SALMONELLA LEVELS FROM TURKEY SKIN

by

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(Under the Direction of Walid Q. Alali)

ABSTRACT

The objective of this study was to determine *Salmonella* levels (presence and numbers) associated with the skin of turkey parts (i.e., drumstick, thigh, and wing). In collaboration with a commercial turkey processor, a total of 20 turkey flocks expected to be highly contaminated with *Salmonella* were sampled. From each flock, 15 samples per part type were collected post-chill and tested for *Salmonella* using the most probable number (MPN) and enrichment methods. Overall *Salmonella* prevalence associated with the skin of drumsticks, thighs, and wings was 13.7%, 19.7% and 25.0%, respectively. *Salmonella* prevalence was significantly higher ($P< 0.05$) from wing skin than that from drumstick skin, but the difference was not significant ($P> 0.05$) when compared to thigh skin. The odds ratio of *Salmonella* presence from thigh skin (odds ratio = 2.4; $P< 0.05$) was significantly higher when this pathogen was also found with wing skin. *Salmonella* mean numbers (logMPN/sample) from skin samples for the three parts were 1.18 logs (drumstick), 1.29 logs (thigh), 1.45 logs (wing) and were not significantly ($P> 0.05$) different. Our findings suggest the high prevalence of *Salmonella* associated with wing skin could be a potential source for ground turkey contamination.

INDEX WORDS: *Salmonella*, ground turkey contamination, skin types, prevalence, MPN method

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TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1. Outbreak of salmonellosis associated with poultry products

The bacteria, *Salmonella*, were named in memory of the pathologist Salmon and his student for their first isolation of this pathogen from porcine intestine (Katscher, 1997). *Salmonella* is a genus belongs to the family of *Enterobacteriaceae*. It is a rod-shaped, gram-negative, non-spore forming, thermolabile, predominantly motile enterobacteria with diameters of approximately 0.7 to 1.5 μm, and lengths from 2 to 5 μm (Fabrega and Vila, 2013). These bacteria are ubiquitous and hardy in nature, including the gastrointestinal tracts of domesticated and wild mammals, birds, reptiles, and insects (Gomez, *et al*., 1996). At present, this genus is divided into two species: *S. enterica* and *S. bongori* (Tortora, 2008).The species *S. enterica* is subdivided into six subspecies which are assigned by taxonomic names. They are *S. enterica* subspecies *enterica* (subspecies I), *salamae* (subspecies II), *arizonae* (subspecies IIIa), *diarizonae* (subspecies IIIb), *houtenae* (subspecies IV) and *indica* (subspecies V) (Tortora, 2008). However, *S. bongori* has only one subspecies (Tortora, 2008). *Salmonella enterica* includes the majority of *Salmonella* strains isolated from humans and warm blooded animals; whereas, *S. bongori* is typically obtained from cold blooded animals (Nataro *et al.*, 2006). Members of the seven subspecies can be serotyped into more than 2,500 different serotypes differentiated by the somatic and flagellar antigens present on the cell surface: H antigen, O antigen, and Vi antigen (Chiu *et al.*, 2005). The H antigen is a flagellar antigen expressed by two flagellin genes (*fli*C and *flj*B) alternatively and is self-interchangeable (Giannella, 1996; Yamamoto

and Kutsukake, 2006); the O antigen is a somatic antigen characterized by the repeating carbohydrate proteins on the outer O-polysaccharide chain (Giannella *et al.*, 1973). The antigen overlays the O antigen is called Vi antigen. It is a capsular polysaccharide which is not found in all serovars. The serovars which own Vi antigen have a high infectious ability through making the immune system less sensitive toward them (Sathyabama *et al.*, 2014; Wain *et al.*, 2005). Among the thousands of serotypes, Typhimurium and Enteritidis have more broad host ranges (FDA, 2012). During the recent few decades, the three most common serotypes isolated from human source in the United States were Typhimurium, Enteritidis and Newport (CDC, 2011a).

Salmonella spp. are common pathogens which cause a broad spectrum of health problems in humans (Chiu *et al.*, 2004). Salmonellosis (i.e., *Salmonella* infection) is one of the most frequent foodborne diseases and is widely distributed in the U.S. Consuming an egg contains 10 to 20 colony forming units (CFU) was sufficient to cause human salmonellosis (Zhang *et al.*, 2011). Salmonellosis can be classified as nontyphoidal salmonellosis and typhoid fever (FDA, 2012). Typhoid fever is caused by *S.* Typhi, which has no known natural host except humans (FDA, 2012). Other serotypes are called nontyphoidal *Salmonella* that primarily transmit through foods, especially poultry meat and eggs (Painter *et al.*, 2007). Normally, the onset of disease symptoms occurs 6 - 72 hours (usually 12 - 36 hours) after ingestion of *Salmonella* through contaminated food, water, and direct contact with animal shedders, and illness can last 4 - 7 days (CDC, 2010). The symptoms of this foodborne disease are usually characterized by abdominal pain, acute onset of fever, diarrhea, nausea, and sometimes vomiting which can range from mild

to severe (WHO, 2013). In a few cases, *Salmonella* can invade sterile sites in human body, such as blood, cerebrospinal fluid, and joints causing severe disease (Barton *et al.*, 2011).

Nontyphoidal *Salmonella* spp. cause an estimated 1.03 million illnesses, 19,336 hospitalizations and 378 deaths in the U.S. annually (Scallan *et al.*, 2011). The Foodborne Diseases Active Surveillance Network (FoodNet) showed that in 2013, the incidence of *Salmonella* infections was 15.2 illnesses per 100,000 people, which was the highest among major foodborne pathogens (CDC, 2011a; CDC, 2014a). A foodborne disease outbreak can be interpreted as no less than 2 cases of a similar illness due to ingestion of a common food (CDC, 2013a). Between 2011 and 2012, 25% of a total 1,632 reported foodborne outbreaks were linked to *Salmonella* (CDC, 2013b). Children, elderly, and immunocompromised patients are the most susceptible to *Salmonella* infections (CDC, 2015a). According to FoodNet data, it was revealed that children less than five years old were the most susceptible to salmonellosis (CDC, 2014b). Generally, children with diarrheal illness are more likely to receive medical care than other groups which may have contributed to higher records of the salmonellosis incidence in this age group (CDC, 2013b).

Due to the major influence of *Salmonella* infections on public health, the U.S. Department of Health and Human Services (HHS) has set a nationwide goal of *Salmonella* incidence reduction by 25% by 2020 (HHS, 2011). Based on the CDC data, the overall incidence of salmonellosis has not declined in the past decade, while the serotypes have substantially fluctuated (CDC, 2011a). Thus, determining sources of *Salmonella* infections in addition to developing effective interventions is vital to reach the 25% targeted reduction in these infections (Jackson *et al.*, 2013).

Live poultry and poultry products continue to be primary reservoirs and vehicles for *Salmonella* spp. (Behravesh *et al.*, 2014). Outbreaks of human salmonellosis related to contact with infected poultry have been reported since 1955 (Pereira and Blaxland, 1955). Based on the FoodNet outbreak surveillance, between 1998 and 2008, 145 (23.5%) of a total 616 foodborne outbreaks (poultry as commodity) were related to *Salmonella* contamination in live poultry and poultry products (Gould *et al.*, 2013). It is important to be aware that *Salmonella* can spread through direct contact with live poultry. Public and animal health officials have investigated 45 outbreaks of human *Salmonella* infections linked to direct contact with live poultry from mail-order hatcheries from 1996 to 2012 (Behravesh *et al.*, 2014). Those outbreaks caused more than 1,581 illnesses, 221 hospitalizations, and 5 deaths (Behravesh *et al.*, 2014). The most recent outbreak in 2014 of *Salmonella* infections linked to live poultry in backyard flocks led to 363 infections in 43 states (CDC, 2014c). Since1984, *Salmonella* outbreaks related to poultry products have been reported and continued to occur almost every year in the U.S. (CDC, 2014d). During the past 3 years, 36.3% (12/33) of the *Salmonella* multistate outbreaks in this country were linked to consumption of poultry products (CDC, 2014c). These outbreaks highlight the need to focus our efforts on better understanding of the *Salmonella* epidemiology as well as developing strategies to control and prevent human salmonellosis associated with poultry contact and consumption from farm to fork (Loharikar *et al.*, 2012).

1.2. Turkey production system in the United States

Demand for turkey in the U.S. has been increasing since mid-1900s (USDA, 1985). In 1983, the annual turkey output was 3.3 billion pounds, which was 4 times higher than that in 1950 (817 million pounds) (USDA, 1985). Recent reports from the U.S. Poultry

Inventory showed that between 1990 and 2010, the annual turkey production was estimated at 5.9 billion pounds (USDA, 2012a; USDA, 2014a; USDA, 2014b). The fast-growing annual turkey meat consumption per capita in the U.S. from 6.1 pounds (in 1960) to 16.4 pounds (in 2012) (USDA, 1985; USDA, 2012a), was mirrored with changes to the poultry industry production system from backyard production to the integrator-grower production (i.e., vertical coordination) which dramatically increased turkey production (Hinrichs and Welsh, 2003; USDA, 2000).

The vertical coordination is the method widely used for poultry production industry. This method is the synchronization of a series stages of a poultry production and marketing system (Martinez, 2002). One type of the vertical coordination called the vertical integration has become the most common form of turkey production in the U.S. (USDA, 2002). It means poultry growers sign a production and marketing contract with integrators that requires them to raise birds owned and supplied by the integrators. Those integrators dictate unified production systems and supply grow out equipment, feedings, veterinarian services, and drugs (Allen *et al.*, 1998).

1.2.1. Breeding and growout stages

The hens (i.e., females) and toms (i.e., male turkeys) are selected for breeding when they reach 24 and 30 weeks, respectively (Frank *et al.*, 2010). Hens are fertilized by artificial insemination 3 times in the first two weeks of the breeding season to aid fertility, and then one time per week throughout a 28 to 30 weeks production period (Saint Jalme *et al.*, 1994). After copulation and laying eggs from breeding birds, fertilized eggs are collected, cleaned, sanitized via fumigation, UV light, spray or washing with sanitizer and shipped to the hatchery (Ernst, 2004). When eggs are hatched into poults, they are shipped

to specially designed barns to be raised into adult turkeys. The barns provide controlled environment that is monitored for temperature, light, air exchange, feed and water (Fanatico, 2007). Moreover, barns are designed with protection from predators, bad weather, and diseases (USDA, 1971).

Feed is formulated to match the growth stages of turkeys. Poults (< 8 weeks) are fed an all-mashed "starter" with no excess calcium supplement (USDA, 1971). As they grow older, the feed is altered to meet a proper balance of nutritional requirements (Forbes and Shariatmadari, 1994). For turkey broilers, they are fed complete, high-energy diet of mixed grains (ground corn, heavy oats and delactosed dried whey), soybean meal, dehydrated alfalfa meal, and vitamin-trace mineral mix (USDA, 1971). Normally, a small percentage (i.e.,6 to 8%) of animal by-products such as fishmeal and meat scrap is added into the feed as major protein content (USDA, 1971). Except for the production of organic turkeys, most ranchers add antibiotics (20g/ton), such as terramycin, aureomycin and bacitracin in turkey feed to improve feed efficiency and protect birds from microbial infections (USDA, 2011). Turkeys have free access to feed and water throughout the growout period (USDA, 1971).

In the U.S., administering hormones or steroids to poultry is banned. These chemicals have been prohibited for over half a century (Czarick and Fairchild, 2012). The superiorly selective breed, better feed formulation, controlled environment, and modern management practices are able to produce larger, more disease resistant turkeys compared to turkeys treated with growth hormones (Czarick and Fairchild, 2012).

1.2.2. Turkey processing and turkey products

When turkeys reach "harvest" time at 24 and 26 weeks for hens and toms, respectively, they are taken off feed 8 hours before slaughter (Doyle, 2013; USDA, 1971). This allows their digestive tracts to empty to reduce the potential for fecal contamination during processing (Doyle, 2013). At harvest, turkeys are caught at night by trained workers (i.e., catching crew) and placed in plastic or wooden transport cages. Birds are then transported to the processing plant by trucks with sufficient ventilation (EFSA, 2004; USDA, 2008a). Upon arriving at the processing plant, birds are removed from the cages and hung up by both legs on continuously moving shackles. Hung turkeys are usually stunned by running their heads through a water bath that is charged with an electric current (USDA, 1971). Stunning causes unconsciousness, but it does not kill the birds (USDA, 2008a). After that, stunned turkeys are killed either by a mechanical rotary knife that cuts through the jugular veins and the carotid arteries of the neck (USDA, 1971). The carcasses are permitted to bleed for around 30 seconds. Following bleeding, the birds go through scalding tanks. These tanks contain hot water (59 to 63°C, pH 9.0) that softens the skin so that the feathers can be removed easier by the pickers (Humphrey and Lanning, 1987; Trampel *et al.*, 2000). The scalding often lasts 1 to 2 minutes (USDA, 2008a).

The carcasses then go through feather-picking machines, which are equipped with rubber "fingers" designed to remove the feathers (Singh and Heldman, 2009). A spray wash is applied on defeathered carcasses prior to evisceration (USDA, 2008a). As carcasses move downstream, heads and legs of the birds are removed by rotary knifes. Thereafter, carcasses are rehung by their hocks onto the eviscerating shackle line. The U.S.

regulations state that scalding and defeathering steps must be separated by a physical wall from the evisceration steps to minimize potential cross-contamination (USDA, 2010).

At evisceration, the oil gland is taken out from the tail and the vent is opened so that the viscera (internal organs) can be removed (Singh and Heldman, 2009; USDA, 2008a). Although broiler processing line is automated, evisceration of turkey carcasses is still performed by hand (Owens *et al.*, 2000). Viscera are removed and placed on one side of the bird. The carcasses are further rinsed via 20 ppm chlorinated water (NCC, 1992; USDA, 2008a). When viscera are separated from the carcasses, edible organs such as heart, liver, and stomach are taken and processed independently (NCC, 1992; USDA, 2008a). The rejected parts, such as trachea, esophagus, and crop are generally dyed (blue-purple) and placed in a container marked "inedible", in order to prevent possible mixing with edible parts (USDA, 2008a). The lungs and kidneys are removed from the other visceral organs using a vacuum pipe (Owens *et al.*, 2000). The carcasses are then washed thoroughly with a sanitizer (e.g.,20 ppm free available chlorine) (USDA, 2008a). Inspections, such as FSIS testing and plant microbial testing, are carried out by the USDA inspectors at this point, in order to inspect the safety and wholesomeness of poultry products and verify good commercial practices throughout slaughter process (USDA, 2013a; USDA, 2014c). Research revealed that chlorine can decrease *Salmonella* load by 30% after the evisceration procedure (Notermans *et al.*, 1980).

After the carcasses have been washed, they are chilled to a temperature below 4.4 °C through water immersion chilling treated with an antimicrobial with a high flow rate (> 1 gallon per bird; pH 6 - 6.5) that can inhibit *Salmonella* growth, reduce the *Salmonella* loads on carcasses as well as cross contamination (USDA, 2008a). Water immersion

chilling is widely used in North America poultry production (James *et al.*, 2006). It includes a pre-chilling step (10 - 15 min) to cool down the carcasses via a countercurrent flow of cold water (7 - 12 °C) (Owens *et al.*, 2000). The carcasses with temperature between 30 °C and 35 °C are then moved into a larger chiller designed with a countercurrent flow and a high flow rate to wash the carcasses for 45 - 115 minutes at 4 °C (Owens *et al.*, 2000). The chilling time depends on the size of the carcasses (Owens *et al.*, 2000). The specified overflow of water with antimicrobial treatment in each tank is a requirement by FDA (2013). For chlorine-based antimicrobials, chlorine, chlorine dioxide and acidified sodium chlorite are widely used in poultry processing plants because of their good water solubility and the enhanced antibacterial functions when dissolved in aqueous solution under the certain pressure (USDA, 2008a). Beside these antimicrobials, lactic acid is also commonly utilized in water chilling. This organic acid with low pH can decrease the *Salmonella* load effectively (USDA, 2008a).

Compared with water chilling, air chilling is less used in the U.S. (Owens *et al.*, 2000). In air chilling, carcasses are hung by shackles and moved through chambers with rapidly moving cold air (-8 °C to -10 °C) for 1 to 3 hours (Veerkamp, 1989). Air chilling is less energy-efficient than water chilling, and birds lose weight due to loss of moisture (Owens *et al.*, 2000). However, air chilling reduces cross contamination between birds since the carcasses are more separated from each other (Owens *et al.*, 2000). Sanchez *et al*. (2002) compared the prevalence of *Salmonella* spp. contamination in broilers after air chilling treatment to that in water chilled broilers. The authors concluded the former treatment led to significantly lower *Salmonella* prevalence than the latter (Sanchez *et al.*,

2002). It was suggested that the occurrence of cross contamination may be more common for broilers during water chilling (Sanchez *et al.*, 2002)

Whole poultry carcasses may be further processed into parts such as wings, backs, necks, drumsticks, thighs, and breasts. These parts (skin-on or skinless) can be sold as is or used for ground poultry products. Ground turkey is one of the most popular turkey products that had the largest growth of consumption in the last 10 years (NTF, 2013). This product is considered a lean source of protein due to it is high protein and low fat content (NTF, 2013). Ground turkey is classified mainly into two types: mechanically separated and non-mechanically ground product. Mechanically separated turkey is produced by grinding bone-in turkey parts like backs, necks, and wings. The crushed meat and bones are continuously pressed against a screen and the edible soft materials are pushed through a metal screen. The resulting minced product has a paste like texture and used generally for cooked products like frankfurters and bologna. Non-mechanically separated ground turkey is produced using skin-on/skinless and boneless parts such as drumstick, thigh, and breast (bones are removed prior to grinding). Skin is normally used in ground turkey as a source of fat. Due to the relatively higher blubbery and spongy fat content and pathogen prevalence, neck skin is not recommended to be used in ground turkey production (USDA, 2008a).

The final temperature of the fresh turkey products before shipment is usually about -5.6 to -4.4 ℃ (22 to 24 °F), just above the freezing point for turkey products. For frozen turkey, the temperature needs to be controlled below -17.8 ℃ (0 °F) and vacuum packaging is required (USDA, 1971). Poultry package is required to be functionalized as

the barrier and sealing agents to protect poultry products from contamination and spoilage (Levine *et al.*, 2001; Owens *et al.*, 2000).

1.3. *Salmonella* contamination in turkey production system (farm-to-processing)

Salmonella is frequently isolated from poultry production systems; during pre-harvest, at harvest, and post-harvest (Bryan and Doyle, 1995; Myint *et al.*, 2004). There is not enough data in the current literature to determine which stage of the production continuum is most directly associated with human salmonellosis (Myint *et al.*, 2004). However, the literature did reveal that the first step of *Salmonella* contamination pathway in the poultry product is infected flocks (Myint *et al.*, 2004). In general, infected birds entering the processing plant and contamination during processing can result in *Salmonella* contamination of finished poultry products (Seligmann and Lapinsky, 1970). Although the number of publications on this pathogen in turkey is low compared to those in broilers, the potential *Salmonella* transmission routes in turkey and broiler production systems are similar (EFSA, 2012; Nde *et al.*, 2007).

1.3.1. Transmission of *Salmonella* in turkey farms

Salmonella contamination routes in turkey production can be classified into vertical and horizontal transmission.

1.3.1.1. Vertical transmission

Salmonella can be transmitted vertically from parents to progeny through internally contaminated eggs or penetration into the forming eggs (Gast and Beard, 1990). The contamination from parents to offspring via internally contaminated eggs is called true vertical transmission or transovarian transmission (EFSA, 2009). This pathway results from *Salmonella* colonization in reproductive organs (ovary or oviduct) or penetration into

the forming eggs, within the body of the hen when it passes down the oviduct and is voided from the cloacae (Keller *et al.*, 1995). Systemic infections of breeding hens with *Salmonella* can contribute to the infection of the reproductive tract, both in experimental and field studies (Cox *et al.*, 2000; EFSA, 2009). To some extent, the shell of hatching eggs from flocks infected with *Salmonella* serves as a barrier to avoid a low level of *Salmonella* invasion (EFSA, 2009). However, when *Salmonella* load is high in hens' upper and/or lower oviduct, the risk of egg contamination probably increases dramatically (Cox *et al.*, 2000). It is reported that *Salmonella* can invade the egg shell, permeate into the vitelline membrane and colonize in the egg content (yolk) with rapid proliferation at warm temperature (Gast *et al.*, 2007).

According to the USDA (2005), most cases of foodborne salmonellosis in the U.S. were associated with the consumption of shell eggs contaminated with *Salmonella* cells that were transferred via vertical transmission. Some particular serovars, including Enteritidis, Typhimurium, Heidelberg, Kentucky and Senftenberg are more commonly found in internal content of fertilized eggs (Byrd *et al.*, 1998). Further experiments showed that these serotypes can be more consistently traced back from breeders to broiler carcasses than are other serovars (Byrd *et al.*, 1998; Kim *et al.*, 2007; Liljebjelke *et al.*, 2005).

1.3.1.2. Pseudo-vertical transmission

The other form of vertical transmission route via externally contaminated eggs is called pseudo-vertical transmission. It is widely believed that egg shells can be contaminated by feces on egg belts, in nest boxes or by handling workers and equipment. Research has demonstrated that the pressure gradient forms during cooling of the egg from body temperature may cause *Salmonella* to enter intact or cracked eggs (Gantois *et al.*,

2009). At hatcheries, eggs are incubated under mild temperature and humidity conditions. This environment leads to rapid multiplication of *Salmonella* inside eggs resulting in hatchery-acquired infection of hatched birds (Gantois *et al.*, 2009). Padron (1990) used several methods to mimic *Salmonella* contamination routes in eggs, such as spraying *Salmonella* solution over *Salmonella* contaminated litter. It turned out that all operations made *Salmonella* penetrate into laid eggs successfully. Thus, systemic infection of breeding hens is not the only route for internally infected eggs. Under the above circumstances, pseudo-vertical transmission showed a similar outcome to true vertical transmission in terms of *Salmonella* infection of fertilized eggs.

1.3.1.3. Horizontal transmission

Horizontal transmission is referred to as transmission of *Salmonella* within and between flocks (EFSA, 2009). *Salmonella* can persist in poultry farm environment and survive for a long time. For instance, this pathogen can survive for more than one year in unused, disinfected poultry houses, and more than two years in poultry feeds (EFSA, 2009). Feed, drinking water, litter, wild animals, rodents, insects, human visitors, and shared equipment are all risk factors for *Salmonella* introduction to breeding stocks and fatting flocks (EFSA, 2009). Areas usually contaminated include feed hoppers and pipes, and ventilation systems (Davies *et al.*, 1998; Davies *et al.*, 1997; Davies and Wray, 1996). Research indicated that disinfection and cleaning often fail to eradicate contamination, even in areas (such as egg handling and storage) perceived to be “clean” (Heyndrickx *et al.*, 2002; Kim *et al.*, 2007). The horizontal transmission at production stage (i.e., breeding flocks, hatchery, grow out, and transportation) at the preharvest level will be discussed next.

1.3.1.3.1. *Salmonella* transmission in breeding flocks

Introduction of *Salmonella* through infected breeders is considered as the upstream of hatchery contamination (Davies and Breslin, 2004; Mueller-Doblies *et al.*, 2013). Once a breeding flock is infected with *Salmonella*, it can be very difficult to eliminate the bacteria from the production and they can spread to other units through horizontal transmission (Corkish *et al.*, 1994; Davies and Breslin, 2004; Liebana *et al.*, 2003). In some cases, breeding birds bought from other rearing farms are prone to importing *Salmonella* infection to the original resident turkeys (Liebana *et al.*, 2002).

1.3.1.3.2. *Salmonella* transmission in hatchery

It is a general notion that the hatchery is another major source of *Salmonella* horizontal transmission. Research has demonstrated that raw poultry products contaminated with *Salmonella* can be traced back to cross contamination of infected eggs and baby chicks in the hatchery (Cox *et al.*, 1990; Cox *et al.*, 1991; Cox *et al.*, 2000). Moreover, a study has revealed that greater than 80% of the chicks around the eggs inoculated with a marker strain of *Salmonella* can be contaminated (Cason *et al.*, 1994). The poor sanitary environment (dust and bioaerosol) for fertilized eggs on arrival to the hatchery incubator is proved to be a control point for enteropathogen spreading to other areas in the hatchery (Mitchell *et al.*, 2002; Russell, 2010). Also, dust carried by airflow can settle on uninfected eggs shells, and is then assumed to follow a fecal–oral route of transmission when hatchlings peck these contaminated egg shells (Kallapura *et al.*, 2014). Other areas of hatcheries that have been identified as being prone to persistent *Salmonella* contamination include incubator door seals, egg transfer machines, egg tray and hatchery/delivery basket washers, chick handling areas and waste processing areas (Davies

and Breslin, 2004; Davies *et al.*, 2003; Kim *et al.*, 2007). Some extrinsic factors, such as temperature, moisture, and storage conditions, were identified as important factors to the trans-shell contamination during the hatchery process (Messens *et al.*, 2005).

1.3.1.3.3. *Salmonella* transmission in growout

Growout is another production stage where *Salmonella* infection and colonization of birds is common, even if the new flock introduced to the rearing house was "*Salmonella* negative" (Russell, 2010). There are several factors that contribute to the horizontal transmission of *Salmonella* during growout. These factors can be summarized as short environmental stresses, contamination by pests (insects, rodents) and contaminated litter.

Short environmental stresses including molting and infection with other pathogens, were common catalysts leading to enhanced *Salmonella* infection susceptibility and an increase of *Salmonella* excretion for birds in the growout stage (Rostagno, 2009). During molting, birds become more susceptible to the stresses, such as food deficiency and nutrient insufficiency. The stresses can induce reduced immunity, delayed gut maturation and increased susceptibility to pathogens (Uni *et al.*, 2003; Yi *et al.*, 2005). Research has shown that molted birds have more severe inflammation in ceca and colon during a forced molt as compared to unmolted birds (Kretzschmar-McCluskey *et al.*, 2008). During this time, most hungry birds search for food on the floor, which may be contaminated (Byrd *et al.*, 2001). Byrd *et al*. (2001) revealed that many birds entering the processing plant had high levels of *Salmonella* in their crops because of the litter pecking. Molting also aggravates the risk of cross contamination within the flock. Therefore, elimination of

Salmonella during growout via clean-up and decontamination of the rearing facility can be difficult.

Insects, especially non-biting flies, and mice are significant vectors and amplifiers of *Salmonella* contamination of rearing house and fattening flocks (Barrow and Ulrich, 2013; Holt, 1993). Featherstone and his colleagues (2010) found that the presence of mice on farms led to higher percentage of *S.* Typhimurium infection in turkey flocks (Featherstone *et al.*, 2010). Dead mice present at turkey house may lead to the greater risk of *Salmonella* transmission since they may contain higher levels of this organism than mice droppings (Featherstone *et al.*, 2010). If the mice bodies are pecked by mature turkeys, *Salmonella* contamination may be spread in the whole rearing house (Hoover *et al.*, 1997; Käsbohrer *et al.*, 2013).

The litter used for multiple consecutive poultry flocks might lead to *Salmonella* colonization in the bird gut (Kallapura *et al.*, 2014; Williams *et al.*, 2012). If *Salmonella* has been detected in the litter of the previously contaminated flock, the new flock raised on the same litter may be infected with *Salmonella* by multiple exposures to this organism through the growout period (Cardinale *et al.*, 2004; Rose N. *et al.*, 2003). Hafez (2010) has shown that *Salmonella* can survive for more than 9 months in the litter after the positive flock are moved out of the house. Also, it is possible that the new flock is infected by bioaerosols formed in the rearing house (Kallapura *et al.*, 2014).

As mentioned earlier, feed withdrawal for mature turkeys before processing is recommended to reduce fecal contamination of carcasses (Northcutt, 2009). The feed withdrawal time needs to be controlled efficiently. Removing feed too late may result in more carcass contamination because the gut will not empty properly and is likely to

rupture during evisceration. If feed is removed too early, the gut could become more fragile. One study showed that feed withdrawal periods greater than 14 hours results in a weaken intestine and gall bladder of birds (Northcutt, 2009). Weakness increases the propensity for them to tear during evisceration.

1.3.1.3.4. *Salmonella* transmission during transportation

Poultry transportation is considered another source of stress risk factor for *Salmonella* dissemination and infection in birds (Kallapura *et al.*, 2014; Volkova *et al.*, 2010). Birds transported in crates are often stressed especially with absence of water and feed. This may lead birds to reduce or stop excretion. Bird's intestine that is not empty is considered a risk factor for carcass cross-contamination during processing. Also, shedding of *Salmonella* by contaminated transport equipment, such as crates and trucks, could results in higher levels of secondary contamination on the birds feathers and consequently carcasses (Volkova *et al.*, 2010).

1.3.2. Transmission of *Salmonella* in turkey processing and products

Salmonella contamination in poultry products at the processing plant is primarily due to: 1) incoming pathogen loads on birds at receiving, and 2) cross contamination of carcasses physical contact during processing because of improper cleaning and disinfection of processing lines, improper chilling, scalding and storage temperatures, poor worker hygiene and infestation with rodents and insects (Lillard, 1990; Trampel *et al.*, 2000). *Salmonella* have been isolated from water, equipment, and carcasses at processing plants (Trampel *et al.*, 2000).

Salmonella in the crops of poultry may spread from carcass to carcass during the crop removal process (Hargis *et al.*, 1995). Studies conducted by Byrd *et al*. (2001)

concluded that crop contents with fluorescent dye transferred to the inside and outside of the whole carcass and were clearly visualized under a black light during processing. Therefore, the commercial croppers may cause a large amount of contamination of the inside and outside of the carcasses.

If the chilling of poultry carcasses is performed properly, microbial populations in carcasses should be reduced (USDA, 2010). However, in many cases, the chilling step might not reduce *Salmonella* contamination and cross contamination. This is due to ineffective concentration of antimicrobials in chilling water, insufficient exposure time to reduce the carcass temperature below 4 °C, and issues with the countercurrent flow of water. As for air chilling, the incidence of such problems is lower (Sanchez *et al.*, 2002). However, if a carcass has a high load of *Salmonella*, the pathogen will remain on the bird and may spread to other carcasses through water droplets (Mead *et al.*, 2000; USDA, 2010).

1.3.2.1. *Salmonella* presence in poultry skin

Research has demonstrated that poultry skin is one of the common surfaces for bacteria attachment; including *Salmonella* (Firstenberg-eden, 1981; Firstenberg-eden *et al.*, 1978). The factors impacting *Salmonella* presence in poultry skin include direct contamination at the poultry farms and cross-contamination during transportation and processing. When birds are raised on farm, their feathers and skin are exposed to *Salmonella* from contaminated litter, feed, water, and/or dust (Featherstone *et al.*, 2010). During transportation, cross-contamination of turkey feathers and skin may occur if the cages are contaminated (Volkova *et al.*, 2010). Slaughtering is a major step that contributes to skin contamination. Although stunning by an electric current is a very brief process to

calm the birds, it can cause excitement to some birds, causing uncontrolled urination and defecation, which might lead to cross contamination on carcasses (Panel, 2012).

Scalding water opens up the feather follicles, which can result in *Salmonella* cells entering the pores (i.e., follicles) (USDA, 2010). After feather picking, feather follicles shrink as carcasses cool downstream, leading to *Salmonella* entrapment (Russell, 2009). Once entrapped inside the feather follicles, *Salmonella* cells are hard to be washed off and can become inaccessible to disinfectants (Kim *et al.*, 1996; Lillard, 1993). Further experiments indicate that higher temperature in the scalding tank makes the carcasses become oily, leading to easier *Salmonella* attachment to the surface of the skin (USDA, 2010). Incomplete sanitation of processing facilities could cause the formation of *Salmonella* biofilm with extracellular matrix (Oscar, 2008). The biofilm can bind to the surface of equipment firmly and lead to continuous source of water contamination (Oscar, 2008; Thomas *et al.*, 1987).

In a previous study from our research group, we found out that *Salmonella* prevalence of stomached neck skin samples (21%, n=299) was significantly higher than that of rinsed samples (2.3%, n=299), which suggested that *Salmonella* is widely present inside chicken neck skin (Wu *et al.*, 2014). Another study showed similar results; 23 % (n=45) of 198 neck skin samples collected at post-chill were *Salmonella* positive (Whyte *et al.*, 2002). Based on the USDA (2012b) data, the estimated national prevalence of *Salmonella* in chicken parts was 24%, which is close to *Salmonella* prevalence from chicken neck skin. Interestingly, in a study conducted by Cox *et al*. (2010), authors suggested that *Salmonella* presence from chicken neck skin may represent contamination status of the whole carcass. As for turkey neck skin, research revealed that *Salmonella*

prevalence from the samples collected at post-evisceration was 42% (n=300) (Cui *et al.*, 2014); this indicates that the inclusion of neck skin in ground turkey production can be a significant contamination source.

Why poultry neck skin is frequently contaminated? The primary contributing factor is related to the manner of processing; carcasses normally hang downward during processing, and fluid from the washing process drips down through the neck skin (Cui *et al.*, 2014; Wu *et al.*, 2014). Kim *et al.* (1996) used confocal scanning laser microscopy to image the surface of the chicken breast skin that was artificially inoculated with the *S.* Typhimurium. Findings indicated that *Salmonella* cells that were attached on the flat part of the skin could be rinsed off easily, but cells presented in the crevices or trapped inside feather follicles remained after washing (Kim *et al.*, 1996). Therefore, the firm attachment and deep entrapment of *Salmonella* in the neck skin may be attributable to the crumpled skin structure and large size of feather pores (Kim *et al.*, 1996). These finding may also help explain why *Salmonella* in neck skin is hard to be inactivated and may contribute to ground poultry contamination when added in as a source of fat. Although neck skin is removed and rendered during ground turkey production, some neck skin leftover pieces might stay intact with the turkey breast skin (Cui *et al.*, 2014). In this way, *Salmonella* levels in neck skin can provide information on potential cross contamination of ground products.

Although turkey neck skin is generally not used in ground production and is being rendered for other purposes, other skin parts (e.g., drumsticks, thighs, and wings) are commonly used as sources of fat in ground turkey products. There is little research available in the literature about *Salmonella* presence and numbers from these skin parts.

1.4. *Salmonella* control in turkey production system

Even though complete eradication of *Salmonella* in turkey processing and products may be impossible, control and prevention of contamination below certain minimum levels is achievable. Food safety systems require control measures implemented at all stages of the food supply chain including production, processing, manufacturing, distribution and preparation of foods at food services and at home. Many systematic preventative approaches such as a supplier management program, Good Manufacturing Practices (GMPs), Hazard Analysis, and Critical Control Points (HACCP) are established and applied to ensure safety of turkey products.

Mint *et al*. (2004) suggested three necessary practices to reduce the risk of human salmonellosis related to the consumption of poultry products. First, have a better understanding of the *Salmonella* serotypes detected from poultry that are responsible for human *Salmonella* infection in order to prevent them from further spreading. Second, develop and validate the detection strategies with higher sensitivity, accuracy and efficiency, in order to identify sources of contamination during pre-harvest, post-harvest and consumption. Third, design and conduct cost-effective prevention and control strategies from farm to fork.

The fact that processing plants have not been able to further reduce the incidence of *Salmonella* in poultry products promote the need for reducing infection and contamination levels in live birds with *Salmonella* before being dispatched to processing plants (USDA, 2008a). The measures taken at this stage are called pre-harvest controls. In order to determine the impact of *Salmonella* prevalence of breeding flocks on their offspring (i.e., fatting flocks), it is necessary to identify *Salmonella* serovars in the two turkey flocks (i.e.,

breeder and fattening turkeys) and compare the results (EFSA, 2012). Research indicated that in some cases there was an overlap between the serovars isolated in breeding flocks and those isolated in fatting flocks; however, some serovars isolated in fattening flocks were not detected in breeding flocks (EFSA, 2012). Thus, other sources of infection are suspected to cause contamination of fatting flocks.

At the early stage, *Salmonella* contaminated eggs are usually sanitized using a variety of methods and agents including formaldehyde based products, chlorine, hydrogen peroxide quaternary ammonium compounds, and polyhexamethylenebiguanide (PHMB) (Berrang and Bailey, 2009). Although these sanitizers can achieve a high kill percentage of *Salmonella*, they can reduce hatchability of eggs. Thus, the usage of these sanitizers is constrained (EFSA, 2009). In Europe, the Health and Safety Policies conducted by poultry companies require the use of less noxious disinfectants, such as amphoteric surfactants, peroxygens or quaternary ammonium compounds which can be inactivated more easily by residual organic matter (EFSA, 2009; Mitchell and Waltman, 2003). Additionally, the disinfectants with low concentration may not be able to inactivate *Salmonella* in hatchery baskets, delivery baskets, and egg trays, which may shed a risk of egg contamination (Davies *et al.*, 2001).

Besides egg sanitation and hatchery, pre-harvest control also consists of monitoring *Salmonella* at the flock level and implementing *Salmonella* reduction measures in infected flocks, which includes pests control, feeding strategy, and strict control (i.e., biosecurity measures) of *Salmonella* in the breeder and growing-finishing poultry supply chain (USDA, 1996). Under these circumstances, efforts to prevent contamination at the source are considered important (USDA, 1996).

For post-harvest process, there are many critical control points including temperature controls (washing treatments and products), chemical interventions, and counter-flow technology in scalders and chillers. Also, the mandatory inspection requirements and increasingly efficient transportation contribute to fresher and safer turkey products (Velhner *et al.*, 2005). The USDA administrators of federal inspection regulate that turkeys and turkey products must be from healthy birds that were handled or processed under strict sanitary conditions; they must be faithfully labeled and not be adulterated (USDA, 2013b). The label includes turkey grading, USDA contract acceptance and certification based on pre-evaluation of several suppliers' turkey products (NTF, 2015). Transportation and food handling at retail outlets and by consumers, like storage at the proper temperature and adequate cooking, are the final critical control points in the farm-to-table continuum (White *et al.*, 1997).

In 2014, FSIS (2015a) updated the performance standards for *Salmonella* in Not-Ready-To-Eat (NRTE) turkey carcasses and ground turkey. The new regulation is for achieving at least a 30% reduction of *Salmonella* illnesses caused by NRTE poultry products (USDA, 2015a). Moreover, the 2015 risk assessment has been conducted by FSIS to determine the relationship between the reduced incidence of *Salmonella* in NRTE turkey products and the predicted illnesses of salmonellosis (USDA, 2015a). The minimum number of samples detected at each visit to the processing plant was determined to be 14 and 10 for turkey carcasses and comminuted turkey, respectively. The *Salmonella* prevalence in turkey carcasses should be < 7.1% (i.e., no more than 4 of 56). As for ground turkey, the maximum acceptable percent of *Salmonella* positive is 13.5% (i.e., no more than 7 of 52) (USDA, 2015a)

1.5. Conclusion

Salmonella contamination in turkeys and turkey products poses a foodborne disease risk. *Salmonella* vertical and horizontal transmission routes during turkey production and processing contribute to potential contamination of finished turkey products. Even though *Salmonella* prevalence in turkey and turkey products has been significantly reduced over the years, outbreaks related to turkey products still occur. The 2011 *S.* Heidelberg multistate outbreak revealed the severer problem of *Salmonella* contamination linked to ground turkey (CDC, 2011b). Intervention practices conducted at turkey processing plants have been effective to reduce *Salmonella* levels on carcasses. However, in ground products, *Salmonella* prevalence is relatively higher. According to the USDA progress report from June 2013 to March 2014, *Salmonella* prevalence in NRTE ground turkey was 20.75%, which was about eleven times higher than that in NRTE turkey carcasses and carcass parts (i.e., 1.85%; 30 out of 1630 samples) (USDA, 2014c). Therefore, identifying potential sources and predictors of *Salmonella* in ground turkey is important to the turkey industry for controlling and preventing contamination, and releasing ground turkey into commerce.

The increase of ground turkey sample size from 25 g to 325 g by USDA (2015a) is anticipated to increase the likelihood of detecting *Salmonella*. It is important for the turkey industry to better understand the relationship of *Salmonella* contamination between ground turkey and its raw ingredients. For raw ground turkey production, various types of turkey meat, along with skin as a source of fat, are used. The USDA (2013b) allows skin to be utilized in ground turkey production. It is well-known that *Salmonella* is present on turkey skin. Therefore, skin can serve as a route of *Salmonella* entry to ground products. A

previous study showed that *Salmonella* prevalence associated with turkey neck skin (i.e., external contamination) samples collected post-evisceration was much higher than that in spleen and bone marrow (i.e., internal contamination/infection) (Cui *et al.*, 2014). The turkey industry is currently minimizing the use of neck skin as source of fat in ground products. However, there is very limited research about *Salmonella* levels in other skin parts that are currently utilized in ground production. Common skin parts used as sources of fat in ground turkey production include drumsticks, thighs, and wings. Because of the different locations and characteristics of these skin parts of a turkey carcass, we expect variation of *Salmonella* contamination levels.

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CHAPTER 2

SALMONELLA LEVELS FROM TURKEY SKIN

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Abstract

The objective of this study was to determine *Salmonella* levels (presence and numbers) associated with the skin of turkey parts (i.e., drumstick, thigh, and wing). In collaboration with a commercial turkey processor, a total of 20 turkey flocks expected to be highly contaminated with *Salmonella* were sampled. From each flock, 15 samples per part type were collected post-chill and tested for *Salmonella* using the most probable number (MPN) and enrichment methods. Overall *Salmonella* prevalence associated with the skin of drumsticks, thighs, and wings was 13.7%, 19.7% and 25.0%, respectively. *Salmonella* prevalence was significantly higher ($P< 0.05$) from wing skin than that from drumstick skin, but the difference was not significant ($P> 0.05$) when compared to thigh skin. The odds ratio of *Salmonella* presence from thigh skin (odds ratio = 2.4; $P< 0.05$) was significantly higher when this pathogen was also found with wing skin. *Salmonella* mean numbers (logMPN/sample) from skin samples for the three parts were 1.18 logs (drumstick), 1.29 logs (thigh), 1.45 logs (wing) and were not significantly ($P> 0.05$) different. Our findings suggest the high prevalence of *Salmonella* associated with wing skin could be a potential source for ground turkey contamination.

2.1. Introduction

Salmonella spp. are considered one of the most common causes of foodborne disease in the United States. It is estimated more than 1.03 million illnesses, 19,336 hospitalizations, and 378 deaths in the U.S. occur annually due to *Salmonella* spp. infections from 2000 to 2008 (Scallan *et al.*, 2011).The incidence of salmonellosis in this country has not changed significantly in the past decade, while the *Salmonella* serotypes responsible for the illnesses have fluctuated somewhat (CDC, 2011a). Additionally, the number of foodborne outbreaks linked to *Salmonella* has increased over the past five years (CDC, 2014d; CDC, 2015b). Poultry products continue to serve as a source for *Salmonella* infections. Between 1998 and 2008, 23.5% of 616 foodborne outbreaks (poultry as commodity) were related to the direct contact of *Salmonella* infected live poultry and consumption of *Salmonella* contaminated poultry products in the U.S. (Gould *et al.*, 2013).

The U.S. is the largest turkey producing country in the world (USDA, 2014d). Turkey production has rapidly increased during the recent few decades (USDA, 2014a). Based on the USDA data, turkey production in 2012 was estimated at 7.5 billion pounds, which was 4.2 times more than the 1.75 billion pounds produced in 1960 (USDA, 2014a). This increase has satisfied the fast-growing annual turkey meat consumption per capita from 6.1 pounds (in 1960) to 16.4 pounds (in 2012) (USDA, 1985; USDA, 2012a). With the greater amount of turkey consumption greater emphasizes should be placed on controlling *Salmonella* in turkey products.

Of the turkey products, ground turkey, has been linked to seven salmonellosis outbreaks between 2008 and 2013 recorded by the CDC (CDC, 2014d). In 2011, a multi-

state foodborne outbreak of *S.* Heidelberg infections linked to ground turkey occurred with 136 cases reported in 34 states (CDC, 2011b). Thirty-nine percent of the illnesses were hospitalized, and one death was reported (CDC, 2011b). This outbreak led to a recall of approximately 36 million pounds of ground turkey products (CDC, 2011b). Intervention practices at turkey processing plants designed to reduce *Salmonella* presence on turkey carcasses have been effective (i.e., 1.8%). However, when it comes to raw ground turkey, *Salmonella* prevalence is still relatively higher (i.e., 20.75%) (USDA, 2014e). Therefore, identifying potential sources of *Salmonella* dissemination to ground turkey is critical to the turkey industry for better control and prevention of turkey product contamination.

Various types of turkey meat along with skin are used in raw ground turkey production. Turkey skin is used in the production as a source of fat in the finished ground products. It has been reported that *Salmonella* is frequently present on turkey skin (Cui *et al.*, 2014; Kim *et al.*, 1996). When contaminated turkey skin is used in ground turkey production, it may serve as a source of *Salmonella* entry to the ground products (Cui *et al.*, 2014). Previous studies revealed that *Salmonella* prevalence and numbers from stomached turkey neck skin samples (n=300) collected pre-chill were 42% and 2.4 (mean log MPN/carcass), respectively (Cui *et al.*, 2014). The authors concluded that *Salmonella* presence at higher levels from neck skin may indicate that the flock has greater probability for *Salmonella* presence in the raw ground turkey (Cui *et al.*, 2014). In another study conducted at a chicken processing plant, *Salmonella* prevalence from stomached chicken neck skin samples (n=299) collected post-chill was 21% compared to 2.3% in rinsed neck skin samples. This may indicate that *Salmonella* was more often firmly attached and/or embedded inside chicken neck skin vs. loose attachment on the skin surface (Wu *et al.*,

2014). Although the use of turkey neck skin as a source of fat is currently minimal, other skin parts (e.g., drumsticks, thighs, and wings) are commonly used in ground turkey production. There is very limited research about *Salmonella* levels (prevalence and numbers) from turkey skin parts that are currently used in ground turkey production. Due to the difference in locations and characteristics of turkey skin of drumsticks, thighs and wings, we hypothesized that levels of contamination will vary by type.

The objective of this study was to determine the prevalence and numbers of *Salmonella* associated with the skin of turkey drumsticks, thighs and wings collected post-chill at a commercial turkey processing plant.

2.2. Materials and Methods

Sample collection. A cross-sectional study was conducted between June 2014 and March 2015 in cooperation with a commercial turkey production company. Three turkey parts: drumstick, thigh, and wing were sampled post-chill from 20 flocks at one processing plant. There was no certain collection frequency for the 20 flocks. Three hundred samples of each turkey part were collected over the study period. For each sample collection interval, 15 turkey carcasses per flock (five carcasses every 30 min) were randomly removed at the exit of the chiller. One part (a drumstick, a thigh, and a wing) from the right half of each carcass was aseptically removed and individually placed in sterile sampling bags (Labplas, Twirl'EM, Ste-Julie, QC, Canada) with corresponding labels. Knifes used for cutting off turkey parts were sanitized with 70% ethanol before harvesting each sample. All samples were placed in coolers with ice packs and shipped promptly overnight to the laboratory (Center for Food Safety, University of Georgia) for *Salmonella* analysis.

Turkey flock selection. The selection of turkey flocks in this study was based on *Salmonella* contamination data from boot-sock testing of the turkey houses as part of the food safety preventive control measures taken by the cooperative turkey company. A turkey flock identified with 3 or 4 *Salmonella* positive boot-sock samples out of 4 samples total per house was classified as 'suspected' highly contaminated flock. This classification was based on the company internal findings. The 'suspected' highly contaminated flock was then processed for sample collection for our study. We hypothesized that turkey skin parts from these flocks would have higher levels of *Salmonella* contamination to be able to detect differences (if present) by part.

Sample preparation. Upon arrival to the laboratory, samples were immediately processed for *Salmonella* analysis. Detailed information of each flock was recorded and logged. As much of the skin as could be removed from the turkey parts (i.e., drumstick, thigh and wing) was stripped off aseptically using scissors, bagged individually in sterile bags (Nasco, Whirl-Pak, Fort Atkinson, WI), and weighted. Scissors were sanitized by 70% ethanol and wiped down with sanitized paper towels between samples. Gloves and sterilized aluminum foil paper used to handle each sample were switched to new ones to avoid cross-contamination. Three hundred milliliters of buffered peptone water (BPW; Difco, Becton Dickenson, Sparks, MD) containing 0.05% Tween 80 (BDH, West Chester, PA) was added to each skin sample and then stomached at high speed for two min (Stomacher 400,Seward Ltd, London, England). The stomached solution was used for *Salmonella* analysis.

***Salmonella* quantitative and qualitative analysis.** The 3-tube 3-dilution most probable number (MPN) method was used to quantify *Salmonella* numbers according to

the USDA, Food Safety and Inspection Service (FSIS) protocol (USDA, 2008b). Additionally, primary (24 h) enrichment and delayed secondary (5 days) enrichment were used to determine the presence of *Salmonella* (USDA, 2013c).

Most probable number method to quantify *Salmonella*. From each sample solution, 9 tubes were used for the pre-enrichment of *Salmonella* with the first three tube-set containing 10 mL of the 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 °C for 24 h. A portion (0.5 mL) of the pre-enrichment culture of each tube was transferred to 10 mL tetrathionate broth (TT; Difco, BD) and then incubated at 42 °C for 24 h. After incubation, a loopful of each TT culture was streaked onto xylose lysine tergito 4 (XLT4; Difco, BD), and then incubated at 37 °C for 22 - 24 h. Up to three presumptive *Salmonella* colonies selected from XLT4 plates were selected and inoculated onto Triple Sugar Iron (TSI; Difco, BD) and Lysine Iron Agar (LIA; Oxoid, Hampshire, England) slants and incubated at 37 °C 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/g value of each sample was acquired using the USDA-FSIS MPN table (USDA, 2008b).

Primary and delayed secondary enrichments for *Salmonella* detection. In addition to *Salmonella* quantification, we enriched the remaining sample solutions to detect low levels of the organism that were undetectable via the MPN method. Thirty milliliters of 11× TT broth was added to each sample solution and then incubated at 42 °C for 24 h (i.e., primary enrichment). After incubation, a loopful of the mixture was streaked

onto XLT4 plates and incubated at 37 °C for 24 h. The remaining isolation and confirmation of *Salmonella* was done as described for the MPN.

To recover injured *Salmonella* cells, a delayed secondary enrichment was performed on all samples by storing the enriched TT broth at room temperature (25 °C) for 5 days. After 5 days, 0.5 mL aliquots were transferred from those samples that were negative on primary enrichment into fresh 10 mL TT broth tubes and incubated (42 °C, 24 h). Afterwards, a loopful of the TT mixture was streaked onto XLT4 plates. The remaining isolation and confirmation of *Salmonella* was done as described for the MPN.

Data Analysis. The outcomes of the study were *Salmonella* prevalence and numbers from skin of drumstick, thigh, and wing samples. Data collected from all samples were tabulated with Microsoft Excel 2007 software. The MPN data/g were adjusted to the original skin masses and dilution factors 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 *Salmonella* per sample) were used in the analysis. *Salmonella* numbers (log MPN/sample) were compared by skin types (drumstick, thigh and wing) using t-test for independent samples in STATA 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 was compared in a similar manner as the log MPN data, but using Chi-square test in STATA software version 10.1. A difference was considered significant at $P < 0.05$. The presence of *Salmonella* from drumstick and thigh skins in relation to the presence of *Salmonella* from

wing skins was assessed using generalized estimating equations models and adjusting for the flock effect in STATA software.

2.3. Results

Overall *Salmonella* prevalence and numbers. A total of 300 skin samples from each turkey part (drumstick, thigh and wing) from 20 turkey flocks were analyzed for *Salmonella* levels. The overall *Salmonella* prevalence and mean logMPN/sample by turkey skin part are shown in Table 1. *Salmonella* prevalence from wing skin was significantly higher ($P< 0.05$) than that from drumstick skin, but the difference was not significant when compared to thigh skin. As for *Salmonella* numbers, there was no significant ($P> 0.05$) difference between the mean logMPN/sample by skin part.

Distribution of *Salmonella* numbers by the three skin parts. The distribution of logMPN of *Salmonella* from the three turkey skin parts is shown in Figure 1. Within *Salmonella* MPN positive samples, drumstick skin (20.7.2%), thigh skin (17.4.2%), and wing skin (23.9%) samples, respectively, fell in the low MPN number interval $\leqslant$ 1.30 logMPN/sample. Moreover, drumstick skin (2.2%), thigh skin (6.5%), and wing skin (12.0%) samples, respectively, fell within 1.51 - 1.70 logs interval. There was no skin sample from drumstick and thigh that had MPN numbers higher than 1.90 logs. However, 7.6% of wing skins with positive MPNs had *Salmonella* numbers between 1.91- 2.10 logs and 1.1% between 2.11 - 2.40 logs.

Figure 2 shows the distribution of number of *Salmonella* positive skin samples of drumsticks, thighs and wings by flock. Two flocks out of the 20 flocks sampled were *Salmonella* negative. Approximately 90% of the 18 *Salmonella* positive flocks had at least

one positive wing skin and/or drumstick skin sample; whereas 78% of the flocks had at least one positive thigh skin sample.

***Salmonella* presence from wing skin in relation to drumstick and thigh skins.** The odds ratio (OR) for *Salmonella* presence from thigh skin was significantly ($P< 0.05$) higher when this pathogen was present in wing skin (OR = 2.4; 95% CI: 1.26 - 4.46). However, the odds ratio for *Salmonella* from drumstick skin in relation to wing skin was not significant (P> 0.05).

2.4. Discussion

In this study, we determined *Salmonella* prevalence and numbers from turkey skin of three parts. Among the 20 flocks sampled, 18 flocks (90%) had at least one *Salmonella* positive sample. The percentage was as similar to the findings reported by Cui *et al.* (2014). It suggests that *Salmonella* contamination of turkey carcasses skin during processing is frequent.

The USDA-FSIS performance standard for *Salmonella* prevalence on turkey carcass and in comminuted turkey is 7.1% (i.e., no more than 4 positives of 56 samples) and 13.5% (i.e., no more than7 positives of 52), respectively (USDA, 2015a). The most recent USDA data from June 2013 to March 2014 showed that *Salmonella* prevalence in NRTE ground turkey was 20.75% (199 out of 959 samples), which was about eleven times higher than that detected from NRTE whole turkey carcasses and carcass parts (i.e., 1.85%; 30 out of 1,630 samples) (USDA, 2014e). Additionally, the overall *Salmonella* prevalence on the turkey carcass (i.e., 1.85%) is significantly lower than the *Salmonella* prevalence from the three skin part types in this study. The FSIS uses a sponge swab sampling method to test for *Salmonella* presence on turkey carcasses (USDA, 2015b); whereas in this study,

we collected specific skin parts and stomached them prior to *Salmonella* testing. In a study conducted in Poland, authors reported *Salmonella* prevalence on turkey carcass collected post-chill was 8.3% (Zdrodowska *et al.*, 2014), which is lower compared to that of skins determined in this study. *Salmonella* presence on poultry carcass collected at retail markets may be more frequent compared to that collected post-chill. In studies conducted in Colombia, China and Vietnam, authors revealed that *Salmonella* prevalence on chicken carcasses sold in markets was 37%, 43.3% and 48.7%, respectively (Donado-Godoy *et al.*, 2014; Ta *et al.*, 2014; Yang *et al.*, 2014).

In this research, *Salmonella* prevalence from turkey wing skin samples (25.0%, n=300) was significantly higher compared to that from drumstick skin (13.7%, n=300). There are several possible reasons that may have contributed to this difference. One possibility is the potential for contaminates to flow from one part to another during processing of the carcass. Carcasses are hung upside down during processing, and contaminated fluids can flow from elevated locations on the carcass (e.g., drumstick and thigh) to the lower parts (e.g., wings). Cross-contamination can also occur during defeathering, which may provide *Salmonella* a route for entering into open feather follicles (Clouser *et al.*, 1995; Kim *et al.*, 1996; Nde *et al.*, 2007). The feather follicles on wing are larger than those on drumstick and thigh (USDA, 1971) and may allow *Salmonella* to enter into these pores easier. A third possibility may relate to the curved structure of wings in that that may provide this pathogen with a protection against water treatments during processing including the chilling step (a guess). *Salmonella* prevalence of turkey thigh skin was not significantly different than wing skin (Table 1). Thighs are closer in proximity to the vent which might lead to the contamination prevalence we observed.

We determined that mean *Salmonella* numbers from skin samples for the three parts collected post-chill were low (⩽ 1.45 logMPN/sample). Other studies have shown the similar results on post-chilled chicken carcasses with skin on (Brichta-Harhay *et al.*, 2008; Wang *et al.*, 2014). In a recent study, authors revealed that the mean number of *Salmonella* from turkey neck skin samples collected post-evisceration was substantially higher (i.e., 2.4 logMPN/sample) (Cui *et al*., 2014). This may indicate the importance of the chilling step in reducing the load of *Salmonella* on the poultry skin.

In spite of the overall low logMPN levels of the three sampled skin parts and lack of statistically significant difference among the logMPN numbers, 12% wing skins had MPN levels higher than 1.70 logMPN/sample (Figure 1), while only 2.2% of the MPN positives which were drumstick and thigh skins had MPN level higher than 1.70 logs. Based on the comparison of both *Salmonella* prevalence and numbers, use of wing skin in ground turkey production could be a potential source for finished product contamination.

Salmonella prevalence and numbers for each skin part varied by flock as shown in Figure 2. The odds of *Salmonella* present from thigh skin correlated with this pathogen's presence from wing skin. Compared with drumstick, the thigh is physically closer to the wing. Thus, during turkey processing, *Salmonella* cross-contamination between wing and thigh skin is more likely to occur than that for drumstick skin at the flock level.

In conclusion, *Salmonella* prevalence from turkey skin is relatively high and varies by parts; whereas numbers of this pathogen were generally low. *Salmonella* contamination of wing skin and its use in ground turkey production might be of a concern to the turkey industry. Nonetheless, other skin parts should be used with caution as potential route for ground product contamination.

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CHAPTER 3

CONCLUSION

Salmonella was prevalent from turkey skin parts collected from post-chill turkey carcasses, but with low numbers. *Salmonella* presence from these skin parts used for ground turkey production can be a source of contamination. Among the three skin parts (i.e., drumstick, thigh and wing) sampled in this study, wing skin seems to be the most heavily contaminated with *Salmonella* and could act as a major source of raw ground product contamination. Therefore, we recommended using the wing skin to make fully cooked turkey products. More research is needed to determine the genotypes of *Salmonella* isolates from this study and their genotypic relatedness, in order to further understand the occurrence of cross-contamination from one skin part to another.

Table1. Overall *Salmonella* prevalence and numbers from skin parts (drumstick, thigh, and wing) from post-chilled turkey carcasses from twenty flocks sampled at a commercial turkey plant[a]

Skin Part	No. of Samples	Prevalence	No. of MPN positive samples	Mean $\log_{10}$ MPN/sample	95% CI
Drumstick	300	13.7%[a]	22	1.18[a]	1.10 - 1.25
Thigh	300	19.3%[ab]	25	1.29[a]	1.21 - 1.37
Wing	300	25.0%[b]	45	1.45[a]	1.35 - 1.54

[a]Comparison within prevalence and mean log MPN: Values followed by the same uppercase letter were not significantly different ($P > 0.05$).Statistical comparisons were based on Chi-square (for prevalence data) and independent t-test (for log MPN data) using STATA software, version10.1 (Stata Corp., College Station, TX).

Figure 1. Percentages of the most probable number (MPN) distribution of *Salmonella* from skin from drumsticks, thighs and wings collected from post-chilled turkey carcasses from twenty flocks sampled at a commercial turkey plant[a] (n = 300 samples/sample type)

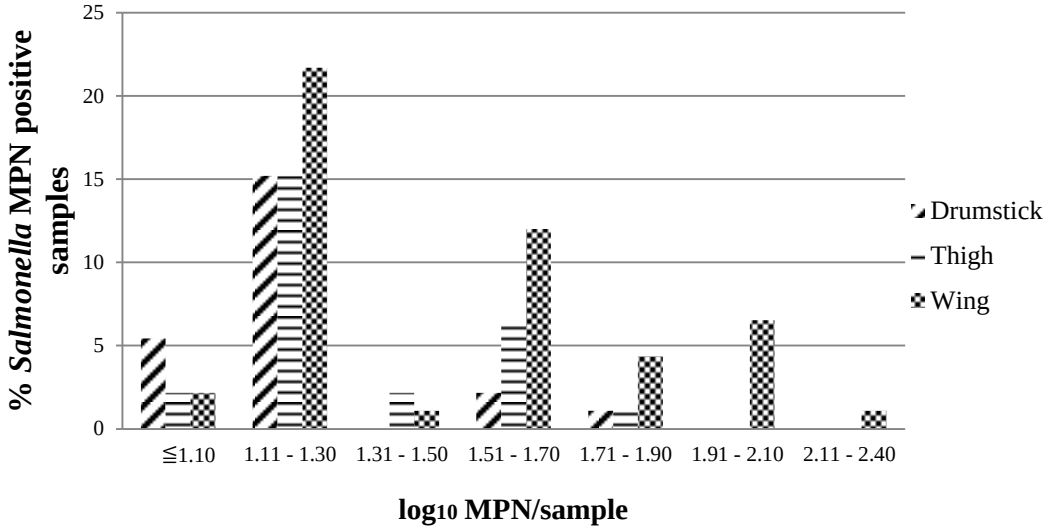

Figure 2. Distribution of number of *Salmonella* positive skin samples from drumsticks, thighs and wings (n=15 samples per part) per flock collected from post-chilled turkey carcasses at a commercial processing plant