SALMONELLA LEVELS IN TURKEY NECK SKIN, BONE, AND SPLEEN IN RELATION TO GROUND TURKEY PRODUCTION

by

YUE CUI

(Under the Direction of Walid Q. Alali)

ABSTRACT

The objective of this study was to determine *Salmonella* levels (presence and numbers) in turkey drumstick bone, spleen, and neck skin samples in relation to *Salmonella* contamination levels in ground turkey. Three hundred samples of each turkey part were collected after evisceration and tested for *Salmonella* using most probable number (MPN) and enrichment methods. The flocks were classified into targeted and non-targeted groups based on the farm/flock historical *Salmonella* contamination data in ground product. Overall *Salmonella* prevalence in bone, spleen, neck skin and ground turkey samples was 9.3%, 6.7%, 42.0%, and 14.5%, respectively. When *Salmonella* was present in spleen and/or bone (at MPN > 1 $\log_{10}$), and in neck skin (MPN > 2 $\log_{10}$), the ground turkey lot was *Salmonella*-positive. Our findings suggested *Salmonella* presence at high levels in neck skin may indicate a highly contaminated flock that results in ground turkey contamination.

INDEX WORDS: *Salmonella*, ground turkey contamination, neck skin, spleen, bone

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YUE CUI

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YUE CUI

Major Professor: Walid Q. Alali

Committee: Mark A. Harrison

Charles L. Hofacre

Electronic Version Approved:

Julie Coffield
Interim Dean of the Graduate School
The University of Georgia
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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Salmonellosis and poultry products

Salmonella spp. belonging to the family of Enterobacteriaceae are small, rod-shaped, gram-negative, non-spore-forming bacteria, which are widely distributed in nature, with humans and animals being their primary reservoirs. The genus *Salmonella* is composed of two species, *Salmonella enterica* and *Salmonella bongori*. The subspecies of *S. enterica* referred to as subspecies I, II, IIIa, IIIb, IV, VI are currently named as *S. enterica* subsp. *enterica*, *S. enterica* subsp. *salamae*, *S. enterica* subsp. *arizonae*, *S. enterica* subsp. *diarizonae*, *S. enterica* subsp. *houtenae*, *S. enterica* subsp. *indica* (CDC, 2011a). Around 2,500 serovars have been identified, most of which are classified under *S. enterica*. *Salmonella* serotype Typhimurium and *Salmonella* serotype Enteritidis are the most common cause of human salmonellosis in the United States (CDC, 2010).

Salmonellosis is a foodborne illness caused by the bacteria *Salmonella*. Most people with salmonellosis develop diarrhea, fever and abdominal cramps 12 to 72 hours after exposure, and these symptoms usually last 4 to 7 days (CDC, 2010). In healthy adults, the symptoms can be mild. However, infants, elderly, and immunocompromised people are more susceptible to infection, causing more severe illness that may need hospitalization. *Salmonella* can travel from the intestines to the blood, and then reach other body organs, which may lead to organ failure and even death (CDC, 2010).

People can be infected with *Salmonella* due to consumption of contaminated foods that is raw, undercooked, and/or cross-contaminated (Scallan *et al.*, 2011; Hale *et al.*, 2012). Contaminated foods usually cannot be distinguished visually from uncontaminated foods. *Salmonella* can contaminate a variety of food commodities such as beef, pork, poultry, milk, and fresh produce (Bonardi *et al.*, 2008; Duggan *et al.*, 2012; Painter *et al.*, 2013).

The Centers for Disease Control and Prevention (CDC) estimated that more than 1.03 million illnesses, 19,500 hospitalizations and 378 deaths are associated with nontyphoidal *Salmonella* infections each year. *Salmonella* is the primary foodborne pathogen causing hospitalizations and death in the U.S. It is estimated that 35% of hospitalizations and 28% of deaths from foodborne pathogens were caused by *Salmonella* (Scallan *et al.*, 2011). According to the CDC Foodborne Diseases Active Surveillance Network (FoodNet), a total of 7,800 laboratory-confirmed *Salmonella* infections were reported in 2012, which equates to 39.9% of the total laboratory-confirmed bacterial and parasitic infections during that year. The incidence of *Salmonella* infection was 16.4 per 100,000 people;1.4 times higher than the National 2020 Health Objective (CDC, 2013). In 2012, the top five *Salmonella* serotypes associated with human salmonellosis were *S.* Enteritidis (16.5%), *S.* Typhimurium (13.4%), *S.* Newport (11.4%), *S.* Javiana (6.4%) and *S.* I 4,[5],12:i:- (2.9%), which totaled 50.6% of the total salmonellosis cases (CDC, 2012b).

Annually, more than 9 million foodborne illnesses are estimated to be caused by major pathogens in the U.S. (Scallan *et al.*, 2011). Based on the food attribution model using foodborne outbreak data from 1998 to 2008, 2.2 million (22%) cases of foodborne

illnesses were attributed to leafy vegetables, followed by dairy (1.3 million [14%]), fruits-nuts (1.1 million [12%]), and poultry and poultry products (900,000 [10%]) (Painter *et al.*, 2013). A total of 877 foodborne outbreaks linked to *Salmonella enterica* were analyzed and 19% were associated with poultry and poultry products. Furthermore, more deaths were attributed to poultry than any other commodity (Painter *et al.*, 2013). Among poultry-associated deaths, 26% were linked to *Salmonella* infections (Painter *et al.*, 2013). Guo et al. (2011) estimated that 17% of reported salmonellosis cases were attributed to turkey products by using: 1) human surveillance data on laboratory-confirmed *Salmonella* infections from the CDC, and 2) *Salmonella* testing data from FSIS from 1998-2003. More recently, between 2011 and 2014, 36.3% (12/33) of the major *Salmonella* multistate outbreaks in U.S was associated with poultry and poultry products (CDC, 2014).

Poultry products continue to be one of the primary sources of human *Salmonella* infections. Live poultry can be carriers, latently infected but rarely show clinical signs. Birds may excrete *Salmonella* in their feces and can be a source of cross-contamination for humans and the environment (Poppe, 2000). Turkey meat is a popular food among U.S. consumers because of its high protein and low fat content. The National Turkey Federation (NTF) reported that turkey consumption in the U.S. has more than doubled since 1970s (NTF, 2014a). In 2013, the turkey consumption in the United States was 16 pounds per person (USDA-ERS, 2013). It has been reported that between 2008 and 2013, 13 outbreaks with 1,022 cases were associated with turkey and turkey products all over the world, with 12 of the outbreaks and 338 cases in the U.S. only (Doyle, 2013). *Salmonella* serotypes that were involved in the outbreaks included *S.* Berta, Enteritidis,

Hadar, Heidelberg, Newport, Saintpaul, Stanley and Subspecies IIIa (Doyle, 2013). Four of the salmonellosis outbreaks linked to turkey meat in the U.S. were multistate outbreaks; three of which (*S.* Berta and *S.* Saintpaul in 2010, and *S.* Heidelberg in 2011) were associated with ground turkey (CDC, 2011b; CDC, 2011c; CDC, 2012a). The *Salmonella* Heidelberg outbreak that occurred in 2011was the largest with 34 states involved, and of the 136 people infected, 39% were hospitalized with one death reported (CDC, 2011c). Approximately 36 million pounds of ground turkey products were recalled, causing millions of economic losses. This 2011 ground turkey *S.* Heidelberg outbreak highlighted the need for better understanding of the transmission routes of *Salmonella* spp. from turkey production system to ground products.

Salmonella **prevalence in turkey and turkey products**

Turkey is consumed year-round in the U.S. although it was once mainly limited to special occasions, such as Thanksgiving. The U.S. is the world's largest turkey producer. Although exports are a major part of the U.S. turkey market, domestic consumption is higher than any other country, estimated at 16 pounds per person in 2013 (USDA-ERS, 2013). Turkey production in the U.S. has increased about 104 percent since 1970 (NTF, 2014a). It is forecasted that turkey meat production in 2014 will be 5.9 billion pounds, slightly higher than in 2011, which was 5.8 billion (AMI, 2011; USDA-ERS, 2014). According to a Marketplace Survey conducted by National Turkey Federation (NTF) in 2007, the top three turkey products were whole birds (24.5%), cooked white meat (13.8%) and ground turkey (10.1%) (NTF, 2012). This survey also revealed that the largest growth of turkey product sales was ground turkey, with over 403 million pounds sold in 2007 compared to 365 million pounds in 2005 (NTF, 2012).

Salmonella can be isolated from live turkey, turkey meat, and ground turkey (Sterzenbach *et al.*, 2010). In 2012, *Salmonella* prevalence in ground turkey was 11%; equating to 5 times higher than the 2.2% prevalence in turkey carcasses (USDA-FSIS, 2013). The most common serotype identified from turkey carcasses was *S.* Hadar (27%), while ground turkey was *S.* III 18:z4,z23:- (23%), respectively (USDA-FSIS, 2012a). Although *S.* Hadar and *S.* III 18:z4,z23:- were commonly isolated from turkey carcasses and ground turkey products, they were not among the top 10 serotypes reported to cause human salmonellosis by the CDC (CDC, 2011a). As for *Salmonella* serotypes from ground turkey only, the other top four serotypes were Hadar, Muenchen, Heidelberg and Newport (USDA-FSIS, 2012a). Three of these serotypes (Muenchen, Heidelberg and Newport) were among the top 10 serotypes reported to cause human salmonellosis (CDC, 2011a).

In 1996, FSIS established pathogen reduction; hazard analysis and critical control point system (PR/HACCP) to develop a better process control for preventing, eliminating, or reducing *Salmonella* and other foodborne pathogens on raw meat and poultry products, including turkey carcasses and ground turkey products (USDA-FSIS, 1996a). After that, several implementations of the PR/HACCP program developed. As a result of this program, the percentage of *Salmonella* positive turkey carcass samples decreased from 19.6% in 1996 to 2.0% in 2012 (USDA-FSIS, 1998; USDA-FSIS, 2013). Additionally, 49.9% of raw ground turkey meat was found to be contaminated with *Salmonella* from federally inspected plants in 1996 (USDA-FSIS, 1996b). This prevalence decreased to 11% in 2012 (USDA-FSIS, 2013).

In recent years, many studies have been conducted to determine the prevalence of *Salmonella* in raw and processed turkey. Nde et al. (2006) reported there were fluctuations in *Salmonella* prevalence on turkey carcasses along a turkey-processing line, with the highest *Salmonella* prevalence of 16.0% (n = 100) in post-defeathering and post-chilled carcasses, 11.0% (n = 99) in post-evisceration carcasses and lowest *Salmonella* prevalence at post-wash stage (1%, n = 100). Another study indicated that the overall prevalence of *Salmonella* in two turkey processing plants was an average of 16.7%, with a greater percentage of the pathogen observed on pre-chill (23%, n = 1201) than post-chill carcasses (10.1%, n = 1192) (Logue *et al.*, 2003). At the processing plants, the defeathering and scalding processing have been identified as major sources of *Salmonella* cross-contamination (Nde *et al.*, 2007).

At retail, according to National Antimicrobial Resistance Monitoring System (NARMS), *Salmonella* prevalence in ground turkey was 12.3% (n = 1320) in 2011 (NARMS, 2012). *Salmonella* Saintpaul, Heidelberg and Hadar were top three common serotypes in ground turkey (NARMS, 2012). Several retail studies showed that *Salmonella* prevalence in ground turkey in the U.S. varied from 7.7% to 36.5% (Rose *et al.*, 2002; Fratamico, 2003; Khaitsa *et al.*, 2007), which had higher *Salmonella* prevalence than in whole raw turkey and turkey parts, such as turkey breast, with the prevalence of 2.2% and 2.6% respectively. (Zhao *et al.*, 2001; Khaitsa *et al.*, 2007). In another study, 30 samples out of 74 (40.5%) retail turkey meats samples (turkey thigh and ground turkey) were found to be *Salmonella* positive (Fakhr *et al.*, 2006).

Salmonella performance standards for turkey

In 2011, USDA-FSIS implemented its performance standard for *Salmonella* in chilled carcasses at turkey slaughter establishments (USDA-FSIS, 2011a). The performance standard stated that, for young turkey, there should be no more than 4 *Salmonella*-positive samples out of 56 turkey carcasses tested. Turkey sponge samples are used for *Salmonella* detection rather than rinse samples due to its large size. Post-chilled turkey carcasses are randomly sampled by USDA employees. Sponge sampling procedure begins by first moistening the sponge with 10 mL of prechilled buffered peptone water (BPW) diluent and then wiping ten times horizontally and ten times vertically over 50 cm^2 turkey back with one side of the sponge. The same procedure is repeated on 50 cm^2 thigh area using the opposite side of the sponge for *Salmonella* detection (USDA-FSIS, 2011a). For ground turkey, FSIS recently reduced the number of the samples required for the *Salmonella* performance standard from 53 to 26, accepting no more than 15 positive samples. FSIS also increased the sample size from 25 g to 325 g (USDA-FSIS, 2012b). The implementation of this new policy would likely increase the probability of detecting *Salmonella*. The change in the sample size is a concern for the turkey industry; whether their ground turkey products will meet the performance standard.

Turkey production system in U.S.

Vertical coordination of the broiler, turkey and egg industries have changed significantly over the last few decades (Martinez, 2002). Vertical coordination, refers to synchronization of successive stages of production and marketing, including open production, contract production and vertical integration (Martinez, 2002). In 2002, it was estimated that production contracts accounted for 56% of turkey production and vertical

integration accounted for 32% in 2002, with the last 12% representing marketing contract (Martinez, 2002). Production contracts mean the grower provides the buildings, equipment, and labor, while the processor provides poults, feed and veterinary services. Vertical integration, which is more prevalent in the turkey industry than in the broiler industry, means a single firm owns all production facilities and hires labor to control the production and processing, from breeding through processing to retail (Mighell, 1963; Martinez, 2002). Recently, turkey companies have become more vertically integrated. By controlling breeding, hatching, growing, processing, shipping and marketing, the industry is able to better produce safer and high-quality products at the lower cost (NTF, 2013). Turkey production in the U.S. is decentralized. The top five turkey production states (by pounds produced) in 2012 were North Carolina, Minnesota, Indiana, Arkansas and Missouri (USDA-NASS, 2013).

Turkey grow out on farm

The quality of turkey meat depends greatly on the genetic selection of pure lineages of male and female birds, which are great grandparent (GGP) and grandparent (GP) flocks, using very precise genetically-influenced criteria, such as growth rate and resistance against disease (EFSA, 2012). Laying turkey hens are artificially inseminated. The hens lay eggs under a controlled environment, with the induction of light. After 28 days of incubation, baby turkeys (poults) are hatched and then transported in controlled climate vehicles to the farms. These turkeys are raised freely in the barn, fed mainly soybean and corn meal with a supplement of vitamins and minerals (USDA-FSIS, 2011b). Fresh water is provided at all times. After 4 to 5 months, the turkeys are ready to be transported to the processing plant. In general, hens take 14 weeks to weigh 17.5

pounds (desired market weight) compared to 18 weeks to weigh 38 pounds for toms turkeys (NTF, 2014b). No hormones are allowed to be given to turkeys, but antibiotics may occasionally be given to prevent diseases or as feed addictive. Feed withdrawal before transporting to processing is required to clean the turkey's intestinal tract and reduce fecal contamination of the turkey carcasses (Doyle and Erickson, 2006; USDA-FSIS, 2011b).

Turkey processing and ground production at processing plant

When turkeys reach the desired market weight, they are transported from the farm to the processing plant. After unloading and holding the birds, turkeys go through the slaughter process. Stunning, which make the birds unconscious, is the first step prior to killing the birds. Chemical stunning (usually CO_2) is commonly used in turkeys due to their large size. Furthermore, this method can reduce struggling and thus result in fewer broken bones and less muscle bruising, compared to electrical and mechanical stunning (Hoen and Lankhaar, 1999; Kang and Sams, 1999). The birds then bleed by a metal knife which is ran via electrical motor. It usually takes 2-5 minutes for birds to bleed out completely before they go to the next step; scalding. Scalding is the process to open up the feather follicles to prepare the carcasses for defeathering. Hot water (normally 138.2-145.4 °F) is used to spray or immerse the turkey carcasses for 50-125 seconds (USDA-FSIS, 2010). After scalding, carcasses typically pass through a picking machine to allow the rubber picking fingers to remove feathers from the carcass. Birds then washed with hot water that may include antimicrobials (e.g., chlorine, and peracetic acid) in preparation for evisceration. Pre-evisceration begins with the removal of the feet at the hock joint, followed by vacuum removal of cloaca. The evisceration process continues by

opening the body cavity and extracting the viscera (USDA inspection point), harvesting giblets, removing and discarding the intestinal tract, air sacs, trachea, crop, and lungs (USDA-FSIS, 2010). Next, the birds go through an inside and outside washing, followed by entering the chilling tanks. Chilling decreases the carcass temperature and inhibits microbial growth by immersing the carcasses in the cold water that may include antimicrobials, such as chlorine. It usually takes about 5 hours before carcasses exit the chiller. Turkey carcasses can be packed and sold as is or go through further processing. During further processing, carcasses are cut into different parts (such as thighs, drumsticks, breast, and wings) and then packaged and shipped to market. Additionally, turkey parts are used for ground turkey products.

Ground turkey and mechanically separated turkey are both produced at the end of the production line. Mechanically separated turkey (MST) is a paste-like turkey product produced by high pressure machinery that separates bone from turkey skeletal muscle tissue and other edible tissue. The bone-in parts are crushed then bones and tissues are forced through a sieve, or a similar screening device (USDA-FSIS, 2011). Unlike MST, in ground turkey production, chilled bone-in, skin-on, or skinless turkey parts must be deboned before entering the grinding machine. Skin is used in the ground turkey as a source of fat; however, neck skin is usually not used in ground turkey. Additionally, the fatty, blubbery, spongy fat and membranes must be removed from the skin covering the crop area before it can be used (USDA-AMS, 2006).

Salmonella transmission throughout the turkey production system

Potential *Salmonella* transmission routes in turkey production system are numerous and similar to that of chicken. There are a large number of publications

reporting *Salmonella* prevalence and transmission routes in chicken production systems; however, the number of studies available on this organism in turkey is limited. *Salmonella* contamination levels need to be controlled at every stage of the production chain in order to reduce contamination of the end product. In general, *Salmonella* can be transmitted through both vertical and horizontal routes.

Vertical transmission

Vertical transmission is transmission of *Salmonella* directly from the parent to an embryo, progeny or poults during hatchery (Liljebjelke *et al.*, 2005). True-vertical transmission happens primarily by reproductive organs such as oviduct or ovarian, or by contacting infected peritoneum or air sacs (Cherrington *et al.*, 1937).This transmission is the most essential route for *S.* Gallinarum and *S.* Pullorum infections (Snoeyenbos, 1991). *Salmonella* Hadar, *S.* Typhimurium, *S.* Enteritis, and *S.* Arizonae can also be transmitted by this route (Lee *et al.*, 1936; Crespo *et al.*, 2004). Within the above mentioned invasive *Salmonella* serovars, a significant variation between the strains and among the same serovars exists. However, there is significant variation between strains within serovars. Iaffaldano et al. (2010) reported the use of contaminated cryopreserved semen could also transmit *Salmonella* to breeder flocks, which could be another route of vertical transmission.

Pseudo-vertical transmission occurs via external contamination of eggs, i.e., *Salmonella* penetrate the shell after the egg has been laid. Some potential contamination sources include fecal material on egg belts, nest boxes, or contacting handling equipment or personnel (EFSA, 2009). *Salmonella* in feces attach to the warm surface of the shell and penetrate inside as it cools, especially when excess moisture is present. It was also

found that white shell turkey eggs were more frequently penetrated by *S*. Typhimurium than normally speckled eggs due to their thinner shells and larger pores (Williams and Dillard, 1969). This pseudo-vertical transmission has a similar result to true vertical transmission in terms of *Salmonella* infection (Hafez, 2010).

Horizontal transmission

Horizontal transmission is the transmission of *Salmonella* between two birds in the same flock, or different flocks, which are not in a parent-offspring relationship. Possible transmission may occur when the birds comes in direct contact with infected birds within the same flock or between flocks, or contact with some contaminated environmental factors such as air, feed, and litter.

Horizontal transmission: hatchery

The hatchery is one of the major sources for early horizontal transmission. Most scientific evidence regarding the infection of poultry with *Salmonella* derives from studies in chickens, and very little information is available on *Salmonella* infection during hatching of turkey eggs. A study in chicken hatcheries has shown that 0.01% - 0.05% of fertile hatching eggs entering the incubator are likely to harbor *Salmonella*, whereas the prevalence of infected chicks after hatchery may reach to 9% (Bailey *et al.*, 1994). This is probably due to the fact that *Salmonella* can spread when contaminated eggs and uncontaminated eggs are incubated together (Cason *et al.*, 1994). A chicken study showed when contaminated fertilized eggs hatch, *Salmonella* can colonize the digestive tract of the baby chick and then introduce *Salmonella* into the rearing farm (Cason *et al.*, 1994). Rapid transmission of *Salmonella* can occur when air circulation carries contaminated fluff and dust throughout the hatchery (Hoover *et al.*, 1997). Besides the

contamination of ventilation ducting, belt slots or door seals in hatchers play an important role in *Salmonella* transmission during hatchery (Mueller-Doblies *et al.*, 2013). It also results from infection and contamination that continuously recycles between hatchers, hatched birds, dust, and crate washing equipment (Davies and Wray, 1994; Davies and Bedford, 2001). It was found that *Salmonella* can survive for long periods in eggshells, meconium, dust, and biofilms and can persist even for many years within the hatchery (Friend and Franson, 1999). During the brooding period, elevated temperature and higher bird density may be conducive to the growth of *Salmonella* and increased horizontal transmission.

Horizontal transmission- rearing

Another form of *Salmonella* horizontal transmission is during rearing (on farm grow out). *Salmonella* can spread directly between infected and uninfected turkey birds and by indirect contact with the environment. Improper sanitation after an infected flock has left the farm can result in infection of the next flock of birds. Nayak (2008) tracked horizontal transmission pathway of *Salmonella* in turkey production environment using *Xba*-I digested pulsed-field gel electrophoresis. The tracking data indicated that once a facility was colonized with *Salmonella*, the bacteria continue to prevail in the environment and horizontally cross-contaminate the facility. Several factors could contribute to the *Salmonella* transmission at farm level, including water, soil, feces, litter, feed, rodent, and farmers' hygiene (Bryan *et al.*, 1968a; Hoover *et al.*, 1997; Arsenault *et al.*, 2007b; Arnold *et al.*, 2009; Aury *et al.*, 2010).

Hoover et al. (1997) reported that *Salmonella* present in drinkers before the placement of the flocks can be a source of infection particularly when birds are young

and most susceptible to colonization (due to the lack of mature gut microflora) (Williams, 1984; Poppe *et al.*, 1986). Aury et al. (2010) reported that the presence of the metering pump in the turkey house can help reduce the *Salmonella* contamination since the farmer can change the chemical parameter of the drinking water (acidification, chlorination) and convey chemical disinfectant by joining such a pump.

Feed and feeders have been found to be a potential *Salmonella* transmission source to the turkey farm (Danguy des Deserts *et al.*, 2011). *Salmonella* contamination of feed depends on *Salmonella* status in its ingredients. Hafez et al. (2010) revealed that nearly every ingredient used in producing poultry feed has been detected to be *Salmonella* positive. In a study, turkey feed was sampled and found that *Salmonella* prevalence ranged from 3% - 18.8% (Bryan *et al.*, 1968a; Hafez *et al.*, 1997; Nayak *et al.*, 2003). The variance might be due to the type of feed tested in these studies. It was reported that the type of the feed may affect the occurrence of *Salmonella* (Harris et al., 1997). Turkey farms normally use pelleted feed or extruded feed; however pelleted feed may pose high risk of *Salmonella* contamination due to lower temperature and pressure used to prepare it, compared to extruded feed. Research conducted by Dutta and his colleagues (2010) showed that turkey feed containing *S.* Enteritidis was responsible for a severe outbreak of gastroenteritis in turkeys which result in 34.4% mortality. Animal feed can also become contaminated at the mill and from delivery vehicles (Whyte *et al.*, 2003). Feeders have been found to be important vehicles of *Salmonella* transmission within a house (Guo *et al.*, 1999). A study suggested that the pan feeding systems showed a lower risk compared with the other feeder types, such as tube feeders or chain feeders.

The possible reason was the pan feeders do not allow accumulation of unused feed and are much easier to clean and disinfect (Danguy des Deserts *et al.*, 2011).

Litter and waste are also vectors for *Salmonella* contamination on turkey farms. *Salmonella* have been reported to survive in turkey litter for up to 9 months after removal of an infected flock (Hafez, 2010). Improper waste (including manure and dead birds) disposal was found to be a high risk of *Salmonella* contamination since it might attract pests that can carry *Salmonella*, and at the same time, increase environmental contamination (Featherstone *et al.*, 2010).

Poor biosecurity in the farm, leading to the transmission of infection between houses and between the house and the environment has been described as an important role in *Salmonella* contamination in the turkey farm (Danguy des Deserts *et al.*, 2011). It was reported that the presence of mice (either live mice, mice droppings, or tracks observed) on a facility was significantly associated with *S.* Typhimurium infection (OR = 4.71) (Featherstone *et al.*, 2010). Infected mice can be a vector of *Salmonella*, amplifying and spreading the organism between houses, consecutive flocks, and possibly even to neighboring units (Featherstone *et al.*, 2010).

Ineffective sanitary practices are a big concern in the turkey farm. Ineffective footbath (lack of disinfectant or not changed regularly) can quickly become a breeding ground for *Salmonella* and increases the risk of *Salmonella* contamination in turkey flocks (Aury *et al.*, 2010). Additionally, elevated temperature and higher bird density may be conducive to the growth of *Salmonella* and increased horizontal transmission (Hoover *et al.*, 1997).

Horizontal transmission – transportation

Moving birds from the farm to the processing plant has a high potential for *Salmonella* horizontal transmission. Cross-contamination during transportation to slaughter via feathers or feet contaminated during rearing can promote *Salmonella* contamination of turkey carcasses (Arsenault et al., 2007a). It is estimated that there can be a 20 - 40% increase in *Salmonella* both inside and outside the birds during the transportation (USDA-FSIS, 2010). Previous broiler chicken studies reported that *Salmonella* can spread through dirty crates, trucks and the catching/pickup crews (Bailey *et al.*, 2001; Corry *et al.*, 2002; Cox and Pavic, 2010). However, a study conducted by Wesley (2006) found that *Salmonella* prevalence of turkey crate swabbed before and after transportation was 47.6% and 39.7%, respectively, suggesting the crate was not associated with an increase in *Salmonella*, which is in contrast with the other broiler studies. Moreover, transportation during low wind speed, and/or closure of truck lateral curtains during transportation can also contribute to *Salmonella* transmission (Arsenault *et al.*, 2007a).

Turkeys are usually held in large open-sided sheds before they are unloaded and slaughtered. During this time, large fans are used to cool the birds and the air currents may distribute dust to the turkeys. It was reported that contaminated dust could serve as a route of rapid airborne transmission of *Salmonella* in turkey prior to slaughter (Harbaugh *et al.*, 2006).

Horizontal transmission – processing plant

Slaughtering and processing can serve as a great route of *Salmonella* horizontal transmission. The contamination of *Salmonella* in a turkey processing plant is very common. Several studies have reported that overall prevalence of *Salmonella* in turkey

processing plants ranged from 10% to 16.7%, with variation in different processing stages (Logue *et al.*, 2003; Nde *et al.*, 2006). It was reported that although many interventions were used, *Salmonella* prevalence on incoming birds was similar to that on finished products (Nde *et al.*, 2006), suggesting that cross-contamination or recontamination might occur. Additionally, Nivaset al. (1973) demonstrated *Salmonella* serotypes introduced into a processing plant spread throughout the plant using serotype analysis, providing valuable information on sources for cross-contamination.

Cross-contamination can occur at any stage in the processing plant when uncontaminated birds come in contact with contaminated birds or contaminated processing facilities. Poor worker hygiene may also increase cross-contamination. It was reported that 25.9% (n = 487) of samples taken from contact-equipment in turkey processing plants were *Salmonella* positive. Automatic tying machines, line tables, conveyors, and saw were contaminated more frequently than other equipment surfaces (Bryan *et al.*, 1968b). Several processes could contribute most to the *Salmonella* transmission at the turkey processing plant, including scalding, defeathering, evisceration, chilling, and further processing.

Scalding is used to break down the protein and open the feather follicle in preparation of defeathering (USDA-FSIS, 2010). High levels of fecal contamination entering the scalding process might be a source of cross-contamination. *Salmonella* is found to survive in scalding water at 60 ^{o}C (Nivas *et al.*, 1973). The same *Salmonella* subtype detected before and after scalding was reported by Nde *et al.* (2007), supporting the roles of scalding in carcass cross-contamination.

Picking process, which is mechanical feather removal by passing through rubber picking fingers, has been reported to be a major source for *Salmonella* cross-contamination (USDA-FSIS, 2010). Mechanism of cross-contamination during picking includes direct contact between contaminated and uncontaminated carcass (Clouser *et al.*, 1995b), aerosols (Allen *et al.*, 2003a; Allen *et al.*, 2003b), and contact with contaminated rubber picking fingers (Nde *et al.*, 2007). Picking could cause extrusions and scattering of fecal material onto the surface of picking fingers and can lead to cross-contamination between the carcasses (Allen, et al., 2003). The isolation of similar *Salmonella* subtypes from the fingers of the picker machines from birds at pre and post defeathering, suggested that picker fingers are a vehicle for cross-contamination during defeathering process (Nde *et al.*, 2007). The prevalence of *Salmonella* on turkey carcasses prescald was relatively low at 7% and increased to 16% after defeathering. Failure to replace the picker fingers, disinfecting them, or build up of feathers in the picking area may increase the cross-contamination level and contaminate *Salmonella*-free flocks when they are processed after a *Salmonella* positive flock.

Evisceration is a procedure to open the turkey cavity and remove viscera (USDA-FSIS, 2010). It was reported to be a critical point for cross-contamination during processing as a result of damage to the intestine as well as contact between intestines and carcasses (Hafez *et al.*, 1997; Hafez *et al.*, 2001). Several broiler studies have reported potential *Salmonella* cross-contamination sources during evisceration, including crop and intestine, and production facilities (Mead *et al.*, 1994; Byrd *et al.*, 2002).

Although chilling is designed to reduce *Salmonella* levels in turkey carcasses, improper dose of disinfectant used in chilling tanks might cause cross-contamination

between *Salmonella* positive and *Salmonella* negative flocks. It was reported that *Salmonella* prevalence in turkey carcass increased from 10% to 16% post chilling, indicating that cross-contamination might occur (Nde *et al.*, 2006).

Further processing refers to a range of further cutting and trimming by a combination of automated and manual lines. Turkey parts or ground turkey may have a higher prevalence of *Salmonella* because of possible cross-contamination between *Salmonella*-positive and *Salmonella*-negative parts during further processing. Morris and Wells (1970) reported the recontamination of carcasses during further processing. It was reported that 30% (n = 70) of the workers' hands and 37.5% (n = 16) of rubber gloves were *Salmonella* positive (Bryan *et al.*, 1968b). Additionally, the serotypes recovered from workers' hands and gloves were similar to those isolated from turkeys and equipment, indicating the workers could serve as mechanical transmitters of *Salmonella*.

According to the published scientific literature on *Salmonella* in turkeys, the main risk factors for *Salmonella* contamination in turkey production system are summarized in Table 1.

Potential sources of *Salmonella* contamination of ground turkey products

Salmonella can multiply in the gastrointestinal tract of birds and contaminate the environment, the bird carcass, and processing equipment due to excretion of the bacteria through feces. The mixing and grinding of turkey parts from several flocks (both contaminated and non-contaminated), and cross-contamination of turkey parts during further processing can be a potential source for ground turkey contamination with *Salmonella*. The source of *Salmonella* in ground turkey can be, in general, an external

contamination of turkey meat and/or internal systemic infection (i.e., internalization) of turkey organs and parts.

Salmonella external contamination: skin and neck skin

The feathers and skin of poultry carcasses are often found to be highly contaminated with *Salmonella*. It might originate from contacting with ingesta or feces (Hargis *et al.*, 1995; Marin *et al.*, 2011) and cross-contamination between two birds and/or between birds and processing facilities on farm, or during transportation and processing (Clouser *et al.*, 1995a; Geornaras *et al.*, 1997; Nde *et al.*, 2006). During scalding, *Salmonella* may enter the feather follicle and be entrapped, especially when the carcasses go through the chilling tank, which makes the follicles tighten up due to the carcasses cooling down. Too much heat in the scalding tank makes the carcasses oily, leading to easier *Salmonella* attachment to the surface of the skin (USDA-FSIS, 2010). The type of defeathering system might affect the *Salmonella* level in turkey skin. Although under Kosher slaughter, carcasses are soaked in cold water to make feather removal easier, this method, as well as the steam spray method, may produce rough skin surfaces in which *Salmonella* become entrapped or embedded, compared to the smooth skin surface to which *Salmonella* are only loosely attached during conventional defeathering (Clouser *et al.*, 1995b). A broiler study showed that once *Salmonella* is entrapped inside the feather follicles, it can become protected from disinfectants (Lillard, 1993). *Salmonella* has been detected in 71% (n = 14) of the turkey skin and feather samples after defeathering and 57% after chilling tanks (n = 14) (Clouser et al., 1995). In another study, 63% (n = 174) of the feather samples were *Salmonella* positive after defeathering (Nde *et al.*, 2007).

Salmonella contamination level on neck skin could represent the external contamination status of the whole carcass. It was revealed that there was no significant difference in *Salmonella* prevalence in chicken neck skin and in whole carcass rinse (Cox *et al.*, 2010). This is mainly because the bird's head hangs downwards during processing and wash fluid drips down through the neck skin. McEvoy *et al.* (2005) compared the efficacy of different sampling methods for *Salmonella* detection of prechilled turkey carcasses and found that 46% (n = 50) of the neck skin excision samples were positive for *Salmonella*. No significant differences have been observed between *Salmonella* recoveries from neck skin excision method and that from two-site swab method sampling (a USDA young turkey carcasses sampling method) (USDA-FSIS, 2011a). This suggests that the neck skin *Salmonella* contamination level may represent the contamination of the whole turkey carcass. Recently, Wu et al. (2014) found that *Salmonella* prevalence of rinsed chicken neck skin samples was 2.3%, which was significantly lower than that in stomached neck skin samples, which was 20.7%. This indicated that *Salmonella* might be firmly attached and/or entrapped inside the skin feather follicles. Turkey feather follicles might entrap more *Salmonella* due to their large size.

Despite that neck skin is removed and rendered during turkey processing, some neck skin leftover pieces might stay intact with the turkey breast skin. In this way, neck skin *Salmonella* level can provide information on potential cross-contamination of ground products.

Salmonella internalization in internal organs including bone marrow and spleen

There is very limited information available in published literature about the *Salmonella* internalization in poultry organs/parts such as spleens and bones. It may be

due to the fact that live poultry can be carriers for most *Salmonella* serotypes, latently infected but rarely showing clinical sign.

Salmonella infection usually begins with colonization of intestinal mucosa, surviving and multiplying in macrophages, spreading to the liver and spleen via the bloodstream or lymphatic system, possibly infecting other inner organ systems (ovary, oviduct, gizzard, yolk sac, or lungs), occasionally even reaching the bone marrow, causing systemic infection (Hafez *et al.*, 1997; Gast, 2007; Hafez, 2010; Mastroeni and Grant, 2011).

Rostagno and colleagues (2006) examined *Salmonella* prevalence in internal organs from six market-age turkey flocks at the processing plant and found that 9% (n = 178) of the spleens, 22.8% (n = 180) of the ceca, and 14.1% (n = 142) of the livers and gallbladders were *Salmonella* positive. In another study, *Salmonella* prevalence in turkey spleen, ceca, and crop during evisceration were 24.9%, 29.7%, and 14.3% respectively. Five serotypes were isolated, including, *S*. Agona, Heidelberg, Newport, Senftenberg and Ohio (Wesley *et al.*, 2006). Hafez *et al.* (1997) inoculated 3-day-old poults orally with 10^6 cfu per birds of *S*. Enteritidis. Samples of liver, lung, spleen, crop, proventriculus and bone marrow were found to be *Salmonella* positive at 21 days of age, with highest detection rates from spleen and ceca. According to a chicken study, the spleen was the first site found to be positive after 12 h post oral inoculation with *S*. Enteritidis, followed by blood (14 h), liver and heart (16 h), pancreas (20 h) and kidney and gallbladder (22 h) (He *et al.*, 2010).

The presence and colonization of *Salmonella* in poultry bone marrow is rarely reported. Hafez et al. reported that *Salmonella* could be present in turkey bone marrow in

21-day old turkey (Hafez *et al.*, 1997). Wu et al. tested chicken drumstick bone as a potential contamination source of ground chicken and found 0.8% (n = 300) of the drumstick bone samples were *Salmonella* positive. In another study, *Salmonella* persisted in the broiler chicken bone marrow until slaughter after orally inoculated with *S.* Enteritidis (Kassem *et al.*, 2012).

Bones crack or break during turkey carcass processing and during deboning (whether it is manual or mechanical deboning). This may result in the release of bone marrow into the meat utilized for ground production.

The spleen, as a part of lymphatic system, can serve as a blood filter (Mebius and Kraal, 2005). Spleens can also serve as potential indicators of bacteremia associated with transport stress or the most recent time the turkeys had been infected (Wesley *et al.*, 2006). Spleens, along with ceca, may provide the highest detection rate for turkey salmonellosis (Hafez *et al.*, 1997). Rostagno et al. (2006), revealed that *Salmonella* was present in 15% (n = 180) of spleen samples in market-age turkeys on farm and 9% (n = 178) at the processing plant. Although turkey spleens are not used in ground turkey production, we hypothesized that spleens can be representative of *Salmonella* systemic infection, which might indicate higher *Salmonella* contamination levels in the external source and in ground turkey at the flock level.

Conclusion

Consumption of *Salmonella* contaminated turkey meat may pose a public health concern when undercooked or under improper handling. *Salmonella* can be transmitted both vertically and horizontally throughout the turkey production system. Main risk factors of *Salmonella* contamination to the final turkey product includes contaminated

egg, resident *Salmonella* in hatchery, contaminated poults, feed, water, litter, rodents on farm, contaminated personnel and equipment, and cross contamination at processing plant.

Ground turkey is highly contaminated with *Salmonella* compared to post chilled turkey carcasses. Meanwhile, the 2011 ground turkey *S.* Heidelberg outbreak highlighted the need for better understanding of the potential contamination source (i.e., neck skin and bone) of *Salmonella* spp. to ground finished products. Turkey skin is used in ground turkey as a source of fat and is found to be highly contaminated with *Salmonella* since the bird's heads hang downwards during processing and wash fluid drips down through the neck skin. Despite that neck skin is removed and rendered during turkey processing, some neck skin leftover pieces might stay intact with the turkey breast skin. This can be a source of ground turkey contamination. Bones crack or break during turkey carcass processing and during deboning, releasing the bone marrow into the meat that may serve as potential contamination source to ground turkey.

FSIS increasing of ground turkey sample size from 25 g to 325 g would increase the probability of detecting *Salmonella*. It is important for the turkey industry to better understand the relationship between *Salmonella* internal infection (inner organs) vs. external contamination (neck skin and other skin parts) and ground turkey *Salmonella*-status. There is very limited information available in the published literature about the *Salmonella* internalization in poultry organs/parts such as spleens and bones. *Salmonella* might reach organs and parts such as spleen and bones when *Salmonella* is a systemically infect turkeys. We also hypothesize that when turkeys are systemically infected,

Salmonella is likely to be present at higher levels in the neck skin and in ground turkey at the flock level.

Table 1. Main risk factors for *Salmonella* contamination in turkey production system

Production State	Risk factor	Reference
Breeder rearing farms	Feed; infected poults	(Hafez *et al.*, 1997; Sivaramalingam *et al.*, 2013)
Commercial hatchery	Contaminated egg; resident *Salmonella*; contaminated crates, dust	(Cherrington *et al.*, 1937; Mueller-Doblies *et al.*, 2013)
Commercial poults rearing farm	Infected poults; water, soil, feces, litter, feed, rodent and farmers' hygiene	(Hoover *et al.*, 1997; Arsenault *et al.*, 2007b; Aury *et al.*, 2010)
Transport to slaughter	Crate, truck, personnel	(Arsenault *et al.*, 2007b; Cox and Pavic, 2010)
Unloading, slaughtering	Dust, personnel, equipment contamination	(Harbaugh *et al.*, 2006)
Scalding	Contaminated water	(Nivas *et al.*, 1973; Nde *et al.*, 2007)
Defeather	Aerosols, fecal material, equipment, personnel	(Allen *et al.*, 2003a; Allen *et al.*, 2003b; Nde *et al.*, 2007)
Evisceration	Intestine/crop, contaminated equipment or workers	(Mead *et al.*, 1994; Hafez *et al.*, 1997; Hafez *et al.*, 2001)
Inside-outside bird washing, trimming	Contaminated equipment, hands or knives	(Bryan *et al.*, 1968b)
Chilling	Chill water, contact between carcasses, aerosols	(Bryan *et al.*, 1968b; Nde *et al.*, 2006)
Further processing	Equipment, tables, knives, hand, gloves	(Bryan *et al.*, 1968b; Morris and Wells, 1970)

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CHAPTER 2

SALMONELLA LEVELS IN TURKEY NECK SKIN, BONE, AND SPLEENS IN RELATION TO GROUND TURKEY PRODUCTION

Cui, Y., Alali, W. Q., Harrison, M.A., and Hofacre, C.L. To be submitted to *Journal of Food Protection*

Abstract

The objective of this study was to determine *Salmonella* levels (presence and numbers) in turkey drumstick bone, spleen and neck skin samples in relation to *Salmonella* contamination levels in ground turkey at the flock level. A total of 300 samples of each turkey part (i.e., neck skin, spleen, drumstick) from 20 flocks were collected at a commercial turkey processing plant after evisceration and tested for the presence and number of *Salmonella* using most probable number (MPN) and enrichment methods. Ground turkey samples were collected and analyzed for *Salmonella* presence and numbers by the cooperating turkey company as part of the routine sampling and testing plans. The flocks were classified as targeted and non-targeted based on the farm/flock historical *Salmonella* contamination data in the ground product. A flock originated from a turkey farm that has produced one or more flock with a high *Salmonella* prevalence (i.e., > 20%) in the ground turkey was labeled as a 'targeted flock'. The outside surface of bone and spleen were sterilized prior to *Salmonella* analysis. The overall *Salmonella* prevalence in bone, spleen, neck skin and ground turkey samples was 9.3%, 6.7%, 42.0%, and 14.5%, respectively. *Salmonella* prevalence in neck skin, spleen, bone and ground turkey from the targeted flocks was significantly higher than those from non-targeted flocks ($P < 0.05$). Within the targeted flocks, *Salmonella* prevalence and numbers in neck skin samples from ground turkey *Salmonella*-positive flocks were significantly higher than those from ground turkey *Salmonella*-negative flocks ($P < 0.05$). When *Salmonella* was present in spleen and/or bone (at MPN > 1 log), and in neck skin (MPN > 2 log), the ground turkey lot was *Salmonella*-positive. Our findings suggested *Salmonella* presence at higher levels in neck skin, but lower internally

in spleen and bone, which may indicate a flock that has greater potential for *Salmonella* in the ground turkey.

Introduction

According to the Centers for Disease Control and Prevention (CDC) data in 2011, *Salmonella enterica* was the second most common identified cause of foodborne disease in the U.S. (Scallan *et al.*, 2011). It is estimated that more than 1.03 million illnesses, 19,500 hospitalizations and 378 deaths are caused by *Salmonella* in U.S. annually (Scallan *et al.*, 2011). *Salmonella* infection (i.e., salmonellosis) can cause mild to moderate gastrointestinal illness and in certain cases; severe disease can occur leading to death (CDC, 2010).

Poultry and poultry products continues to be one of the primary sources of human salmonellosis. According to the National Turkey Federation (NTF), turkey meat consumption in the U.S. has doubled since 1970s (NTF, 2014). In the U.S., 877 foodborne outbreaks linked to *Salmonella* occurred during 1998-2008, of which 19% were associated with poultry and poultry products (Painter *et al.*, 2013). Interestingly, 17% of reported salmonellosis cases in the U.S. between 1998 and 2003 were attributed to the consumption of turkey products (Guo *et al.*, 2011). Moreover, between 2008 and 2013, 12 salmonellosis foodborne outbreaks were associated with turkey and turkey products in the U.S.; three of them were associated with ground turkey (CDC, 2011; CDC, 2012; Doyle, 2013). The 2011 *S.* Heidelberg outbreak linked to ground turkey was the largest with 136 cases reported in 34 states, 39% of cases were hospitalized, and one death was reported (CDC, 2011). Approximately 36 million pounds of ground turkey products were recalled due to this outbreak, causing millions of dollars in economic losses. The 2011 ground turkey *S.* Heidelberg outbreak highlighted the need for better understanding of the transmission routes of *Salmonella* spp. to ground finished products.

According to the U.S. Department of Agriculture, Food Safety and Inspection Services (USDA-FSIS) 2012 annual progress report on *Salmonella* testing of raw meat and poultry products, *Salmonella* prevalence in ground turkey was 11%; equating to 5 times higher than the 2.2% prevalence on turkey carcasses (USDA-FSIS, 2013a). Ground turkey is produced by grinding boneless turkey meat (e.g., boneless drumsticks, thighs, breasts, necks, and wings) with skin-on and/or skinless (USDA-FSIS, 2011). Although it is known that *Salmonella* could be present externally on the surface of a carcass (i.e., on and in the skin), there are limited data on *Salmonella* contamination numbers in the skin. Moreover, it is currently unknown: 1) if *Salmonella* is present in internal organs or parts and at what levels, and 2) if external and internalized *Salmonella* levels (presence and numbers) contributes to ground turkey contamination, at the flock level.

Turkey skin is used in ground turkey as a source of fat. It has been reported that skin and feathers of turkey carcasses have been found highly contaminated with *Salmonella* (Clouser *et al.*, 1995a). This mainly due to carcass direct contact with ingesta and feces during processing, as well as cross-contamination between birds as well as between birds and the processing equipment (Clouser *et al.*, 1995a; Geornaras *et al.*, 1997; Nde *et al.*, 2006). During scalding, the high temperature of water opens up the skin feather follicles in preparation for defeathering at the pickers. Open skin feather follicles are sites for *Salmonella* to enter and get entrapped especially after carcasses cool down further in the process (USDA-FSIS, 2010). *Salmonella* was detected in 71% (n = 14) of the turkey skin samples after defeathering and in 57% post chilling (n = 14) (Clouser et al., 1995). In another study, 63% (n = 174) of the skin samples were *Salmonella* positive after defeathering (Nde *et al.*, 2007). It was revealed that *Salmonella* recovery from

turkey neck skin excision samples did not differ from those obtained by modified carcass rinse method and two sites swabbing methods, which was USDA-FSIS method to detect *Salmonella* on turkey carcass (McEvoy *et al.*, 2005; USDA-FSIS, 2011a). This is mainly because the bird's head hangs downwards during processing and wash fluid drips down through the neck skin. Despite that turkey neck skin is removed and rendered during processing, traces of neck skin might stay intact with the turkey breast skin. This can be a source of ground turkey contamination.

There is very limited information available in the published literature about *Salmonella* internalization in poultry organs/parts such as spleens and bones. We hypothesized that when *Salmonella* causes a systemic infection in turkeys, it may reach internal organs and parts such as spleen and bones. We also hypothesized that when *Salmonella* causes a systemic infection, it likely to be present at higher levels in the neck skin and in ground turkey at the flock level.

Bones crack and break during turkey carcass processing and deboning (whether it is manual or mechanical deboning). This may result in the release of bone marrow into the meat utilized for ground production. In a recent study chicken study, authors reported that *Salmonella* prevalence in drumstick bones was 0.7% (n = 300)(Wu et al., 2014). Another study by Rostagno et al. (2006) revealed that *Salmonella* was present in 15% (n = 180) of spleen samples in market-age turkeys on farm.

The USDA-FSIS is implementing a change in the ground turkey sample size (from 25 g to 325 g) to test for *Salmonella* (USDA-FSIS, 2012). The implementation of this new policy will likely increase the probability of detecting *Salmonella*. Thus, the change in the sample size is a concern for the turkey industry; whether their ground

turkey products will meet the FSIS *Salmonella* performance standard. It is important for the turkey industry to better understand the relationship between *Salmonella* internal infection vs. external contamination and ground turkey *Salmonella*-status.

The objective of this study was to determine *Salmonella* levels (presence and numbers) in turkey drumstick bone, spleen and neck skin samples in relation to *Salmonella* contamination levels in ground turkey at the flock level.

Materials and Methods

Sample collection. A cross-sectional study was conducted between June 2013 and March 2014 in cooperation with a commercial turkey production company. Turkey parts (i.e., drumstick, neck skin, and spleen) were sampled from 20 flocks at the processing plant. A total of 300 samples of each turkey part were collected over the study period. Fifteen turkey carcasses per flock (five carcasses every 30 min) were randomly pulled off the processing line after evisceration and the USDA-FSIS inspection point. From each carcass, one part (a drumstick, a neck skin, and a spleen) was collected and bagged individually in sterile bags (Nasco, Salida, CA). Sampling of a carcass started with removing the neck skin with a knife, sanitizing the knife with 70% ethanol, then harvesting the spleen and the drumstick. All samples were shipped overnight on ice to the laboratory (Center for Food Safety, University of Georgia) and were processed immediately upon arrival for *Salmonella* presence and numbers. Ground turkey samples (25 g, n = 6 samples on average per flock) were collected and analyzed for *Salmonella* presence and numbers by the cooperating turkey company as part of the routine sampling and testing plans using BAX - polymerase chain reaction (PCR) assay system according to USDA microbiology laboratory guidebook protocol (USDA-FSIS, 2013c).

Turkey flock selection. The selection of turkey flocks to include in this study was based on historical *Salmonella* contamination data of ground turkey provided by the cooperative turkey company. A flock originated from a turkey farm that has produced one or more flock with a high *Salmonella* prevalence (i.e., > 20%) in the ground turkey was labeled as a 'targeted flock'. Other turkey farms/flocks with a history of low or no *Salmonella*-positives in the ground turkey product were labeled as 'non-targeted flocks'. In this study, 13 flocks were targeted and seven were non-targeted. We hypothesized that turkey parts from the targeted flocks would have higher levels of *Salmonella* compared to non-targeted flocks.

Sample preparation for *Salmonella* analysis. Upon arrival at the laboratory, all sample information was logged and recorded**.** The turkey neck skin samples were quite large (~600 g). Therefore, a composite sample of 100 g that consisted of four pieces from the four corners of the neck skin and one from the middle were sampled. Gloves were changed and cutting scissors were sanitized with 70% ethanol between each sample to prevent cross contamination. A 500 mL buffered peptone water (BPW; Difco, Becton Dickenson, Sparks MD) containing 0.05% Tween 80 (BDH, West Chester, PA) was added to the 100 g neck skin composite sample and then stomached at high speed for two min (Stomacher 400, Seward Ltd, London, England). The stomached solution was used for *Salmonella* analysis.

For the turkey drumstick samples, the outside surface meat and the cartilage at ends of the samples were carefully removed. Membrane covering the surface of the bone was removed to have the least amount of tissues left on the bone for better surface sterilization. After that, the outside surface of the extracted bone was sterilized by

soaking it in 80% ethanol (200 mL per bone) for 30 s, followed by dipping into boiling water (200 mL per bone) for 15 s. This procedure was repeated for 3 times to eliminate any *Salmonella* cell on the outside surface of the bone without killing any *Salmonella* inside the bone (if present). This procedure was an efficient method to sterilize chicken drumstick bone surfaces without killing *Salmonella* inside the bone (Wu *et al.*, 2014). We also tested the sterilization procedure experimentally on turkey bone and was found to be effective (data not shown). The sterilized bones were placed in double Whirl-Pak bags (Nasco, Salida, CA) and crushed into pieces with a rubber mallet to release the bone marrow. A 500 mL of BPW containing 0.05% Tween 80 was added to the crushed bone sample and mixed well by shaking the bag for 30 s. The bone-BPW solution was used for *Salmonella* analysis.

Each spleen sample was immersed in boiling water for 5 s to sterilize the outside surface without killing *Salmonella* inside the organ if present. This sterilization protocol was based on our preliminary experimental sterilization studies (data not shown). The sterilized spleen was placed in Whirl-Pak bag, smashed by hand and then 100 mL BPW containing 0.05% Tween 80 was added. The spleen with the solution was macerated in a stomacher for 1min at high speed and then used for *Salmonella* analysis.

***Salmonella* quantitative and qualitative analysis.** The 3-tube 3-dilution most probable number (MPN) method was used to quantify *Salmonella* according to USDA-FSIS methods (USDA-FSIS, 2008). Additionally, primary and a delay secondary enrichment was used for detection of *Salmonella* (USDA-FSIS, 2013b; Wu *et al.*, 2014).

Most probable number method to quantify *Salmonella*. For each sample solution, nine tubes were used for the pre-enrichment of *Salmonella* with first three tube-

set containing 10 mL of the original sample solution and the remaining second and third sets of three tubes containing 9 mL BPW and 9.9 mL BPW, respectively. One milliliter and 0.1 mL of the sample solution were added to the second and the third sets of tubes, respectively. All nine tubes were incubated at 37 ℃ for 24 h.

A portion (0.5 mL) of the pre-enrichment culture was transferred to 10 mL tetrathionate broth (TT; Difco, BD) and then incubated at 42 ℃ for 24 h. After incubation, a loopful of each TT culture was streaked onto xylose lysine tergito 4 (XLT4; Difco, BD) plates which were incubated at 37 ℃ for 22 – 24 h. Up to three presumptive *Salmonella* colonies from XLT_4 plates were selected and inoculated onto triple sugar iron (TSI; Difco, BD) and lysine iron agar (LIA; Oxoid, Hampshire, England) slants that were incubated at 37 ℃ for 24 h. Isolates with typical *Salmonella* reactions on TSI and LIA were then confirmed by the agglutination *Salmonella* Poly O A-I & Vi antiserum test (Difco, BD). The MPN/mL value of each sample was acquired using the USDA-FSIS MPN table (USDA-FSIS, 2008).

Primary and a delay secondary enrichment for *Salmonella* detection. In addition to *Salmonella* quantification, the samples were enriched to detect low *Salmonella* levels that are undetectable via the MPN method. Seven milliliters of 11X TT broth was added to spleen samples and 47 mL of 11X TT broth was added to bone and skin samples which were incubated at 42 ℃ for 24 h (i.e., primary enrichment). After incubation, a loopful of the mixture was streaked onto XLT_4 plates and incubated (37 ℃, 24 h). The remaining isolation and confirmation of *Salmonella* was done as described for MPN.

To recover injured *Salmonella* cells, a delayed secondary enrichment was performed on all samples by holding the enriched TT at room temperature for 5 days. After 5 days, 0.5 mL aliquots were transferred from those samples negative on primary enrichment into a fresh 10 mL TT broth tubes and were incubated (42 ℃,24 h). A loopful of the mixture was streaked onto XLT_4 plates. The remaining isolation and confirmation of *Salmonella* was done as described for MPN.

Data analysis. The outcomes of the study were the prevalence and numbers of *Salmonella* on neck skin, spleen, bone, and ground turkey samples. The MPN data per ml were adjusted to the original weight or volume per sample and then $\log_{10}$ transformed to approximate normality. Only MPN per sample values that met or exceeded the limit of detection (i.e., 12 salmonellae per sample) were used in the analysis. *Salmonella* mean numbers were compared between sample types by: 1) flock type (targeted and non-targeted), and 2) within the targeted flocks by ground turkey status (i.e., positive or negative flocks based on the ground turkey testing) using t-test for independent sample in STATA statistical software version 10.1 (Stata Corp., College Station, TX). A difference was considered significant at $P < 0.05$.

A sample was considered *Salmonella* positive if the organism was detected via MPN, primary enrichment, or delayed secondary enrichment. The prevalence data were compared in a similar manner as the MPN data, but using Chi-square test in STATA software. A difference was considered significant at $P < 0.05$.

Results

Overall *Salmonella* prevalence and numbers. A total of 300 samples of each turkey parts (spleen, drumstick bone, and neck skin) representing 20 flocks were

collected and tested. Ninety percent (18/20) of the flocks had at least one *Salmonella*-positive sample. Data on *Salmonella* presence and numbers from 117 ground turkey samples representing the 20 flocks were provided by the cooperating turkey producing company. The overall *Salmonella* prevalence and mean $\log_{10}$MPN/sample in turkey parts and ground turkey is shown in Table 2. The overall *Salmonella* prevalence in bone, spleen, neck skin and ground turkey samples was 9.3%, 6.7%, 42.0%, and 14.5%, respectively.

***Salmonella* prevalence and numbers by flock type.** *Salmonella* prevalence and mean $\log_{10}$ numbers in turkey parts and ground turkey by flock type is shown in Table 3. *Salmonella* prevalence in neck skin, spleen, bone and ground turkey from the targeted flocks were significantly higher than those from non-targeted flocks ($P < 0.05$). As for *Salmonella* numbers, there was no significant differences ($P > 0.05$) between those in neck skin samples in targeted flocks compared to the numbers in non-targeted flocks. *Salmonella* numbers were undetectable via the MPN method in turkey bone, spleen and ground product samples from the non-targeted flocks. The distribution of the mean $\log_{10}$ MPN of *Salmonella* in turkey parts by flock type is shown in Figure 1. Within *Salmonella* MPN positive samples, 91% of *Salmonella* MPN numbers of bone samples and 50% of that in spleen samples in the targeted flocks fell in the low number interval (i.e., 0.5-1.5 $\log_{10}$ MPN/sample). For skin samples, 39% of *Salmonella* numbers in targeted flocks and 41% of *Salmonella* numbers in non-targeted flocks fell in the 1.6-2.5 logs interval. The percentage of *Salmonella* numbers in skin samples from targeted flocks that fell in the high interval (3.6-3.9 logs) were 10 times higher than that in skin samples from non-targeted flocks.

Salmonella prevalence and numbers within the targeted flocks by ground turkey *Salmonella*-status is shown in Table 4. Among the 13 targeted flocks collected, five flocks resulted in ground turkey lots being *Salmonella*-positive (i.e., at least one positive sample). *Salmonella* prevalence and numbers of neck skin samples in ground turkey *Salmonella*-positive flocks were significantly higher than those in ground turkey *Salmonella*-negative flocks ($P < 0.05$). There was no significant difference between *Salmonella* prevalence of turkey bone and spleen samples in ground turkey *Salmonella*-positive and negative flocks ($P > 0.05$). At the flock level, when *Salmonella* was internalized in spleen and/or bone, it was likely to be present at higher prevalence (46.7% - 100%) in the neck skin. When *Salmonella* was present in spleen and/or bone (at MPN > 1 $\log_{10}$), and in neck skin (MPN > 2 $\log_{10}$), the ground turkey lot was *Salmonella*-positive (data not shown). Additionally, at the flock level, when *Salmonella* was internalized in spleen and/or bone, it likely to be present at higher prevalence (46.7% - 100%) in the neck skin (data not shown).

Discussion

Salmonella contamination of turkey flocks during processing was common; 90% (18/20) of the flocks had at least one *Salmonella*-positive sample. The high prevalence might be due to the cross-contamination at the processing plant (neck skin). Furthermore, 13 of the flocks were targeted for sampling as they were expected to have higher *Salmonella* contamination levels compared to non-targeted. This result is similar to another study where all 24 turkey flocks surveyed at the processing plant were found to be *Salmonella* positive (Hafez *et al.*, 2001).

Our study showed that *Salmonella* present in turkey neck skin samples (42%, n = 300) was at a higher prevalence compared to that in spleen and bones of turkeys (Table 2). This prevalence is similar to a study that reported 46% (n = 100) of neck skin excisions at pre-chill stage were *Salmonella*-positive (McEvoy *et al.*, 2005). The high prevalence in neck skin might be due to the following: 1) the cross-contamination during processing (Clouser *et al.*, 1995a; Nde *et al.*, 2007); and 2) *Salmonella* entrapped in neck skin feather follicle (Kim and Doores, 1993; Kim et al., 1996). A recent chicken study found that 20.7% (n = 300) of stomached neck skin samples were *Salmonella*-positive at post-chilled stage (Wu *et al.*, 2014). Since turkey feather follicles are larger than chickens, they might entrap more *Salmonella* especially when sample collection was conducted at post-evisceration compared to post-chill as in the Wu et al. study.

In this study, we revealed that *Salmonella* could be present internally (i.e., internalized) in turkey's organs/parts such as spleen and bone, but in general at low levels. *Salmonella* internalization of naturally infected turkey is rarely reported. In one study, Rostagno et al. (2006) revealed that *Salmonella* was present in 9% (n = 180) of spleen samples in market-age turkeys at a processing plant, which is slightly higher than the 6.7% prevalence in our study. Although the authors mentioned that spleen samples were collected aseptically, they did not report if the outside surface of spleen was sterilized. Recently, Wu et al. (2014) assessed *Salmonella* prevalence in chicken drumstick bones as a potential source of ground chicken contamination. The authors found that 0.8% (n = 300) of the chicken drumstick samples were *Salmonella* positive, which is much lower than the 9.3% prevalence in our study. It might due to the reason that turkey grow out period is longer than that for chickens which could increase the

probability of systemic infection with *Salmonella*. Furthermore, turkey drumstick bones are much larger than chicken drumstick bones allowing for more possible internalization. Additionally, the turkey's immune system and disease susceptibility are different than chickens (Hafez, 2010).

Salmonella could be colonized and transmitted to the inner organs through different ways, such as the invasion-associated type III secretion and macrophage survival (Jones *et al.*, 2007; Sterzenbach *et al.*, 2010). According to the MPN distribution data (Fig 1), *Salmonella* numbers in the spleen had a wider distribution (0.6 - 3.0 logs) compared to the numbers in bones (1.2 - 1.7 logs). This might be due to the fact that the spleen acts as a primary blood filter as a part of lymphatic system. *Salmonella* can colonize the bird intestinal tract during hatching from the egg and may cross the intestinal barrier and get engulfed by the macrophages (Williams, 1984; Brown et al., 2005). Once inside the macrophages, *Salmonella* has the ability to evade lysis, potentially leading to systemic *Salmonella* infection in birds (Bohez et al., 2006; Jones et al., 2007). The spleen can harbor these macrophages carrying *Salmonella* at higher prevalence and numbers than other organs (Rostagno *et al.*, 2006). This finding agrees with a previous study that spleen may provide the highest detection percentage for poultry salmonellosis (Hafez *et al.*, 1997).

According to the 2013 USDA-FSIS progress report on *Salmonella* testing of raw meat and poultry product, 11% (n = 1,155) of the ground turkey samples were *Salmonella* positive, which is slightly lower than our findings. This may be due to the inclusion of targeted turkey flocks in this study. Furthermore, it might due to the difference in detection methods. The cooperating company used a polymerase chain

reaction (PCR)-based method to detect *Salmonella* in order to get rapid results. However, it might cause false-positive results compared to the traditional culture based method. Koyuncu (2010) compared the accuracy of commercial PCR-based methods for *Salmonella* detection and found that many positive results could not be confirmed by *Salmonella* isolation.

Enumeration of *Salmonella* in different turkey parts as well as ground turkey can provide data for the industry to develop effective interventions and also may be used to assess risk and progress with regard to their food safety systems. To the best of our knowledge, this is the first study that provided data on *Salmonella* quantities in turkey samples in relation to ground turkey production. Low numbers of *Salmonella* present in bone (mean =1.3 logs) and spleen (mean = 1.5 logs) may suggest a lower risk *Salmonella* numbers in ground turkey compared to neck skin. The higher numbers of *Salmonella* in skin (mean = 2.4 logs), especially with 9.8% of the neck skin samples in targeted flock had high levels (i.e., 3.5- 3.9 logs), may pose a high risk for *Salmonella* numbers in ground turkey. High numbers of *Salmonella* in neck skin could serve as source of *Salmonella* cross-contamination at the processing plant, and may indicate highly contaminated flocks.

At the flock level, *Salmonella* prevalence in neck skin, spleen, bone and ground turkey from the targeted flocks was significantly higher than those from non-targeted flocks ($P < 0.05$) (Table 3). This may suggest that *Salmonella* contamination in the final product (i.e., ground turkey) is more likely in targeted flocks vs. non-targeted flocks. Although there was no significant differences between *Salmonella* means in neck skin samples in targeted flocks (2.5; 95% CI: 2.3-2.7) compared to that in non-targeted flocks

(2.0; 95% CI: 1.6-2.4) (Table 3), only 17 samples were used to calculate the mean in non-targeted flocks compared to the 80 samples in targeted flock. This indicates that there were more neck skin samples with countable MPN values in targeted flocks compared to samples in non-targeted flocks.

In the targeted flocks, both the prevalence and numbers of *Salmonella* from neck skin samples from ground turkey *Salmonella*-positive flocks were significantly higher than those from ground turkey *Salmonella*-negative flocks; whereas, no significant differences were observed in bone and spleen samples (Table 4). This finding suggested that neck skin samples could be a potential source for *Salmonella* contamination in ground product. Although turkey neck skin is generally not utilized in ground production, pieces of it can be still attached to the skin of breast meat serving as a potential source for *Salmonella* contamination in ground product.

Since we sampled at post-evisceration, the skin goes through washing, chilling and further processing steps that may impact *Salmonella* contamination levels before it reaches the grinder. According to Morris *et al.* (1970), authors reported that further processing steps resulted in recontamination of the carcass with *Salmonella*. Manual deboning after chilling has been reported to increase *Salmonella* levels on the skin and muscles of turkey carcass parts (Hafez *et al.*, 1997). Additionally, *Salmonella* was recovered from 40% of the workers' hands in a chicken processing plant, suggesting a possible route for cross contamination (Wit and Kampelmacher, 1982).

In our study, we found that at the flock level, when *Salmonella* was internalized in spleen and/or bone, it likely to be present at higher prevalence (46.7% - 100%) in the neck skin.

Additionally, we observed in this study that when *Salmonella* was present in spleen and/or bone (at MPN > 1 $\log_{10}$), and in neck skin (MPN > 2 $\log_{10}$), the ground turkey lot was *Salmonella*-positive. This might be due to when *Salmonella* causes systemic infection in turkeys, we hypothesized that a concurrent higher levels of fecal shedding of *Salmonella* occurred leading to higher levels of this organism in the neck skin, resulting in higher probability of cross-contamination of skin and other parts, leading to contamination of the final ground product. It is noteworthy that this trend was observed when the sample size of ground turkey testing was 25 g.

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CHAPTER 3

CONCLUSION

Salmonella present in external surface of turkey neck skin and internalized sources of spleen and bone may contribute to *Salmonella* contamination of ground turkey. However, neck skin appears to be more significant risk to ground turkey contamination. *Salmonella* presence at quantifiable numbers internally in spleen and bone and in neck skin may indicate a highly contaminated flock that resulted in ground turkey contamination. This study provides a possible explanation of the higher *Salmonella* prevalence in ground turkey compared to that on turkey carcass. Further research is needed to characterize the isolates from this study using serotyping and the molecular subtyping to better understand how *Salmonella* becomes internalized in turkey parts/organs and if the strains detected in the parts are similar to those in ground turkey.

Table 2: Overall *Salmonella* prevalence and numbers in drumstick bones, spleens, and neck skins from commercially processed turkey carcasses

Sample type	No. of samples	No. of Positive	Prevalence	Mean log_{10}MPN/sample[a]	95% CI[a]
Bone	300	28	9.3%	1.3	1.2-1.4
Spleen	300	20	6.7%	1.5	1.0-2.0
Neck skin	300	126	42.0%	2.4	2.2-2.6
Ground turkey	117	17	14.5%	1.9	1.1-2.6

[a] Mean log most probable number (MPN) of *Salmonella* per sample and its 95% confidence interval (CI).

Table 3: *Salmonella* prevalence and numbers in drumstick bones, spleens, and neck skins from commercially processed turkey carcasses by flock type[a]

Sample type	Targeted flocks (n=195 carcass)				Non-targeted flocks (n=105 carcass)			
	Prevalence	95% CI	Mean	95% CI	Prevalence	95% CI	Mean	95% CI
Bone	13.8%[A]	9.7%-19.4%	1.3	1.2-1.4	1.0%[B]	0.17%-5.19%	-[b]	
Spleen	10.3%[A]	6.7%-15.3%	1.5	1.0-2.0	0%[B]		-	
Neck skin	51.3%[A]	44.3%-58.2%	2.5	2.3-2.7	24.8%[B]	17.5%-33.8%	2.0	1.6-2.4
Ground turkey	18.8%[A]	11.9%-28.4%	1.9	1.1-2.6	3.0%[B]	0.5%-15.3%	-	

[a] Comparison of prevalence: Values within the same row followed by the same uppercase letter were not significantly different ($P > 0.05$).Means of neck skin samples were not significantly different ($P > 0.05$). Statistical comparisons were based on Chi-square (for prevalence data) and independent t-test (for mean data) using STATA statistical software version 10.1 (Stata Corp., College Station, TX).

[b]"-"*Salmonella* numbers were undetectable via MPN method.

Table 4: *Salmonella* prevalence and numbers in drumstick bones, spleens, and neck skins from commercially processed turkey carcasses by ground turkey *Salmonella*-status in targeted flocks[a]

Sample type	Ground turkey *Salmonella*-positive flocks(n=75carcasses)				Ground turkey *Salmonella*-negative flocks (n=120 carcass)			
	Prevalence	95% CI	Mean	95% CI	Prevalence	95% CI	Mean	95% CI
Bone	21.3%[A]	13.6%-31.9%	1.3	1.2-1.4	9.2%[A]	5.2%-15.7%	-[b]	-
Spleen	14.7%[A]	8.4%-24.4%	1.5	1.0-2.0	7.5%[A]	4.0%-13.6%	-	-
Neck skin	86.7%[A]	77.2%-92.6%	2.6	2.4-2.9	40.8%[B]	32.4%-49.8%	1.9	1.5-2.2

[a] Comparison of prevalence: Values within the same row followed by the same uppercase letter were not significantly different (P > 0.05).Means of neck skin samples were significantly different ($P < 0.05$). Statistical comparisons were based on Chi-square (for prevalence data) and independent t-test (for mean data) using STATA statistical software version 10.1 (Stata Corp., College Station, TX). .

[b] "-" *Salmonella* numbers were undetectable via MPN method.

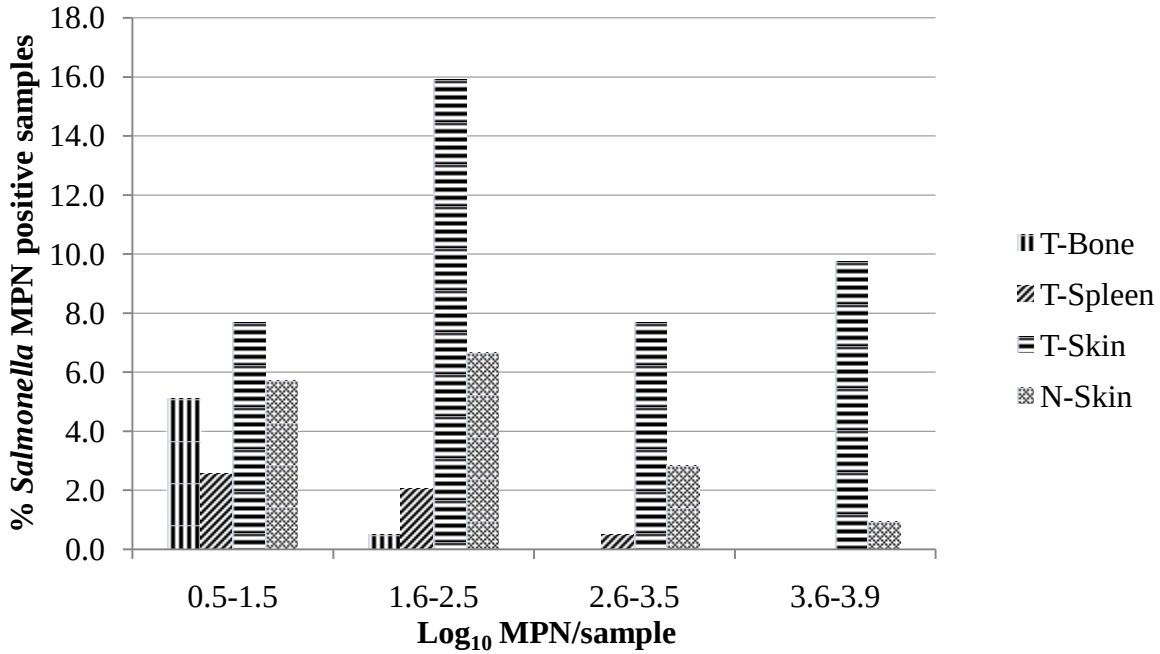

Figure 1. Percentage bar chart illustrating the log Most Probable Number (MPN) distribution of *Salmonella* on turkey bone, spleen and neck skin in targeted and non-targeted flocks. *Salmonella* numbers were undetectable on turkey spleen and bone from non-targeted flocks. T: Targeted flocks, N: Non-targeted flocks