, pistachios, shake and meal products, and dairy bull calves (*73, 76, 77, 78, 79, 80*).

It is clear that the sources of *Salmonella* infections are fairly diverse. However, some of

the more prominent sources are poultry and poultry products; this includes eggs, broilers,

layers, and turkeys. Connection of poultry with *Salmonella* has been documented as far back as

the 1960's. In a report issued by the Interagency Food Safety Analytics Collaboration (IFSAC),

based on outbreak data between 1998 and 2012, chicken, eggs and turkey accounted for 10%,

12% and 7%, respectively, of source attribution to *Salmonella* illnesses (*37*). The Center for

Science in the Public Interest (*11*), listed chicken as the second highest risk for causing serious illness with turkey as the fourth riskiest meat and poultry product in a review of outbreak data between 1998 and 2010.

The largest *Salmonella* outbreak in recent history can be traced to Foster Farms Brand produced chicken (including rotisserie chickens and raw product) of California that was contaminated with multidrug resistant *Salmonella* Heidelberg (*74*). The nearly yearlong outbreak between 2013 and 2014 was linked to 634 reported illnesses and 240 (77%) hospitalizations in 29 states and Puerto Rico; fortunately, there were no deaths linked to this outbreak (*74*). Interestingly, 15% of those who developed salmonellosis from exposure developed blood infections, where normally only 5% of people develop blood infections from *Salmonella* infection, this would suggest that strains involved with this outbreak were more virulent than normal strains of NTS (*35, 74*). Of the 68 clinical isolates that were collected from ill persons infected with the seven different genetic strains of *Salmonella* Heidelberg, 35% (24 of 68) were not resistant to any of the antibiotics used in the NARMS panel (*74*). However, 65% (44 of 68) of isolates were resistant to at least one antibiotic and 35% (24 of 68) of the total number of clinical isolates were resistant to one or more antibiotics in three or more drug classes (*74*). While these isolates were not resistant antibiotics used to typically treat salmonellosis, they were resistant to a combination of the following antibiotics: ampicillin, chloramphenicol, gentamicin, kanamycin, streptomycin, sulfisoxazole, and tetracycline (*74*). However, some of these antibiotics are critically important to human medicine, as listed by the World Health Organization (*100*), as they are used to treat other infections including multidrug resistant tuberculosis.

Between 2011 and 2017, seven other outbreaks linked poultry products as the primary source for *Salmonella* infections (*82*). Two outbreaks in 2011 related to turkey (turkey burgers and ground turkey) resulted in 148 reported infections, 40 hospitalizations and one death – in addition to the recall of more than 16.3 million kg of product (*62*, *83*, *84*). In 2012, the CDC identified an outbreak of *Salmonella* Heidelberg that was linked to "Kosher Broiled Chicken Livers," which resulted in 154 cases and 30 hospitalizations (*86*). Experts believed that the majority of the outbreak cases were linked to incorrect preparation of product that appeared to be ready-to-eat, but, it was in fact only partially cooked and therefore needed to be fully cooked before consuming (*86*).

Between June of 2012 and May of 2013, Foster Farms was linked to an outbreak of *Salmonella* Heidelberg that spread across 13 states and resulted in 134 cases and 33 hospitalizations (*75*). While this outbreak was only limited to product produced at two processing facilities in the upper northwest, the particular strain of *Salmonella* Heidelberg responsible for this outbreak would later be found in the multistate outbreak for Foster Farms over the next year (*74*, *75*). In 2014 there was another outbreak of *Salmonella* Heidelberg associated with chicken, only this was mechanically separated chicken from a Tyson processing facility resulting in 9 illnesses and two hospitalizations at a correctional facility in Tennessee (*85*). Interestingly, only two isolates exhibited antibiotic resistance to ceftriaxone, which is considered medically important as it is commonly used to treat serious cases of salmonellosis (*85*). During the fall of 2015, there were two unrelated outbreaks of *Salmonella* Enteritidis linked to raw, frozen, stuffed chicken entrees produced by two separate companies that

resulted in 20 reported illnesses, six hospitalizations and over 1.9 million kg of product recalled (*72*, *81*).

Painter et al. (*58*) studied to which foods that illness, hospitalizations and deaths from food commodities could be attributed using U.S. outbreak data between 1998 and 2008. They reported that *Salmonella* was responsible for the highest number of reported outbreaks and outbreak-associated illnesses, as well as estimated illnesses, hospitalizations, and deaths among bacterial etiologic agents (*58*). Furthermore, poultry was established as the source of contamination for an estimated 17.9% of all bacterial illnesses, 16.2% of bacterial hospitalizations, and 30.4% of bacterial deaths, which was the largest commodity source for bacterial infection deaths (*58*). It was important to note that results for poultry as a source for bacterial contamination did include other bacteria, such as *Bacillus cereus, Campylobacter* spp., *Clostridium perfringens, Listeria monocytogenes,* and *Staphylococcus aureus* (*58*).

A 2012 study by Batz et al. (*5*) evaluated the disease burden of U.S. food sources between 1999 and 2008, determined that poultry accounted for 20.9% of all non-typhoidal *Salmonella* outbreaks. When eliciting the opinion of leading food safety scientific experts on their best estimate of the percentage of foodborne illnesses associated with consumption of food in various categories, poultry was listed as being responsible for 35.1% of NTS illnesses (*5*). In both cases, outbreak data and expert elicitation, poultry was labeled with the highest percentage across all food sources for non-typhoidal *Salmonella* attribution. The food group title "poultry" for this study included chicken, turkey, other intact poultry and poultry dishes (*5*).

Salmonella contamination of live poultry originates from many different sources; these sources of contamination can be divided into two forms of transmission, horizontal and vertical (*31*). Vertical transmission, which is of lesser concern to modern poultry production due to extensive management, involves the transfer of bacteria through the breeding program from the parent flock to the day-old chicken leaving the hatchery (*31*). Horizontal transfer, however, is of greater concern due to the wide range of variables that have the ability to contaminate and infect the bird during the rearing stage (*31*). Published scientific literature, it has repeatedly indicated that feedstuffs and water can become a harbor for *Salmonella* to survive in the gastrointestinal tract and eventually infect the live bird (*25*, *31*, *39*). This gastrointestinal infection may cause the live animal to act as a carrier, therefore spreading the pathogen in its fecal matter (*25*). Other sources of horizontal contamination during the rearing of the birds include inadequate cleaning and disinfecting of facilities in between flocks and poor building hygiene, such as contaminated feeders and ventilation systems (*31*). Heyndrickx et al. (*31*) showed that, even when live birds test negative for *Salmonella* before leaving barns, some have tested positive for *Salmonella* at the processor; this may be attributed to contaminated transporting crates or stress induced shedding.

Within the processing facilities, several opportunities exist for contamination. The comingling of birds from different sources provides opportunity for contamination at every step of production. When birds enter the facility, they may have feathers, skin, crop or cloaca highly contaminated with *Salmonella* (*23*, *41*). The scalding process may become the first opportunity for cross-contamination in the harvesting process if the water is not at high enough temperatures to inactivate the pathogen, fecal matter builds up in the tank, or the water isn't

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agitated or moved in the direction opposite of the birds (*23*). Post scalding, other major sources

of contamination can be the head pulling and evisceration steps were crop leakage and

intestinal rupture may occur (*23*). Once harvesting of poultry carcasses is complete, most

undergo chilling via immersion chilling. It has been demonstrated that, even with antimicrobials

present, immersion chilling vats may act as vectors for *Salmonella* contamination between

carcasses (*38*, *59*).

Recovery of antimicrobial-resistant *Salmonella* in foods of animal origin has raised

concerns about treatment failures in cases of human salmonellosis; this stems from the fact

that antimicrobial-resistant strains appear to be more often associated with severe disease

than are susceptible isolates (*59*). Through selective pressure, horizontal gene transfer and co-

genetic selection, bacteria are able to display resistance through one of multiple mechanisms.

These mechanisms include ability to produce enzymes that inactivate antimicrobial agents

through degradation or structural modification, reduction of bacterial cell permeability to

antibiotics, activation of antimicrobial efflux pumps, and modification of the cellular target for

the antimicrobials (*24*, *98*).

While a recent topic of discussion, antimicrobial resistance is not a new phenomenon. In

2011, D'Costa et al. (*19*) discovered a diverse collection of 30,000-year-old genes in the Alaskan

permafrost that encoded for antimicrobial-resistance to, among others, β-lactam, tetracycline

and glycopeptide antibiotics. Some of the earliest published literature on antibiotic resistance

in *Salmonella* was by Edwards and others (*14*, *44*, *52*, *61*), while evaluating *Salmonella*

resistance to tetracycline and chloramphenicol, they found that *Salmonella* Typhimurium

displayed a higher frequency for the specific resistance than any other serotypes.

Today the National Antimicrobial Resistance Monitoring System (NARMS) utilizes nine different classes of antimicrobials for testing susceptibility of *Salmonella* isolates of animal origin, using Minimum Inhibitory Concentration (MIC) breakpoints established by the Clinical and Laboratory Standards Institute (CLSI; *87*, *88*). Antimicrobial classes of interest to *Salmonella* resistance are as follows, these include most broad-spectrum antimicrobials: Aminoglycosides, β-lactam/β-lactamase Inhibitor Combinations, Cephems, Folate Pathway Inhibitors, Macrolides, Penicillins, Phenicols, Quinolones, and Tetracyclines (*87*, *88*). For the 2,217 human non-typhoidal *Salmonella* isolates collected by CDC in 2014 (every 20th isolate submitted from participating public health laboratories), streptomycin, tetracycline, and sulfizoxazole were the most common antimicrobial resistance expressed (*87*, *88*). Meanwhile, among over 1,000 chicken isolates, collected from retail product and regulatory carcass rinsates, the most common resistance patterns were streptomycin, tetracycline, and sulfizoxazole resistance (*87*, *88*). Retail product sampling for chicken is primarily restricted to skin-on, bone-in chicken breasts, but can include wings, legs and thighs if chicken breasts are not available at sampling location (*88*). Similar to trends seen in chicken, the most common antimicrobial resistance types among 430 turkey isolates collected from retail ground product and regulatory carcass swabs were tetracycline, streptomycin, and ampicillin (*87*, *88*). While potentially associated, antimicrobial resistance patterns in livestock species cannot be classified as causative to resistance patterns in human medicine.

In an effort to control presence of *Salmonella* and other pathogens in the food supply, the USDA-FSIS developed the *Salmonella* verification program in 1996 as part of the Pathogen Reduction; Hazard Analysis and Critical Control Point (PR/HACCP) Systems Final Rule (*94*). This

final rule was established, using national baseline studies, to provide *Salmonella* performance standards which are used to verify process control in meat and poultry slaughter (*94*). As processes change and improve to better manage and control pathogens, the initial standards have become outdated.

During 2012, USDA-FSIS (*92*) conducted the Nationwide Microbiological Baseline Data Collection Programs: Raw Chicken Parts Baseline Survey to establish new performance standards for *Salmonella* and *Campylobacter* for raw chicken parts (legs, wings and breasts) as over 80% of raw chicken is sold as chicken parts, therefore establishing a need for increased inspection. Maximum acceptable number of positives for *Salmonella* are set for five categories; broiler carcasses (7.5%, 5 of 51), turkey carcasses (1.7%, 4 of 56), comminuted chicken (25.0%, 13 of 52), comminuted turkey (13.5%, 7 of 52), and chicken parts (15.4%, 8 of 52; *94*). Performance standards for *Salmonella* are independent of *Campylobacter*; failing to meet performance standards for one pathogen does not indicate a fail to meet for the other pathogen. These performance standards are based on samples that USDA-FSIS collected during a 52-week moving window; within higher volume facilities, samples are collected up to once per week, with less frequency in lower volume facilities (*94*). It is important to note that these performance standards only apply to a measure of effectiveness of the establishment's overall process controls rather than individual products, in order to measure effectiveness of slaughter and grinding processes in minimizing contamination (*94*). Based on moving window data between April of 2016 and June of 2017, 82.5% of broiler establishments were meeting the standards, but only 67.2% of establishments could reach the standards for chicken parts (*95*).

During 2014, the USDA-FSIS published the Modernization of Poultry Slaughter

Inspection; Final Rule (Federal Register Docket No FSIS-2011-0012) to facilitate pathogen

reduction in poultry products, improve effectiveness of poultry slaughter inspection, make

better use of agency resources, and to remove unnecessary regulatory obstacles to innovation

(*94*). This federal docket resulted in the update to performance standards in 2015 with the goal

of protecting consumers from contaminated products by verifying that each establishment

meets the new performance standards (*94*). In relation to *Salmonella* and its global impact, the

USDA-FSIS has developed two strategic goals; 1) The "All-Illness Measure" tracks and sets

quarterly goals for reducing the total number of illnesses caused by *Salmonella, Escherichia coli*

O157:H7, and *Listeria monocytogenes* as they are commonly attributed to USDA-FSIS regulated

products and (2) the percentage of young chicken establishments meeting the new *Salmonella*

performance standards (*94*). These standards are designed to prompt industry to control for

major foodborne pathogens associated with USDA-FSIS products, reduce potential for human

exposure to said pathogens, and to help the agency meet the Healthy People 2020 goals (*94*).

1.5 *Salmonella* in Joint Health

Healthy skeletal joints are important for sustained movement resulting in increased

physical welfare. A key component of the well-being of skeletal joints is the synovial joint

capsule that forms part of the seal that keeps lubricating synovial fluid in position, provides

passive stability by limiting joint movements, provides active stability via its proprioceptive

nerve endings, and additionally form articular surfaces (*60*). The synovial joint capsule consists

of dense fibrous connective tissue, lined with synovium, that forms a sleeve around the

articulating bones to which it is attached (*60*). Furthermore, the capsule contains nerves and

blood vessels that pass through the capsule, as well as potential accessory ligaments to help restrict unwanted movement (*60*). Synovial fluid encapsulated within the synovial joint has two main functions: (1) to aid in nutrition of articular cartilage by acting as a transport medium for nutritional substances, such as glucose, and (2) to aid in mechanical function of joints by lubricating articulating surfaces (*56*).

Among rheumatic diseases, one area of concern is septic arthritis, which develop as a result of infection to the vascular synovial membrane due to a bacteremic episode (*67*). It may also occur secondary to penetrating trauma or after trauma to a joint without an obvious break in the skin (*67*). While almost every bacterial organism has been shown to cause septic arthritis, the two most commonly isolated bacteria from human patients are *Staphylococcus aureus* and *Streptococcus* spp. (*22*, *67*). Other bacteria of interest that have been isolated from both human and animal joint samples include *Enterococcus* spp., *Salmonella* spp., *Shigella*, *Escherichia coli*, and *Pseudomonas* spp. (*9*, *22*, *50*, *53*, *70*). Gram-negative bacilli account for approximately 10 to 20% of cases, with higher prevalence in patients with a history of intravenous drug abuse, extremes of age, or immunocompromising conditions (*67*).

One of the many forms of septic arthritis is reactive arthritis; a result of a microbial infection at a distant site, such as the gastrointestinal system, that results in joint inflammation without traditional evidence of sepsis (*67*). As defined by Chaurasia et al. (*13*), reactive arthritis (ReA) presents as acute asymmetrical lower limb arthritis, with or without the inflammation of tendon/ligament and bone junctions, within two to four weeks of enteric infections. As a member of the heterogeneous group called seronegative spondyloarthropathies (SSA), ReA has a strong association with the human leucocyte antigen (HLA) B27 (*13*, *67*). The human leucocyte

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antigen is a major histocompatibility complex in humans and it is thought that HLA-B27 may affect elimination of the infecting bacteria or an individual's immune response (*50*). While HLA-B27 does not appear to predispose initial infection itself, it increases risk of developing arthritis that is more likely to be severe and prolonged (*43*, *47*, *50*). A case study conducted by Leirisalo-Repo (*43*), in which hospital records between 1970 and 1986 of 63 patients with reactive arthritis were reviewed, 88% of patients were found to exhibit the HLA-B27 genotype.

Among pathogenic triggers for reactive arthritis, the most common have been *Salmonella* Typhimurium and *Salmonella* Enteritidis (*21*, *43*, *47*, *70*). However, other *Salmonella* serovars have been isolated from human cases of ReA. Leirisalo-Repo et al. (*43*) determined a total of 17 different *Salmonella* serotypes were isolated from 63 individuals who had experienced gastroenteritis induced ReA. Scallan et al. (*64*) estimated that 8% of all salmonellosis cases result in ReA. While normally self-limiting, and lasting between a few weeks and a few months, severe, prolonged polyarticular reactive joint disease can occur after intestinal salmonellosis and is not altered by long-term antibiotic therapy (*35*, *47*). Additionally, *Salmonella* may not need to be viable to cause ReA; Calin et al. (*10*) reported ReA after individuals were vaccinated with heat killed *Salmonella* Typhi.

In animal science literature, case studies involving *Salmonella* joint infections have been reported as far back as 1944; Higgins et al. (*32*) observed *Salmonella* Enteritidis associated with arthritis in turkeys. Poultry with inflamed arthritic joints associated with *Salmonella* infections have been noted several times in published literature (*9*, *15*, *51*, *53*, *99*) normally leading to lameness, leaving an observable and impactful challenge to the industry. One of the more prominent, and not fully understood, sequelae of these localized bacterial infections is bacterial

chondronecrosis with osteomyelitis (BCO; femoral head necrosis; *9, 99*). Wideman and Prisby

(*99*) described bacterial chondronecrosis with osteomyelitis as necrotic degeneration and

bacterial infection primarily within the proximal head of the femur and tibia, but also in the

growth plates of other bones that are subjected to severe torque and shear stress, such as the

fourth thoracic vertebrae. While *Salmonella* has been found to be the sole infectious agent

when culturing material from BCO, it is often found in association with *Escherichia coli* or

Staphococcus aureus (*9*). Butterworth (*9*) noted that BCO is a common cause of lameness in

broilers between 25 and 45 days of age and may be exacerbated by conformation, rapid

growth, and heavy weights. Not only can *Salmonella* infect broiler limbs, but as noted by

Kramer et al. (*42*), the lymphatic system may be unable to fully clear *Salmonella* in a systemic

infection, as *Salmonella* can invade and replicate within macrophages, B-cells and T-cells,

therefore supporting persistence and harborage within the bird.

  Poultry are not the only species in which *Salmonella* have been documented in the

skeletal system. Barcel Oliver et al. (*4*) isolated *Salmonella* Typhimurium from the coxofemoral

joint of a foal which exhibited signs of lameness and was later euthanized for chronic intestinal

salmonellosis and renal failure. After orally inoculating pigs with a strain of *Salmonella*

Typhimurium resistant to novobiocin and nalidixic acid, Broadway et al. (*8*) isolated their strain

of *Salmonella* from three different synovial joints derived from the same pig 72 hours post

inoculation. Finally, Frizzo et al. (*26*) also isolated a novobiocin and nalidixic acid resistant strain

of *Salmonella* Dublin from synovial fluid of an orally inoculated dairy calf. With the published

literature highlighting the ability of *Salmonella* to be harbored within the skeletal system of

humans and animals, it's important that further research is conducted to understand

mechanisms behind its survival and mitigation as they apply to food safety.

REFERENCES

1.   Aberle, E. D., J. C. Forrest, D. E. Gerrard, and E. W. Mills. 2012. Principles of Meat

Science, 5th ed.

2.   Arthur, T. M., D. M. Brichta-Harhay, J. M. Bosilevac, M. N. Guerini, N. Kalchayanand, J. E.

Wells, S. D. Shackelford, T. L. Wheeler, and M. Koohmaraie. 2008. Prevalence and

Characterization of *Salmonella* in Bovine Lymph Nodes Potentially Destined for Use in

Ground Beef. *J. Food Prot.* 71:1685–1688.

3.   Barbut, S. 2015. Primary processing of poultry. The Science of Poultry and Meat

Processing.

4.   Barcel Oliver, F., T. M. Russell, K. L. Uprichard, K. M. Neil, P. J. Pollock, and C. Francina

Barcel Oliver. 2017. Treatment of septic arthritis of the coxofemoral joint in 12 foals.

Vet. Surg. 46:530–538.

5.   Batz, M. B., S. Hoffmann, and J. G. J. Morris. 2012. Ranking the disease burden of 14

pathogens in food sources in the United States using attribution data from outbreak

investigations and expert elicitation. *J. Food Prot.* 75:1278–1291.

6.   Batz, M., S. Hoffmann, and J. G. Morris. 2014. Disease-Outcome Trees, EQ-5D Scores,

and Estimated Annual Losses of Quality-Adjusted Life Years (QALYs) for 14 Foodborne

Pathogens in the United States. *Foodborne Pathog. Dis.* 11:395–402.

7.   Brenner, F. W., R. G. Villar, F. J. Angulo, R. Tauxe, and B. Swaminathan. 2000. *Salmonella*

nomenclature. *J. Clin. Microbiol.* American Society for Microbiology (ASM) 38:2465–7.

8.   Broadway, P. R., J. A. Carroll, J. C. Brooks, J. R. Donaldson, N. C. Burdick Sanchez, T. B. Schmidt, T. R. Brown, and T. R. Callaway. 2015. *Salmonella* prevalence of lymph nodes and synovial fluid of orally inoculated swine. *Agric. Food Anal. Bacteriol.* 5:6–14.

9.   Butterworth, A. 1999. Infectious components of broiler lameness: A review. *Worlds. Poult. Sci. J.* 55:345–352.

10.   Calin, A., N. Goulding, and D. Brewerton. 1987. Reactive arthropathy following *Salmonella* vaccination. *Arthritis Rheum.* John Wiley & Sons, Inc. 30:1197.

11.   Center for Science in the Public Interest (CSPI). 2013. Risky Meat: A CSPI Field Guide to Meat and Poultry Safety.

12.   Chan, K., S. Baker, C. C. Kim, C. S. Detweiler, G. Dougan, and S. Falkow. 2003. Genomic Comparison of *Salmonella enterica* serovars and *Salmonella bongori* by Use of an S. enterica serovar Typhimurium DNA Microarray. *J. Bacteriol.* 185:553–563.

13.   Chaurasia, S., A. K. Shasany, A. Aggarwal, and R. Misra. 2016. Recombinant *Salmonella* Typhimurium outer membrane protein A is recognized by synovial fluid CD8 cells and stimulates synovial fluid mononuclear cells to produce interleukin (IL)-17/IL-23 in patients with reactive arthritis and undifferentiated spon. *Clin. Exp. Immunol.* 185:210–218.

14.   Cherubin, C. E. 1981. Antibiotic Resistance of *Salmonella* in Europe and the United States. *Source Rev. Infect. Dis.* 3:1105–1126.

15.   Cosgrove, A. S., and P. R. Lindenmaier. 1961. Case Report: Hock Swellings as a Primary Lesion of *Salmonella* Pullorum Infection in Broiler Chickens. *Source Avian Dis.* 5:144–146.

16.   Crump, J. A., S. P. Luby, and E. D. Mintz. 2004. The global burden of typhoid fever. *Bull. World Health Organ.* 82:346–53.

17.   Crump, J. A., F. M. Medalla, K. W. Joyce, A. L. Krueger, R. M. Hoekstra, J. M. Whichard, E. J. Barzilay, and E. I. P. N. W. Emerging Infections Program NARMS Working Group. 2011. Antimicrobial resistance among invasive nontyphoidal *Salmonella* enterica isolates in the United States: National Antimicrobial Resistance Monitoring System, 1996 to 2007. *Antimicrob. Agents Chemother.* American Society for Microbiology (ASM) 55:1148–54.

18.   D'Aoust, J.-Y. 1991. Pathogenicity of foodborne *Salmonella*. *Int. J. Food Microbiol.* 12:17–40.

19.   D 'costa, V. M., C. E. King, L. Kalan, M. Morar, W. W. L. Sung, C. Schwarz, D. Froese, G. Zazula, F. Calmels, R. Debruyne, G. B. Golding, H. N. Poinar, and G. D. Wright. 2011. Antibiotic resistance is ancient. *Nature* 477:457–461.

20.   Dawoud, T. M., Z. Shi, Y. M. Kwon, and S. C. Ricke. 2017. Overview of Salmonellosis and Food-borne *Salmonella*: Historical and Current Perspectives, p. 113–138. *In* Producing Safe Eggs.

21.   Dworkin, M. S., P. C. Shoemaker, M. J. Goldoft, and J. M. Kobayashi. 2001. Reactive Arthritis and Reiter's Syndrome Following an Outbreak of Gastroenteritis Caused by *Salmonella* Enteritidis 33:1010-1014.

22.   Fihman, V., D. Hannouche, V. Bousson, T. Bardin, F. Lioté, L. Raskine, J. Riahi, M. J. Sanson-Le Pors, and B. Berçot. 2007. Improved diagnosis specificity in bone and joint infections using molecular techniques. *J. Infect.* 55:510–517.

23.   Finstad, S., C. A. O'Bryan, J. A. Marcy, P. G. Crandall, and S. C. Ricke. 2012. *Salmonella* and broiler processing in the United States: Relationship to foodborne salmonellosis. *Food Res. Int.* 45:789–794.

24.   Foley, S. L., and A. M. Lynne. 2008. Food animal-associated *Salmonella* challenges: Pathogenicity and antimicrobial resistance. *J. Anim. Sci* 86:173–187.

25.   Foley, S. L., T. J. Johnson, S. C. Ricke, R. Nayak, and J. Danzeisen. 2013. *Salmonella* pathogenicity and host adaptation in chicken-associated serovars. *Microbiol. Mol. Biol. Rev.* American Society for Microbiology 77:582–607.

26.   Frizzo, L. S., M. V. Zbrun, L. P. Soto, E. Bertozzi, G. J. Sequeira, L. E. Marti, M. L. Signorini, R. Rodríguez Armesto, and M. R. Rosmini. 2012. Pathogen translocation and histopathological lesions in an experimental model of *Salmonella* Dublin infection in calves receiving lactic acid bacteria and lactose supplements. *J. Vet. Sci.* 13:261–270.

27.   Gal-Mor, O., E. C. Boyle, and G. A. Grassl. 2014. Same species, different diseases: How and why typhoidal and non-typhoidal *Salmonella* enterica serovars differ. *Front. Microbiol.* 5:391

28.   Gragg, S. E., G. H. Loneragan, K. K. Nightingale, D. M. Brichta-Harhay, H. Ruiz, J. R. Elder, L. G. Garcia, M. F. Miller, A. Echeverry, R. G. R. Porras, and M. M. Brashears. 2013. Substantial within-Animal Diversity of *Salmonella* Isolates from Lymph Nodes, Feces, and Hides of Cattle at Slaughter. *Appl. Environ. Microbiol.* 79:4744–4750.

29.   Hendriksen, R. S., M. Mikoleit, V. P. Carlson, S. Karlsmose, A. R. Vieira, A. B. Jensen, A. M. Seyfarth, S. M. DeLong, F.-X. Weill, D. M. A. Lo Fo Wong, F. J. Angulo, H. C. Wegener, and F. M. Aarestrup. 2009. WHO Global Salm-Surv external quality assurance system for

serotyping of *Salmonella* isolates from 2000 to 2007. *J. Clin. Microbiol.* American Society for Microbiology 47:2729–36.

30.   Hendriksen, R. S., A. R. Vieira, S. Karlsmose, D. M. A. Lo, F. Wong, A. B. Jensen, H. C. Wegener, and F. M. Aarestrup. 2011. Global Monitoring of *Salmonella* serovar distribution from the World Health Organization Global Foodborne Infections Network Country Data Bank: Results of quality assured laboratories from 2001 to 2007. *Foodborne Pathog. Dis.* 8:887–900.

31.   Heyndrickx, M., D. Vandekerchove, L. Herman, I. Rollier, K. Grijspeerdt, and L. De Zutter. 2002. Routes for *Salmonella* contamination of poultry meat: epidemiological study from hatchery to slaughterhouse. *Epidemiol. Infect*. Cambridge University Press 129:253–265.

32.   Higgins, W. A., J. B. Christiansen, and C. H. Schroeder. 1944. A *Salmonella* Enteritidis Infection Associated with Leg Deformity in Turkeys. *Poult. Sci.* 23:340–341.

33.   Hoffmann, S., M. B. Batz, and A. J. G. Morris. 2012. Annual Cost of Illness and Quality-Adjusted Life Year Losses in the United States Due to 14 Foodborne Pathogens. *J. Food Prot.* 75:1292–1302.

34.   Hoffmann, S., B. Maculloch, and M. Batz. 2015. Economic Burden of Major Foodborne Illnesses Acquired in the United States. Available at: https://www.ers.usda.gov/webdocs/publications/43984/52807_eib140.pdf?v=42136. Accessed June 12, 2017.

35.   Hohmann, E. L. 2001. Nontyphoidal Salmonellosis. *Clin. Infect. Dis.* 32:263–239.

36.   Hulebak, K. L., and W. Schlosser. 2002. Hazard Analysis and Critical Control Point (HACCP) History and Conceptual Overview. *Risk Anal.* 22:547–552.

37.   Interagency Food Safety Analytics Collaboration (IFSAC). 2015. Foodborne Illness Source

        Attribution Estimates for *Salmonella*, *Escherichia coli* O157 (*E. coli* O157), *Listeria*

        monocytogenes (Lm), and *Campylobacter* using Outbreak Surveillance Data. Available at:

        https://doc-0g-78-apps-

        viewer.googleusercontent.com/viewer/secure/pdf/56hvu1v4n58j623c5h105va98rtuhie

        5/p8aa8omv10mfa5bua9nar2rf7ijsf863/1495406100000/lantern/044780360499922620

        30/ACFrOgBb0xXWxl9wM6J82GnARJZmpCQzof3mTkVMwnXtTtsoTS6zE83-

        _9VGpA5BZngUkz2XJCSP. Accessed May 21, 2017.

38.   Izat, A. L., C. D. Driggers, M. Colberg, M. A. Reiber, and M. H. Adams. 1989. Comparison

        of the DNA Probe to Culture Methods for the Detection of *Salmonella* on Poultry

        Carcasses and Processing Waters. *J. Food Prot.* 52:564–570.

39.   Jones, F. T., R. C. Axtell, D. V. Rives, S. E. Scheideler, F. R. J. Tarver, R. L. Walker, and M. J.

        Wineland. 1991. A Survey of *Salmonella* Contamination in Modern Broiler Production. *J.*

        Food Prot. 54:502–507.

40.   Jones, T. F., L. A. Ingram, P. R. Cieslak, D. J. Vugia, M. Tobin-D'Angelo, S. Hurd, C. Medus,

        A. Cronquist, and F. J. Angulo. 2008. Salmonellosis outcomes differ substantially by

        serotype. *J. Infect. Dis.* 198:109–114.

41.   Kotula, K. L., and M. E. Davis. 1999. Broiler skin sampling for optimum recovery of

        Salmonella spp. *J. Food Prot.* 62:284–6.

42.   Kramer, J., A. H. Visscher, J. A. Wagenaar, and S. H. M. Jeurissen. 2003. Entry and

        survival of *Salmonella enterica* serotype Enteritidis PT4 in chicken macrophage and

        lymphocyte cell lines. *Vet. Microbiol.* 91:147–155.

43. Leirisalo-Repo, M., P. Helenius, T. Hannu, A. Lehtinen, J. Kreula, M. Taavitsainen, and S. Koskimies. 1997. Long term prognosis of reactive *Salmonella* arthritis. *Ann Rheum Dis* 56:516–520.

44. Ligon, B. L. 2004. Penicillin: Its Discovery and Early Development. *Semin. Pediatr. Infect. Dis.* 15:52–57.

45. MacDonald, J. M. 2014. Technology, Organization, and Financial Performance in U.S. Broiler Production. Available at: https://www.ers.usda.gov/webdocs/publications/43869/48159_eib126.pdf?v=41809. Accessed June 5, 2017.

46. Majowicz, S. E., J. Musto, E. Scallan, F. J. Angulo, M. Kirk, S. J. O 'brien, T. F. Jones, A. Fazil, and R. M. Hoekstra. 2010. The Global Burden of Nontyphoidal *Salmonella* Gastroenteritis. *Clin. Infect. Dis.* 50:882–889.

47. Mäki-Lkola, O., and K. Granford. 1992. *Salmonella*-triggered Reactive Arthritis. *Scand. J. Rheumatol.* 21:265–270.

48. Marcus, S. L., J. H. Brumell, C. G. Pfeifer, and B. B. Finlay. 2000. *Salmonella* pathogenicity islands: big virulence in small packages. *Microbes Infect.* 2:145–156.

49. Marder, E. P., P. R. Cieslak, A. B. Cronquist, J. Dunn, S. Lathrop, T. Rabatsky-Ehr, P. Ryan, K. Smith, M. Tobin-D'Angelo, D. J. Vugia, S. Zansky, K. G. Holt, B. J. Wolpert, M. Lynch, R. Tauxe, and A. L. Geissler. 2017. Incidence and Trends of Infections with Pathogens Transmitted Commonly Through Food and the Effect of Increasing Use of Culture-Independent Diagnostic Tests on Surveillance — Foodborne Diseases Active Surveillance Network, 10 U.S. Sites, 2013–2016. *Morb. Mortal. Wkly. Rep.* 66:397–403.

50.   Marler, B. 2015. *Salmonella* Bacteria Induced Reactive Arthritis or Reiter's Syndrome

        Available at: http://www.foodpoisonjournal.com/food-poisoning-resources/salmonella-

        induced-reactive-arthritis-or-reiters-syndrome/#.wvrcvjpmzvo. Accessed June 28, 2017.

51.   McNamee, P. T., and J. A. Smyth. 2000. Bacterial chondronecrosis with osteomyelitis

        ("femoral head necrosis") of broiler chickens: a review. *Avian Pathol.* 29:253–270.

52.   McWhorter, A. C., M. C. Murrell, and P. R. EDWARDS. 1963. Resistance of *Salmonella*

        isolated in 1962 to chloretetracycline. *Appl. Microbiol.* 11:368–370.

53.   Nairn, M. E. 1973. Synovitis of the Turkey. *Avian Dis.* 17:504–517.

54.   National Chicken Council. 2017. Per Capita Consumption of Poultry and Livestock, 1965

        to Estimated 2016, in Pounds. Available at:

        http://www.nationalchickencouncil.org/about-the-industry/statistics/per-capita-

        consumption-of-poultry-and-livestock-1965-to-estimated-2012-in-pounds/. Accessed

        May 26, 2017.

55.   National Research Council. 1987. Poultry Inspection: The Basis for a Risk-Assessment

        Approach.

56.   Newton, C. D., and D. M. Nunamaker. 1985. Synovial Fluid Textbook of Small Animal

        Orthopedics. J.B. Lippincott Company.

57.   O'brien, S. J. 2013. The "Decline and Fall" of Nontyphoidal *Salmonella* in the United

        Kingdom. *Clin. Infect. Dis.* 56:705–710.

58.   Painter, J. A., R. M. Hoekstra, T. Ayers, R. V Tauxe, C. R. Braden, F. J. Angulo, and P. M.

        Griffin. 2013. Attribution of foodborne illnesses, hospitalizations, and deaths to food

commodities by using outbreak data, United States, 1998-2008. *Emerg. Infect. Dis.* Centers for Disease Control and Prevention 19:407–15.

59.   Parveen, S., M. Taabodi, J. G. Schwarz, T. P. Oscar, J. Harter-Dennis, and D. G. White. 2007. Prevalence and Antimicrobial Resistance of *Salmonella* Recovered from Processed Poultry. *J. Food Prot.* International Association for Food Protection 70:2466–2472.

60.   Ralphs, J. R., and A. M. Benjamin. 1994. The joint capsule: structure, composition, ageing and disease. *J. Anat* 184:503–509.

61.   Ramsey, C. H., and P. R. Edwards. 1961. Resistance of Salmonella isolated in 1959 and 1960 to tetracyclines and chloramphenicol. *Appl. Microbiol.* 9:389–391.

62.   Routh, J. A., J. Pringle, M. Mohr, S. Bidol, K. Arends, M. Adams-Cameron, W. T. Hancock, B. Kissler, R. Rickert, J. Folster, B. Tolar, S. Bosch, C. Barton Behravesh, I. T. Williams, and L. Gieraltowski. 2015. Nationwide outbreak of multidrug-resistant *Salmonella* Heidelberg infections associated with ground turkey: United States, 2011. *Epidemiol. Infect.* 143:3227–3234.

63.   Rowe, B., M. L. Hall, L. R. Ward, and J. D. de Sa. 1980. Epidemic spread of *Salmonella* Hadar in England and Wales. *Br. Med. J.* 280:1065–1066.

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

65.   Scallan, E., R. M. Hoekstra, F. J. Angulo, R. V Tauxe, M.-A. Widdowson, S. L. Roy, J. L. Jones, and P. M. Griffin. 2011. Foodborne illness acquired in the United States--major pathogens. *Emerg. Infect. Dis.* Centers for Disease Control and Prevention 17:7–15.

66.   Schultz, M. 2008. Theobald Smith. *Emerg. Infect. Dis.* 14:1940–1942.

67.   Shirtliff, M. E., and J. T. Mader. 2002. Acute Septic Arthritis. *Clin. Microbiol. Rev.* 15:527–544.

68.   The National Chicken Council. 2012. How Broilers are Marketed. Available at: http://www.nationalchickencouncil.org/about-the-industry/statistics/how-broilers-are-marketed/. Accessed June 6, 2017.

69.   The National Chicken Council. 2012. U.S. Chicken Industry History. Available at: http://www.nationalchickencouncil.org/about-the-industry/history/. Accessed May 31, 2017.

70.   Tuompo, R., T. Hannu, L. Mattila, A. Siitonen, and M. Leirisalo-Repo. 2013. Reactive arthritis following *Salmonella* infection: a population-based study. *Scand. J. Rheumatol.* 42:196–202.

71.   U.S. Centers for Disease Control and Prevention. 2016. Eight Multistate Outbreaks of Human *Salmonella* Infections Linked to Live Poultry in Backyard Flocks. Available at: https://www.cdc.gov/salmonella/live-poultry-05-16/index.html. Accessed June 26, 2017.

72.   U.S. Centers for Disease Control and Prevention. 2015. Multistate Outbreak of Drug-Resistant *Salmonella* Enteritidis Infections Linked to Raw, Frozen, Stuffed Chicken Entrees Produced by Barber Foods (Final Update). Available at: https://www.cdc.gov/salmonella/frozen-chicken-entrees-07-15/index.html. Accessed June 26, 2017.

73. U.S. Centers for Disease Control and Prevention. 2017. Multistate Outbreak of Multidrug-Resistant *Salmonella* Heidelberg Infections Linked to Contact with Dairy Bull Calves (Final Update). Available at: https://www.cdc.gov/salmonella/heidelberg-11-16/index.html. Accessed June 26, 2017.

74. U.S. Centers for Disease Control and Prevention. 2014. Multistate Outbreak of Multidrug-Resistant *Salmonella* Heidelberg Infections Linked to Foster Farms Brand Chicken. Available at: https://www.cdc.gov/salmonella/heidelberg-10-13/. Accessed May 26, 2017.

75. U.S. Centers for Disease Control and Prevention. 2013. Multistate Outbreak of *Salmonella* Heidelberg Infections Linked to Chicken. Available at: https://www.cdc.gov/salmonella/heidelberg-02-13/index.html. Accessed June 26, 2017.

76. U.S. Centers for Disease Control and Prevention. 2016. Multistate Outbreak of *Salmonella* Infections Linked to Alfalfa Sprouts from One Contaminated Seed Lot. Available at: https://www.cdc.gov/salmonella/muenchen-02-16/index.html. Accessed June 26, 2017.

77. U.S. Centers for Disease Control and Prevention. 2016. Multistate Outbreak of *Salmonella* Montevideo and *Salmonella* Senftenberg Infections Linked to Wonderful Pistachios. Available at: https://www.cdc.gov/salmonella/montevideo-03-16/index.html. Accessed June 26, 2017.

78. U.S. Centers for Disease Control and Prevention. 2016. Multistate Outbreak of *Salmonella* Oranienburg Infections Linked to Shell Eggs. Available at:

https://www.cdc.gov/salmonella/oranienburg-10-16/index.html. Accessed June 26, 2017.

79.   U.S. Centers for Disease Control and Prevention. 2016. Multistate Outbreak of *Salmonella* Reading and *Salmonella* Abony Infections Linked to Alfalfa Sprouts. Available at: https://www.cdc.gov/salmonella/reading-08-16/index.html. Accessed June 26, 2017.

80.   U.S. Centers for Disease Control and Prevention. 2016. Multistate Outbreak of *Salmonella* Virchow Infections Linked to Garden of Life RAW Meal Organic Shake & Meal Products. Available at: https://www.cdc.gov/salmonella/virchow-02-16/index.html. Accessed June 26, 2017.

81.   U.S. Centers for Disease Control and Prevention. 2015. Outbreak of *Salmonella* Enteritidis Infections Linked to Raw, Frozen, Stuffed Chicken Entrees Produced by Aspen Foods (Final Update). Available at: https://www.cdc.gov/salmonella/frozen-chicken-entrees-part2-07-15/index.html. Accessed June 26, 2017.

82.   U.S. Centers for Disease Control and Prevention. 2017. Outbreaks Involving *Salmonella.* Available at: https://www.cdc.gov/salmonella/outbreaks.html. Accessed June 26, 2017.

83.   U.S. Centers for Disease Control and Prevention. 2011. *Salmonella* Hadar Infections Associated with Turkey Burgers. Available at: https://www.cdc.gov/salmonella/2011/turkey-burger-4-4-2011.html. Accessed June 26, 2017.

84.   U.S. Centers for Disease Control and Prevention. 2011. *Salmonella* Heidelberg Infections Linked to Ground Turkey. Available at: https://www.cdc.gov/salmonella/2011/ground-turkey-11-10-2011.html. Accessed June 26, 2017.

85.   U.S. Centers for Disease Control and Prevention. 2014. *Salmonella* Heidelberg Infections Linked to Tyson Brand Mechanically Separated Chicken at a Correctional Facility. Available at: https://www.cdc.gov/salmonella/heidelberg-01-14/index.html. Accessed June 26, 2017.

86.   U.S. Centers for Disease Control and Prevention. 2012. *Salmonella* Infections Linked to Kosher Broiled Chicken Livers. Available at: https://www.cdc.gov/salmonella/2011/chicken-liver-1-11-2012.html. Accessed June 26, 2017.

87.   U.S. Food and Drug Administration. 2016. NARMS Integrated Report: 2014 The National Antimicrobial Resistance Monitoring System: Enteric Bacteria Data Tables. Available at: https://www.fda.gov/downloads/AnimalVeterinary/SafetyHealth/AntimicrobialResistance/NationalAntimicrobialResistanceMonitoringSystem/UCM528751.pdf. Accessed May 1, 2017.

88.   U.S. Food and Drug Administration. 2016. NARMS Integrated Report: 2014 The National Antimicrobial Resistance Monitoring System: Enteric Bacteria Methods. Available at: https://www.fda.gov/downloads/AnimalVeterinary/SafetyHealth/AntimicrobialResistance/NationalAntimicrobialResistanceMonitoringSystem/UCM529808.pdf. Accessed August 1, 2017.

89.   U.S. Food and Drug Administration. 2012. *Salmonella* species. Bad Bug Book Handbook of Foodborne Pathogenic Microorganisms and Natural Toxins.

90.   USDA Agricultural Marketing Service. 2017. Broiler Market News Report. Available at: https://www.ams.usda.gov/mnreports/pytbroilerfryer.pdf. Accessed May 5, 2017

91. USDA Agricultural Marketing Service. 2002. United States Classes, Standards, and Grades for Poultry. Available at: https://www.ams.usda.gov/sites/default/files/media/Poultry_Standard%5B1%5D.pdf. Accessed June 7, 2017.

92. USDA Food Safety and Inspection Service. 2016. Docket No. FSIS-2014-0023.

93. USDA Food Safety and Inspection Service. 2015. FSIS History. Available at: https://www.fsis.usda.gov/wps/portal/informational/aboutfsis/history/history. Accessed June 1, 2017.

94. USDA Food Safety and Inspection Service. 2016. Pathogen Reduction – *Salmonella* and *Campylobacter* Performance Standards Verification Testing. Available at: https://www.fsis.usda.gov/wps/wcm/connect/b0790997-2e74-48bf-9799-85814bac9ceb/28_IM_PR_Sal_Campy.pdf?MOD=AJPERES. Accessed June 27, 2017.

95. USDA Food Safety and Inspection Service. 2017. *Salmonella* and *Campylobacter* Verification Testing Program Monthly Reports. Available at: https://www.fsis.usda.gov/wps/portal/footer/!ut/p/a1/zvnnbwihep0tpewryd0psbdnv1lbmcy26njpwbyqw8lkurf21xe9nanvjh7karh4vjl5zeacl5ayulwsemun1xub5g_zy_gutp_qbe-sw_q4v4nz8swz3s-hcaejjmz41q9gktrvawan58zhsixvgr52dvumqt62inurqqmnaau1z3svgjoaon5a58nlo5izlblayl2eoq. Accessed May 26, 2017.

96. USDA Foreign Agricultural Service. 2017. Livestock and Poultry: World Markets and Trade. Available at: https://apps.fas.usda.gov/psdonline/circulars/livestock_poultry.pdf. Accessed June 2, 2017.

97.   USDA National Agricultural Statistics Service. 2017. United States Department of

Agriculture National Agricultural Statistics Service Poultry - Production and Value.

Available at: http://usda.mannlib.cornell.edu/usda/current/PoulProdVa/PoulProdVa-04-

28-2017.pdf. Accessed May 26, 2017.

98.   Van Hoek, A. H. A. M., D. Mevius, B. Guerra, P. Mullany, A. P. Roberts, and H. J. M. Aarts.

2011. Acquired antibiotic resistance genes: an overview. *Front. Microbiol.* 2:203.

99.   Wideman, R. F., and R. D. Prisby. 2013. Bone circulatory disturbances in the

development of spontaneous bacterial chondronecrosis with osteomyelitis: a

translational model for the pathogenesis of femoral head necrosis. *Front. Endocrinol.*

(Lausanne). 3:183.

100.   World Health Organization. 2017. Critically Important antimicrobials for human

medicine. 5th rev. Geneva. Available at:

http://apps.who.int/iris/bitstream/10665/255027/1/9789241512220-eng.pdf?ua=1.

Accessed July 20, 2017.

CHAPTER 2 - *SALMONELLA* CONTAMINATION IN POULTRY—ARE WE MISSING A POTENTIAL RESERVOIR?

2.1 Summary

The objective of this study was to assess presence and characteristics of *Salmonella enterica* found in synovial fluid of broiler carcasses. Synovial fluid of three individual joints from 500 broiler carcasses was individually sampled (1,500 total samples) from five broiler processing facilities located in the Southeast and Western U.S. The external surface of broiler carcasses was decontaminated prior to sampling of the shoulder, coxofemoral, and tibiofemoral joints. Individual samples were enriched, composited, and subjected to rapid PCR-based detection of *Salmonella*. Individual samples from any positive composites were also enriched before determination of *Salmonella* presence in the same manner. Positive individual samples were subjected to secondary enrichment before plating onto selective agar for isolation of *Salmonella. Salmonella* isolates were serotyped before determination of antimicrobial susceptibility. Overall, 1.00% (5 out of the 500 broiler carcasses) of composite samples, and 0.47% (7 out of 1,500 samples) of individual samples were positive for *Salmonella.* Five of the seven isolates were susceptible to all drugs and determined to be *Salmonella* Enteritidis. The remaining two isolates, identified as *Salmonella* Typhimurium, were resistant to streptomycin. To our knowledge, no previous assessments of *Salmonella* in synovial fluid of broilers has been reported; however, results of the present study suggested that *Salmonella* may be present in synovial fluid of broilers. Although low prevalence, this information provides valuable insight into potential poultry contamination pathways and warrants further exploration.

2.2 Introduction

While advancements have been made in securing safe food for the global population, reduction of *Salmonella enterica* remains one of the biggest areas of concern. Non-Typhoidal *Salmonella* is a leading cause of foodborne illnesses worldwide with an estimated 93.8 million cases and 155,000 deaths each year (*18*). These infections can stem from exposure to food and water that have been contaminated with fecal matter from an infected individual or animal (*9*).

Salmonella infection have been attributed to a wide variety of foodstuffs, with the most common sources within the U.S. being poultry, eggs, and fresh produce (*9, 11*). Between 1998 and 2012, the Interagency Food Safety Analytics Collaboration (*15*) determined that poultry meat products accounted for 17% (chicken products 10%; turkey products 7%) of all *Salmonella* outbreaks; the highest of any meat protein source. This is alarming considering that in the United States, domestic consumers eat more poultry than any other meat protein with an estimated 49 kg of total poultry products consumed as compared to 48 kg of total red meat products (*9, 22*).

Nonetheless, the poultry industry has made tremendous efforts to reduce presence of *Salmonella enterica.* According to the United States Department of Agriculture Food Safety and Inspection Service (USDA-FSIS), results from the third quarter of 2015 (July 1 to September 30) indicated the prevalence of *Salmonella* sampled from young chicken carcasses was only 1.4%; however, prevalence associated with chicken parts remained alarmingly high at 22.1% (*36*). Presently, based on 52-week moving window data between April 2016 and June 2017, 82.45% of broiler establishments met the maximum acceptable *Salmonella* prevalence performance standards, however only 67.20% of establishments met the standards set for chicken parts (*35*).

The high prevalence in chicken parts compared to whole bird samples suggests that the pathogen is somehow evading current carcass intervention strategies prior to processing—yet, limited information exists about this phenomenon. While not the conventional means for *Salmonella* contamination in poultry, several studies have isolated *Salmonella* from the skeletal system (*5*, *7*, *13*, *20*, *21*, *37*). Higgins et al. (*13*) noted the presence of *Salmonella* in association with joint inflammation in turkeys. While its role is not fully understood, *Salmonella* has also been associated as either the sole bacterium genus or as part of a cocktail of bacteria present in cases of bacterial chondronecrosis with osteomyelitis (BCO), commonly known as femoral head necrosis (*5*, *20*). Furthermore, *Salmonella* has been documented in the skeletal systems of cattle (*12*), horses (*3*), and pigs (*4*). Beyond zoonotic cases, *Salmonella* has been identified as a contributor to joint health issues in humans. Scallan et al. (*24*), estimated that 8% of all cases of non-Typhoidal *Salmonella* gastroenteritis result in a condition known as reactive arthritis. In such cases, the individual will develop joint inflammation and infection as a result of a microbial infection at a distal site (*26*). While normally being self-limiting and only lasting for a short period of time, published literature (*14*, *19*) suggests reactive arthritis can result in severe, prolonged polyarticular reactive joint disease which has been shown to be unresponsive to long-term antibiotic therapy.

In regard to commercial poultry production, the ability for *Salmonella* to persist within a joint may provide one pathway by which comminuted poultry products or parts have a greater *Salmonella* prevalence than the carcass. Thus, the objective of this study was to assess presence of *Salmonella enterica* in joint synovial fluid of commercially produced broilers and, further, to characterize any *Salmonella* isolated from those joints.

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2.3 Material and Methods

Pre-Trial Carcass Decontamination Work. While the broiler carcasses used in the study came from processing facilities that utilized various carcass decontamination interventions, carcass contamination—and the potential to inadvertently introduce carcass microbial contamination into the synovial joint during sampling—was a concern. Thus, a pre-trial study was performed to develop a method which could effectively reduce any potential carcass surface *Salmonella* contamination and thereby reduce the potential for translocation of carcass surface *Salmonella* to a *Salmonella*-free joint capsule.

For pre-trial work, 12 whole skin-on chicken wings (including the humerus, radius, ulna, and phalanges [wing tip]) and leg quarters were inoculated by pipetting 500 µl of inoculum only on the skin surface at 6.29 ± 0.01 log CFU/mL, with a novobiocin and nalidixic acid-resistant strain of *Salmonella* Typhimurium FFSRU ST NN (provided by USDA-ARS). Inoculum cells were allowed to attach for 1 h at 4°C for maximum cell attachment to skin surfaces. Inoculated parts (n = 3) were then randomly divided among three different decontamination procedures: (i) immersion in 100% ethanol for 2 min, flame sterilization, skinning, then immersion in boiling water (95°C+, 10 s), (ii) immersion in boiling water, skinning, then re-immersion in boiling water, and (iii) immersion in 100% concentrated bleach (8.25% sodium hypochlorite) for 2 min, skinning, then immersion in boiling water. All samples were rinsed in 200 ml of buffered peptone water (BPW; Difco, Becton, Dickinson and Company [BD], Sparks, MD). Using methodology described by Scott et al. (*25*), rinsing was performed by vigorously shaking samples by hand with a strong downward force for 1 min to recover cells from the chicken parts. Rinsates were serially diluted 10-fold in 0.1% BPW. Appropriate dilutions (0.1 or 1 ml)

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were then surface plated onto tryptic soy agar (Acumedia-Neogen Corp., Lansing, MI)

supplemented with nalidixic acid (20 µg/ml; Sigma-Aldrich, St. Louis, MO) and novobiocin (25

µg/ml; Sigma-Aldrich). Results of pre-trial work (Table 2.1) determined that ethanol immersion,

followed by flame sterilization, skinning of the joint area and subsequent immersion in boiling

water was most effective ($P = 0.07$) at reducing surface contamination before synovial joint

exposure. This decontamination procedure resulted in 1.53 ± 0.27 log CFU/mL survival of

Salmonella Typhimurium inoculum.

      Broiler Procurement and Processing. A brief schematic outlining broiler procurement

and processing, synovial fluid sampling, and microbiological assessment is available in Figure

2.1. A total of 1,500 synovial fluid samples from three unique true joints (shoulder,

coxofemoral, and tibiofemoral; 500 samples per joint) of 500 broiler carcasses was collected.

Broiler carcasses (n = 500) were obtained from three conventional and two antibiotic-free

broiler processing facilities (100 broilers per facility) located in the Southeast and Western U.S.

(Figure 2.2) immediately following immersion chilling in varying levels of peracetic acid (6.56

ppm to 34 ppm) or free chlorine (1.9 ppm to 2.7 ppm). Skeletal defect-free, A quality broiler

carcasses were collected post-chilling to allow for effectiveness of in-plant interventions. Broiler

carcasses were shipped overnight to Colorado State University (CSU; Fort Collins, CO) for

processing and testing for presence of *Salmonella*. Each broiler processing location was

sampled twice (50 broiler carcasses per collection) during the study, with at least three weeks

between collections of samples. Facilities used in this study processed daily, on average,

162,356 broilers (range of 148,063 to 218,418 broilers) and an average live weight of 3.04 kg

(range of 2.97 to 3.26 kg). Broiler carcasses were transported in insulated liners placed in

shipping boxes. Temperature recorders (DeltaTrak, Modesto, CA) placed within the shipping

containers recorded an average shipping temperature of 5.3°C, with a range of -4.8°C to 16.8°C.

High temperatures were due to temperature recorders being placed on the top of chicken

carcasses in poorly packaged shipments, these were isolated to one facility.

Upon receipt, broilers were split into equal left and right halves using a bandsaw (AEW

Thurne 400, Marel Meat Processing, Lenexa, KS) for ease of handling and randomization of

sampling. Broiler halves were then transferred to the Center for Meat Safety & Quality

Microbiology Laboratory (CSU, Department of Animal Sciences) and stored at 4°C for up to

three h until processing.

Broiler carcass halves were individually immersed in 100% ethanol (Fisher Chemical, Fair

Lawn, NJ) for 2 min, then flame sterilized for approximately 30 s. Following flame sterilization,

the skin from each broiler half was aseptically removed around the shoulder, coxofemoral, and

tibiofemoral joints using a sterile scalpel (Bard-Parker Carbon Steel #21 Surgical Blade; Aspen

Surgical, Caledonia, MI). As previous work has suggested, *Salmonella* may harbor on the skin of

broilers; therefore, removal of the skin surrounding the joint reduced likelihood of synovial fluid

cross contamination (*16*) Each carcass half was then immersed in rolling boiling water (95°C+,

10 s) before sampling of the synovial fluid. Internal joint temperatures were collected during all

decontamination steps on eight chicken pieces before the present study with a maximum of

34°C and an average of 22°C. Additionally, raw meat color around the joint capsule indicated

little change in the raw tissue due to heat.

Synovial Fluid Sampling. After sterilizing the external surface, each joint was exposed

using an air cooled, flame sterilized scalpel. Scalpels were flame sterilized before and after

every incision to reduce likelihood of cross contamination. Synovial fluid samples were collected from each joint (n = 3 joints per broiler carcass) using a sterile cotton swab sampler pre-moistened and immersed in 10 mL of BPW (3M Swab Sampler; Maplewood, MN). Swabbing was only done on the exposed bones of the joints, as this would be the most consistent location for sampling that provided a surface for *Salmonella* colonization. As the synovial fluid present was estimated to be less than 10 µl, it is likely that any bacteria collected was primarily the result of mechanical swabbing. Swabs were subjected to primary enrichment for 22 h at 35°C.

Microbiological Assessment. Following primary enrichment, 1 mL aliquots from individual synovial fluid enrichments were composited to form a representative sample for each broiler carcass (i.e., 1 mL from each of the three synovial fluid joint enrichments per carcass; 3 mL total). Composited synovial fluid enrichments were then subjected to a rapid polymerase chain reaction-based *Salmonella* detection assay (BAX; DuPont, Wilmington, DE; AOAC 100201) following MLG 4C.07 of the USDA-FSIS Laboratory Guidebook (*33*). If the composite sample was positive for *Salmonella*, individual enriched synovial fluid samples from the original composite were then also assessed for the presence of *Salmonella* using the same PCR-based assay.

Any individual synovial fluid samples that tested positive for *Salmonella* were subjected to secondary enrichment and subsequent plating on selective agars using protocols described in the USDA-FSIS Microbiology Laboratory Guidebook, version 4.09 (*34*). More specifically, aliquots of 500 µL from the individual enriched samples were transferred to 10 mL of tetrathionate-Hajna (TT) broth (Difco, BD). Additionally, a second aliquot of 100 µL from the same sample was transferred to 10 mL of Rappaport-Vassiliadis (RV) broth (Difco, BD). The TT

and RV enrichment broths were incubated at 42°C for 23 ± 2 h. Following secondary enrichment, aliquots (10 μL) of TT and RV were streak-plated onto brilliant green sulfa agar (BGS; Difco, BD) and xylose lysine Tergitol 4 agar (XLT-4; Difco, BD) plates and incubated at 35°C for 18 to 24 h for isolation of *Salmonella* colonies. Representative colonies (n=3) were selected randomly from each selective agar and purified by performing two successive streaks on BGS or XLT-4 agar. Pure isolates were prepared and stored at -80°C in Tryptic Soy Broth (TSB; Acumedia-Neogen Corp., Lansing, MI) with 16% glycerol (EMD Chemicals, Inc., San Diego, CA).

Presumptive *Salmonella* isolates (n = 84; 12 per presumptive positive) were shipped to the National Veterinary Services Laboratory in Ames, IA for serotyping and assessment of antimicrobial susceptibility. Genus identification was determined using matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) with serotyping based on Kauffmann-White scheme according to O antigens determined by Luminex xMAP (Luminex Corporation, Austin, TX) microbead-based suspension array. All isolates were evaluated for susceptibility to amoxicillin, clavulanic acid, ampicillin, azithromycin, cefoxitin, ceftiofur, ceftriaxone, chloramphenicol, ciprofloxacin, gentamicin, nalidixic acid, streptomycin, sulfisoxazole, tetracycline, trimethoprim and sulphamethoxazole using broth microdilution methods (Sensititre NARMS Gram Negative Plates; Trek Diagnostics, Oakwood Village, OH). Susceptibility and resistance classifications were established using breakpoints established by the Clinical Laboratory Sciences Institute (4).

Statistical Analysis. Pre-trial work was designed as a paired comparison with a total of n = 3 per treatment. Bacterial populations recovered were analyzed to determine the effects of treatment on inoculated populations compared to populations recovered from control samples.

Bacterial counts were converted to base-10 logarithms and expressed as bacterial populations with differences of least square means analyzed using a significance level of α = 0.05.

Fisher's Exact Test for count data was used to compare prevalence rates between production systems (antibiotic-free vs conventional) and regions (Western vs Southeastern) due to the low prevalence rates. For comparison of joint *Salmonella* prevalence, Exact McNemar test (with central confidence intervals) was used to account for paired data in a 2x2 table with the following comparisons: shoulder joint vs. coxofemoral joint, coxofemoral joint vs. tibiofemoral joint, and shoulder joint vs. tibiofemoral joint. A significance level of 0.05 was used for all analyses. Exact binomial test was used to determine 95% confidence intervals based on number of positive samples vs. the number of trials. All statistical analyses were performed in R: A language and environment for statistical computing (*23*) version 3.3.1.

2.4 Results and Discussion

Overall, prevalence of *Salmonella* among joints for all broiler carcasses (Table 2.2) was 0.47% (7 out of 1,500 samples; 95% Confidence Level [95% CI]: 0.19 to 0.96%). Prevalence of *Salmonella* in synovial fluid was 1.00% (synovial fluid samples from 5 out of 500 broiler carcasses; 95% CI: 0.32 to 2.32%). This information may help to bridge a gap in explaining the difference between regulatory prevalence of *Salmonella* in samples of entire young chicken carcasses versus chicken parts as synovial fluid exposed during processing may cross-contaminate poultry parts.

Between production systems (Table 2.2), *Salmonella* prevalence was numerically higher (*P* > 0.05) in joints of conventionally-raised broilers (0.67%; 6 out of 900 samples; 95% CI: 0.25 to 1.45%) compared to joints of broilers raised without use of antibiotics (0.17%; 1 out of 600

samples; 95% CI: 0.00 to 0.92%). Likewise, a similar tendency was observed in prevalence of

Salmonella in synovial fluid from conventionally-raised broilers (1.33%; 4 out of 300 broiler

carcasses; 95% CI: 0.36 to 3.37%) compared to broilers raised without antibiotics (0.50%; 1 out

of 200 broiler carcasses; 95% CI: 0.01 to 2.75%).

Similar to the present study, Lestari et al. (*17*) were unable able to detect a statistical

difference between conventional products and those raised without the use of antibiotics in a

survey of *Salmonella* prevalence in retail chicken product from Louisiana. However, Cui et al. (*8*)

described seeing higher prevalence of *Salmonella* in retail chicken products raised without

antibiotics (61%) vs. conventionally raised chicken products (44%) from retail stores in

Maryland. Meanwhile, Alali et al. (*1*) discussed a significantly higher odd (*P* < 0.05) of

Salmonella prevalence on broiler farms producing conventional broilers vs. those that house

broilers raised without antibiotics in North Carolina. These results suggest that *Salmonella*

prevalence between antibiotic-free and conventional broilers may be driven by factors other

than the presence/absence of antibiotics in poultry operations.

Between regions (Table 2.2), *Salmonella* prevalence tended to be higher for joints of

broilers from the Southeast (0.83%; 5 out of 600 samples; 95% CI: 0.27 to 1.93%) compared to

joints of broilers from the West region (0.22%; 2 out of 900 samples; 95% CI: 0.02 to 0.80%).

Moreover, prevalence of *Salmonella* in synovial fluid was higher in the Southeast (1.50%; 3 out

of 200 broiler carcasses; 95% CI: 0.31-4.32%) than the West (0.67%; 2 out of 300 broiler

carcasses; 95% CI: 0.08-2.39%). Among all comparisons, there were no statistical differences (*P*

> 0.05). Altekruse et al. (*2*) displayed a regional variation in the presence of *Salmonella*

Enteritidis among broiler carcass samples collected by USDA-FSIS between 2000 and 2005,

however this data may be skewed as it does not normalize for the larger number of broilers

processed in the Southeast U.S. compared to any other region. Characterizing *Salmonella*

isolates from two distinctly different produce growing regions of the U.S., Strawn et al. (*27*)

found regional differences with little overlap (n = 3) in 40 different pulsed-field gel

electrophoresis (PFGE) types collected. These studies indicate that particular *Salmonella*

isolates may be more prevalent in certain regions of the U.S. than other regions.

As shown in Table 2.3 and Figure 2.3, *Salmonella* prevalence varied among joints.

Prevalence was higher in the shoulder joint (0.80%; 4 out of 500 samples; 95% CI: 0.21 to 2.03)

compared to the coxofemoral (0.40%; 2 out of 500 samples; 95% CI: 0.04 to 1.44) and

tibiofemoral (0.20%; 1 out of 500 samples; 95% CI: 0.00 to 1.11) joints. This dissimilarity in joint

prevalence suggested that *Salmonella* in the synovial joint is not a result of systemic infection,

but rather, it is likely isolated. This hypothesis is further supported by the fact that only two

broiler carcasses had more than one joint which contained *Salmonella.* An isolated incident,

such as an injury, may have led to the introduction of *Salmonella* into the synovial joint capsule.

Characterization of all *Salmonella* isolates revealed presence of only two serotypes:

Typhimurium and Enteritidis. These data coincide with retail and regulatory serotype

surveillance, which also identify *Salmonella* Typhimurium and Enteritidis as two of the most

common serotypes isolated from poultry (*10*, *31*). Interestingly, all isolates from joints of

broilers that originated from the Southeastern region were identified as *Salmonella* Enteritidis

while those from the Western region were *Salmonella* Typhimurium. It was surprising however

not to see the presence of serovars *Salmonella* Kentucky or Heidelberg as these are two other

serotypes commonly found among both retail and regulatory testing of chicken products (*32*).

In recent years, *Salmonella* Heidelberg has been linked to several outbreaks involving chicken products, with the largest being the Foster Farms outbreak of 2013 through 2014 resulting in 634 illnesses (*28*, *29*, *30*).

Assessment of antimicrobial susceptibility revealed that all *Salmonella* Enteritidis isolates were susceptible to all medications tested, while *Salmonella* Typhimurium isolates were resistant to only streptomycin. All isolates (n = 12) from *Salmonella*-positive individual joint synovial fluid samples displayed the same antimicrobial susceptibility profile, independent of selective media used for isolation and purification. Interestingly, although the streptomycin-resistant *Salmonella* Typhimurium isolates were from the shoulder joints of two separate broilers from the Western region; one broiler originated from a conventional production system, while the other originated from an antibiotic-free production system. While not an antibiotic used to commonly treat salmonellosis, streptomycin has been listed as a critically important antibiotic by the World Health Organization (*38*). In 2014, among samples collected from retail chicken products, streptomycin was the second most commonly expressed antibiotic resistance phenotype (31.5%) behind tetracycline resistance (47.6%; *32*). Although resistance to streptomycin is a concern, it is not uncommon for *Salmonella* to exhibit resistance to at least one antimicrobial drug (*32*).

Previous research and outbreaks have demonstrated presence of antimicrobial resistant *Salmonella* in broilers and other poultry. Most recently, as reported by the 2015 National Antimicrobial Resistance Monitoring System (NARMS; *32*), more than 59% of *Salmonella* isolated from retail chicken product testing exhibited some form of resistance to antimicrobials, with over 20% displaying resistance to three or more antimicrobial classes. The most common

resistance phenotypes among *Salmonella* isolates seen in sampled retail chicken products

included tetracycline (47.6%), streptomycin (31.5%), and sulfisoxazole (30.8%) resistance (*32*).

Absence of tetracycline and sulfisoxazole (a sulfonamide) resistance shown in the present study

was surprising due to use of the broad-spectrum antibiotics in the poultry industry. While no

previous investigations of resistance among *Salmonella* isolated from the synovial fluid has

been reported, present data coincide with the commonality of *Salmonella* that are resistant to

streptomycin in reports from retail and regulatory sampling (*32*).

As presence of *Salmonella* in ground poultry and poultry parts remains problematic,

alternative sources for *Salmonella* contamination should be evaluated to allow poultry

processors the ability for better pathogen control. *Salmonella* prevalence has also been noted

in other species of livestock including bovine, equine, and porcine. Although this is the first

study of its kind to assess the presence of *Salmonella* in the synovial fluid of broilers, previous

research has documented its presence in the synovial fluid of bovine and porcine specimens

following oral inoculation (*4*, *12*). Similarly, Barcel Oliver et al. (*3*) isolated *Salmonella* from a

coxofemoral joint of a foal that later required euthanasia because of chronic salmonellosis.

Although the mechanism for its manifestation in the synovial fluid is unknown, these results

indicate that *Salmonella* may be present in synovial fluid of broilers, although at a relatively low

rate. This provides potential evidence for a novel pathway in which *Salmonella* could evade

current decontamination strategies—and furthermore, result in increased prevalence in

comingled poultry products. The increased potential for contaminated product with the

comingling of products poses a health risk to consumers as many are unaware of how to

properly handle and prepare raw or not-ready-to-eat partially cooked products. We believe

further investigations regarding the mode of entry and potential mitigation strategies are

warranted.

Table 2.1. Pre-trial survival of *Salmonella* Typhimurium from three different decontamination processes on broiler parts[a,b]

Treatment	Mean Survival Log CFU/mL
Control	6.29 [a]
Treatment 1	1.53 [b]
Treatment 2	2.77 [b]
Treatment 3	2.76 [b]

[a,b]Within a column, numbers followed by the same letter are not different ($P > 0.05$)

Table 2.2. Prevalence of *Salmonella enterica* from broiler joint synovial fluid between Production Systems and Regions[a]

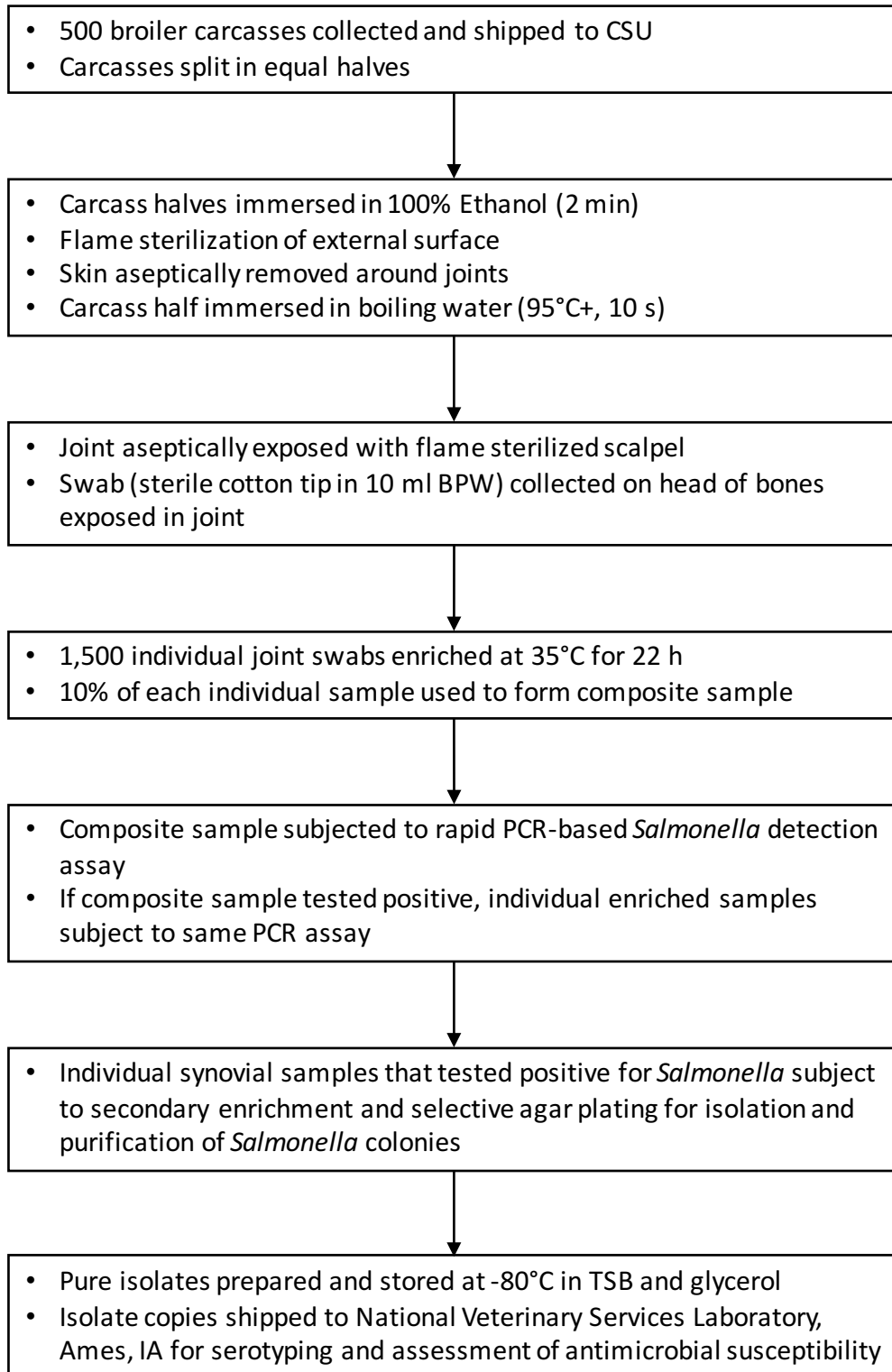| Broiler Production	Broilers			Joints		
	No. of Samples	Prevalence (%)	95% CI	No. of Samples	Prevalence (%)	95% CI
Overall Prevalence	500	5 (1.00)	0.32-2.32	1,500	7 (0.47)	0.19-0.96
Production System						
Antibiotic-Free	200	1 (0.50) [a]	0.01-2.75	600	1 (0.17) [a]	0.00-0.92
Conventional	300	4 (1.33) [a]	0.36-3.37	900	6 (0.67) [a]	0.25-1.45
Production Region						
Southeast	200	3 (1.50) [x]	0.31-4.32	600	5 (0.83) [x]	0.27-1.93
West	300	2 (0.67) [x]	0.08-2.39	900	2 (0.22) [x]	0.02-0.80

[a]Within production system within a column, numbers followed by the same letter are not different ($P > 0.05$)

[x]Within production region within a column, numbers followed by the same letter are not different ($P > 0.05$)

Table 2.3. Prevalence of *Salmonella enterica* in synovial fluid of **individual joints** from commercially produced broilers[a]

Individual Broiler Joints	No. of Samples	Prevalence (%)	95% CI
Shoulder	500	4 (0.80)[a]	0.21-2.03
Coxofemoral	500	2 (0.40)[a]	0.04-1.44
Tibiofemoral	500	1 (0.20)[a]	0.00-1.11

[a]Within a column, numbers followed by the same letter are not different ($P > 0.05$)

- 500 broiler carcasses collected and shipped to CSU
- Carcasses split in equal halves

↓

- Carcass halves immersed in 100% Ethanol (2 min)
- Flame sterilization of external surface
- Skin aseptically removed around joints
- Carcass half immersed in boiling water (95°C+, 10 s)

↓

- Joint aseptically exposed with flame sterilized scalpel
- Swab (sterile cotton tip in 10 ml BPW) collected on head of bones exposed in joint

↓

- 1,500 individual joint swabs enriched at 35°C for 22 h
- 10% of each individual sample used to form composite sample

↓

- Composite sample subjected to rapid PCR-based *Salmonella* detection assay
- If composite sample tested positive, individual enriched samples subject to same PCR assay

↓

- Individual synovial samples that tested positive for *Salmonella* subject to secondary enrichment and selective agar plating for isolation and purification of *Salmonella* colonies

↓

- Pure isolates prepared and stored at -80°C in TSB and glycerol
- Isolate copies shipped to National Veterinary Services Laboratory, Ames, IA for serotyping and assessment of antimicrobial susceptibility

Figure 2.1. Illustration of synovial sampling procedure and *Salmonella* detection.

Figure 2.2. Broiler carcass sampling regions.
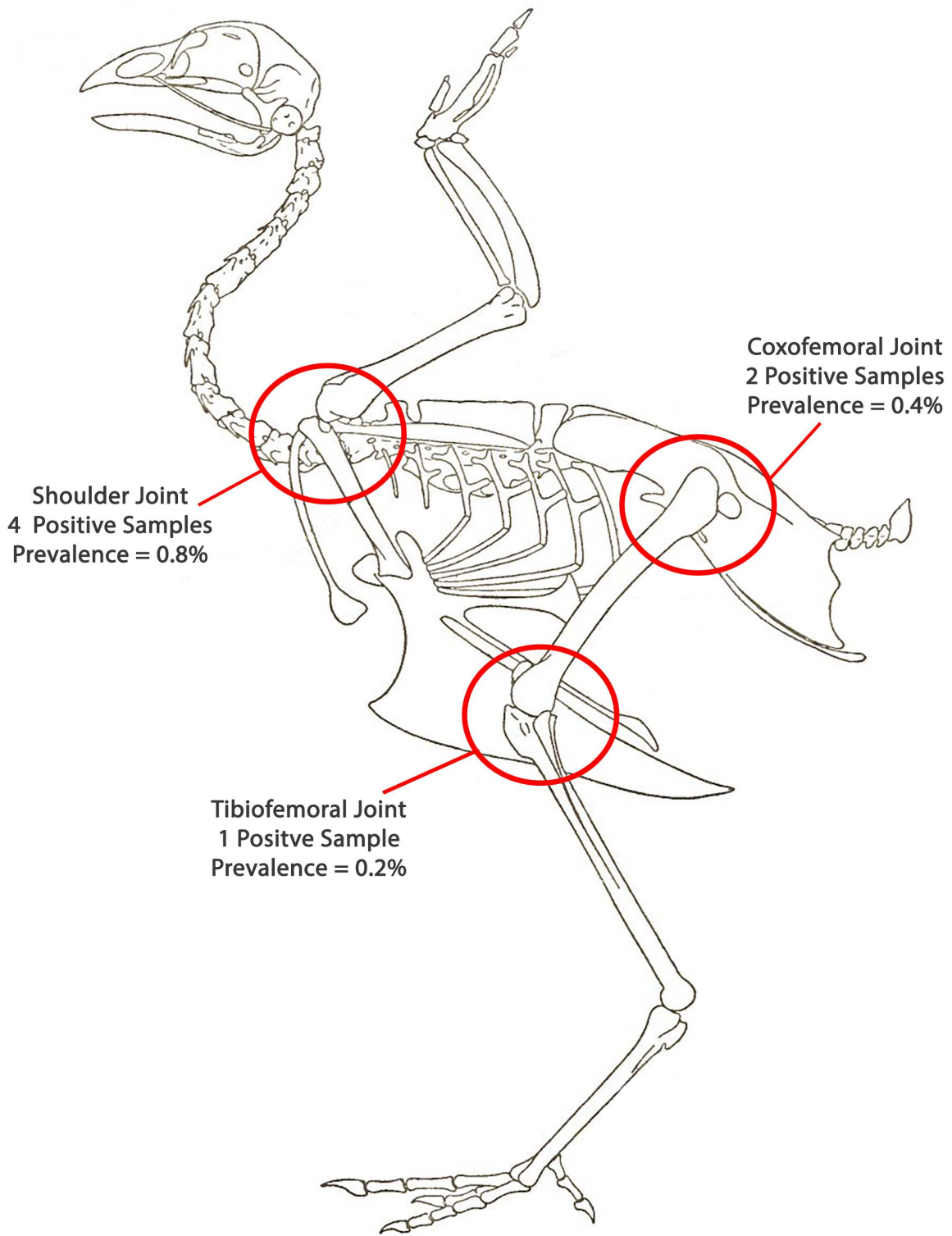
Figure 2.3. Illustration of *Salmonella* prevalence from joint synovial fluid among sampled joints.

REFERENCES

1. Alali, W. Q., S. Thakur, R. D. Berghaus, M. P. Martin, and W. A. Gebreyes. 2010. Prevalence and Distribution of *Salmonella* in Organic and Conventional Broiler Poultry Farms. *Foodborne Pathog. Dis.* 7:1363–1371.

2. Altekruse, S. F., N. Bauer, A. Chanlongbutra, R. DeSagun, A. Naugle, W. Schlosser, R. Umholtz, and P. White. 2006. *Salmonella* Enteritidis in broiler chickens, United States, 2000-2005. *Emerg. Infect. Dis.* Centers for Disease Control and Prevention 12:1848–52.

3. Barcel Oliver, F., T. M. Russell, K. L. Uprichard, K. M. Neil, P. J. Pollock, and C. Francina Barcel Oliver. 2017. Treatment of septic arthritis of the coxofemoral joint in 12 foals. *Vet. Surg.* 46:530–538.

4. Broadway, P. R., J. A. Carroll, J. C. Brooks, J. R. Donaldson, N. C. Burdick Sanchez, T. B. Schmidt, T. R. Brown, and T. R. Callaway. 2015. *Salmonella* prevalence of lymph nodes and synovial fluid of orally inoculated swine. *Agric. Food Anal. Bacteriol.* 5:6–14.

5. Butterworth, A. 1999. Infectious components of broiler lameness: A review. *Worlds. Poult. Sci. J.* 55:345–352.

6. Clinical and Laboratory Standards Institute. 2016. Performance Standards for Antimicrobial Susceptibility Testing, 27th ed.

7. Cosgrove, A. S., and P. R. Lindenmaier. 1961. Case Report: Hock Swellings as a Primary Lesion of *Salmonella* Pullorum Infection in Broiler Chickens. *Source Avian Dis.* 5:144–146.

8. Cui, S., B. Ge, J. Zheng, and J. Meng. 2005. Prevalence and Antimicrobial Resistance of *Campylobacter* spp. and *Salmonella* Serovars in Organic Chickens from Maryland Retail

Stores. *Appl. Environ. Microbiol.* 71:4108–4111.

9.   Dawoud, T. M., Z. Shi, Y. M. Kwon, and S. C. Ricke. 2017. Overview of Salmonellosis and

Food-borne *Salmonella*: Historical and Current Perspectives, p. 113–138. *In* Producing

Safe Eggs.

10.   Finstad, S., C. A. O'Bryan, J. A. Marcy, P. G. Crandall, and S. C. Ricke. 2012. *Salmonella* and

broiler processing in the United States: Relationship to foodborne salmonellosis. *Food

Res. Int.* 45:789–794.

11.   Foley, S. L., and A. M. Lynne. 2008. Food animal-associated *Salmonella* challenges:

Pathogenicity and antimicrobial resistance. *J. Anim. Sci* 86:173–187.

12.   Frizzo, L. S., M. V. Zbrun, L. P. Soto, E. Bertozzi, G. J. Sequeira, L. E. Marti, M. L. Signorini,

R. Rodríguez Armesto, and M. R. Rosmini. 2012. Pathogen translocation and

histopathological lesions in an experimental model of *Salmonella* Dublin infection in

calves receiving lactic acid bacteria and lactose supplements. *J. Vet. Sci.* 13:261–270.

13.   Higgins, W. A., J. B. Christiansen, and C. H. Schroeder. 1944. A *Salmonella* Enteritidis

Infection Associated with Leg Deformity in Turkeys. *Poult. Sci.* 23:340–341.

14.   Hohmann, E. L. 2001. Nontyphoidal Salmonellosis. *Clin. Infect. Dis.* 32:263–239.

15.   Interagency Food Safety Analytics Collaboration (IFSAC). 2015. Foodborne Illness Source

Attribution Estimates for *Salmonella*, *Escherichia coli* O157 (*E. coli* O157), *Listeria

monocytogenes* (Lm), and *Campylobacter* using Outbreak Surveillance Data. Available at:

https://doc-0g-78-apps-

viewer.googleusercontent.com/viewer/secure/pdf/56hvu1v4n58j623c5h105va98rtuhie5

/p8aa8omv10mfa5bua9nar2rf7ijsf863/1495406100000/lantern/04478036049992262030

/ACFrOgBb0xXWxl9wM6J82GnARJZmpCQzof3mTkVMwnXtTtsoTS6zE83-
_9VGpA5BZngUkz2XJCSP. Accessed May 21, 2017

16.   Kotula, K. L., and M. E. Davis. 1999. Broiler skin sampling for optimum recovery of
        Salmonella spp. *J. Food Prot.* 62:284–6.

17.   Lestari, S. I., F. Han, F. Wang, and B. Ge. 2009. Prevalence and Antimicrobial Resistance of
        Salmonella Serovars in Conventional and Organic Chickens from Louisiana Retail Stores. *J.
        Food Prot.* International Association for Food Protection 72:1165–1172.

18.   Majowicz, S. E., J. Musto, E. Scallan, F. J. Angulo, M. Kirk, S. J. O ʼbrien, T. F. Jones, A. Fazil,
        and R. M. Hoekstra. 2010. The Global Burden of Nontyphoidal *Salmonella*
        Gastroenteritis. *Clin. Infect. Dis.* 50:882–889.

19.   Mäki-Lkola, O., and K. Granford. 1992. *Salmonella*-triggered Reactive Arthritis. *Scand. J.
        Rheumatol.* 21:265–270.

20.   McNamee, P. T., and J. A. Smyth. 2000. Bacterial chondronecrosis with osteomyelitis
        (“femoral head necrosis”) of broiler chickens: a review. *Avian Pathol.* 29:253–270.

21.   Nairn, M. E. 1973. Synovitis of the Turkey. *Avian Dis.* 17:504–517.

22.   National Chicken Council. 2017. Per Capita Consumption of Poultry and Livestock, 1965
        to Estimated 2016, in Pounds. Available at:
        http://www.nationalchickencouncil.org/about-the-industry/statistics/per-capita-
        consumption-of-poultry-and-livestock-1965-to-estimated-2012-in-pounds/. Accessed
        May 26, 2017.

23.   R Core Team. 2016. R: A Language and Environment for Statistical Computing. 3.3.1. R
        Foundation for Statistical Computing, Vienna, Austria.

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

25. Scott, B. R., X. Yang, I. Geornaras, R. J. Delmore, D. R. Woerner, J. O. Reagan, J. B. Morgan, and K. E. Belk. 2015. Antimicrobial Efficacy of a Sulfuric Acid and Sodium Sulfate Blend, Peroxyacetic Acid, and Cetylpyridinium Chloride against *Salmonella* on Inoculated Chicken Wings. *J. Food Prot.* 78:1967–1972.

26. Shirtliff, M. E., and J. T. Mader. 2002. Acute Septic Arthritis. *Clin. Microbiol. Rev.* 15:527–544.

27. Strawn, L. K., M. D. Danyluk, R. W. Worobo, and M. Wiedmann. 2014. Distributions of *Salmonella* subtypes differ between two U.S. produce-growing regions. *Appl. Environ. Microbiol.* 80:3982–91.

28. U.S. Centers for Disease Control and Prevention. 2014. Multistate Outbreak of Multidrug-Resistant *Salmonella* Heidelberg Infections Linked to Foster Farms Brand Chicken. Available at: https://www.cdc.gov/salmonella/heidelberg-10-13/. Accessed May 26, 2017.

29. U.S. Centers for Disease Control and Prevention. 2013. Multistate Outbreak of *Salmonella* Heidelberg Infections Linked to Chicken. Available at: https://www.cdc.gov/salmonella/heidelberg-02-13/index.html. Accessed June 26, 2017.

30. U.S. Centers for Disease Control and Prevention. 2014. *Salmonella* Heidelberg Infections Linked to Tyson Brand Mechanically Separated Chicken at a Correctional Facility. Available at: https://www.cdc.gov/salmonella/heidelberg-01-14/index.html. Accessed

June 26, 2017.

31. U.S. Department of Health and Human Services. 2015. Foodborne Disease Active

Surveillance Network 2015 Surveillance Report (Final Data). Available at:

https://www.cdc.gov/foodnet/pdfs/FoodNet-Annual-Report-2015-508c.pdf. Accessed

May 4, 2017.

32. U.S. Food and Drug Administration. 2016. NARMS Integrated Report: 2014 The National

Antimicrobial Resistance Monitoring System: Enteric Bacteria Data Tables. Available at:

https://www.fda.gov/downloads/AnimalVeterinary/SafetyHealth/AntimicrobialResistanc

e/NationalAntimicrobialResistanceMonitoringSystem/UCM528751.pdf. Accessed May 1,

2017.

33. USDA Food Safety and Inspection Service. 2017. FSIS Procedure for the Use of a

Polymerase Chain Reaction (PCR) Assay for Screening *Salmonella* in Meat, Poultry, Egg,

and Siluriformes (Fish) Products and Carcass and Environmental Sponges. Available at:

https://www.fsis.usda.gov/wps/wcm/connect/b7f78261-7977-4d00-9c1f-

ded224560058/MLG-4C.pdf?MOD=AJPERES. Accessed May 8, 2017.

34. USDA Food Safety and Inspection Service. 2017. Laboratory Guidebook Notice of Change

Title: Isolation and Identification of *Salmonella* from Meat, Poultry, Pasteurized Egg, and

Siluriformes (Fish) Products and Carcass and Environmental Sponges. Available at:

https://www.fsis.usda.gov/wps/wcm/connect/700c05fe-06a2-492a-a6e1-

3357f7701f52/MLG-4.pdf?MOD=AJPERES. Accessed May 8, 2017

35. USDA Food Safety and Inspection Service. 2017. *Salmonella* and *Campylobacter*

Verification Testing Program Monthly Reports. Available at:

https://www.fsis.usda.gov/wps/portal/footer/!ut/p/a1/zvnnbwihep0tpewryd0psbdnv1lb

mcy26njpwbyqw8lkurf21xe9nanvjh7karh4vjl5zeacl5ayulwsemun1xub5g_zy_gutp_qbe-

sw_q4v4nz8swz3s-

hcaejjmz41q9gktrvawan58zhsixvgr52dvumqt62inurqqmnaau1z3svgjoaon5a58nlo5izlblay

l2eoq. Accessed May 26, 2017.

36.   USDA Food Safety and Inspection Service. 2015. USDA FSIS Quarterly Progress Report on

Salmonella and *Campylobacter* Testing of Raw Meat and Poultry Products, July 2015 to

September 2015. Available at: https://www.fsis.usda.gov/wps/wcm/connect/d0e57a3b-

765a-4e98-be9d-7ba915513061/q3-cy2015-salmonella-testing.pdf?mod=ajperes.

Accessed May 4, 2017.

37.   Wideman, R. F., and R. D. Prisby. 2013. Bone circulatory disturbances in the development

of spontaneous bacterial chondronecrosis with osteomyelitis: a translational model for

the pathogenesis of femoral head necrosis. *Front. Endocrinol. (Lausanne).* 3:183.

38.   World Health Organization. 2017. Critically Important antimicrobials for human medicine.

5th rev. Geneva. Available at:

http://apps.who.int/iris/bitstream/10665/255027/1/9789241512220-eng.pdf?ua=1.

Accessed July 20, 2017.