Evaluation of *Salmonella* detection and enumeration throughout the ground turkey supply chain.

by

Marvin Obed Tzirin Patal

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Major Professor
Dr. Jessie Vipham

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Abstract

Non-typhoidal *Salmonella enterica* (hereafter referred to as *Salmonella*) is a significant foodborne pathogen worldwide and is one of the major causes of diarrheal disease. Salmonellosis, the enteric disease caused by *Salmonella*, can have fatal effects on people's health, especially for vulnerable populations. Sources of *Salmonella* contamination in food have a wide range; however, poultry products (including chicken and turkey) are known to be primary reservoirs. Some of the challenges associated with controlling *Salmonella* in poultry arise from the complexity of the supply chain and the different sources of *Salmonella* at each stage within it. Reducing the risk of salmonellosis associated with poultry products, particularly ground turkey, is currently a priority for the United States Department of Agriculture Food Safety and Inspection Services (USDA-FSIS). Therefore, the turkey industry is in need of holistic approaches to reduce *Salmonella* that consider the sources of contamination along the supply chain and its impact on final product contamination.

The objective of this study is to evaluate the *Salmonella* detection and quantification throughout the turkey supply chain to better understand *Salmonella* contamination in ground turkey. Thus, sixty-six commercial turkey barns were sampled at four levels of the supply chain (i.e., farm, early harvest, processing, and final product). Various sample types were considered, including boot swabs, feathers, and fan dust swabs at the farm level; trailer swabs, paw swabs, and vent swabs at the early harvest level; and livers, lungs, ceca, and ground turkey at the processing and final product levels. Samples were tested for *Salmonella* using the BAX® System Real-Time PCR Assay, and those that tested positive were quantified using the BAX System SalQuant® assay. Growers filled out a questionnaire with 15 questions regarding barn

management, and 19 health variables were monitored during each barn processing to document risk factors.

Overall, *Salmonella* was found in 21.6% of the samples (812 out of 3,766). The empirical prevalence levels varied across sample types, ranging from 2.9% from ceca to 51.2% from vent swabs. Notably, ground turkey had a contamination rate of 28.2%. Results found significant differences in the probability of *Salmonella* detection across sample types and sampling locations along the supply chain. Early harvest had the highest estimated probability of detection at 38.9% (95% confidence interval, CI [28.3, 50.6]), while processing had the lowest at 2.4% (95% CI [1.4, 4.1]). The quantification dataset posed a challenge as 44.7% of the observations were below the limit of quantification, likely due to technical limitations of analytic assay. For quantifiable samples, the overall *Salmonella* level mean was 2.19 log CFU/unit (SD 1.34) with varying levels across sample types. Results showed that *Salmonella* prevalence fluctuates along the turkey supply chain. These findings provide meaningful insights for the turkey industry to understand better *Salmonella* dynamics along the supply chain and its impact on the safety of ground turkey.

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Dedication

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Chapter 1 - Literature review

1.1. *Salmonella enterica*

1.1.1. *Salmonella* characteristics and biology

Salmonella, a genus of bacteria commonly associated with human foodborne illness, consists of gram-negative, facultatively anaerobic, and flagellated bacilli (Coburn et al., 2007). This genus includes two species, *Salmonella bongori*, and *Salmonella enterica*, which primarily differ in their ecological niches, antigenic characteristics, and potential for causing disease. *S. bongori* is rarely linked to human illness as it predominantly colonizes cold-blooded animals, such as lizards and snakes. In contrast, *S. enterica* is a significant cause of human foodborne disease and is typically found in the gastrointestinal tract of mammals, birds, and reptiles (Brenner et al., 2000). *S. enterica* is divided into over 2,500 serotypes, each characterized by a combination of O (somatic) and H (flagellar) antigens (Brenner et al., 2000; Lahiri et al., 2010). These serotypes can be classified into two categories based on human immune responses. Typhoidal *Salmonella enterica*, such as serotypes Typhi and Paratyphi, are known to cause typhoid fever, a life-threatening infection (Buckle et al., 2012). On the other hand, non-typhoidal *Salmonella enterica* (hereafter referred to as *Salmonella*), such as serotypes Typhimurium and Enteriditis, can cause gastroenteritis and are the leading group of concern in the food industry (Ao et al., 2015; Fazza et al., 2021). Additionally, there is a distinction between invasive and non-invasive serotypes within the non-typhoidal group. For instance, the most common serotypes associated with human disease (invasive *Salmonella*) include Typhimurium, Enteritidis, Newport, Heidelberg, and Javiana, accounting for 61% of clinical isolates from 1996 to 2006 in the US (Jones et al., 2008). In contrast, serotype Kentucky is commonly found in poultry production establishments (USDA-FSIS, 2024b) but rarely associated with human

disease (non-invasive *Salmonella*) in the US (Shah et al., 2017; Siceloff et al., 2022). This immense genetic diversity is one of the many challenges in public health when controlling salmonellosis.

Salmonellosis is a gastrointestinal infection caused by *Salmonella*. It is the third leading cause of foodborne illness worldwide (Lee & Yoon, 2021) and the first cause of diarrheal disease deaths (WHO, 2015). Salmonellosis symptoms range from mild to severe; the most common symptoms include nausea, diarrhea, abdominal cramps, vomiting, and fever (Ehuwa et al., 2021). Contaminated food and water are the primary sources of infection, particularly poultry, meat, and egg products, which are well known for being reservoirs of bacteria. However, *Salmonella* has been associated with outbreaks in almost every food category and can often be found in fruits and vegetables (CDC, 2019b, 2024b). Salmonellosis usually resolves on its own without medical intervention (i.e., antibiotics). However, in vulnerable populations, such as infants or people with weakened immune systems, additional care is necessary (Chen et al., 2013).

1.1.2. Public health impact of *Salmonella*

The World Health Organization (WHO) estimates that annually, 600 million people get sick, and 420,000 die because of foodborne diseases globally; about 79 million cases and 59,000 deaths are attributed to *Salmonella* (WHO, 2015). The Centers for Disease Control and Prevention (CDC) estimates that *Salmonella* causes 1.35 million infections, 16,500 hospitalizations, and 420 deaths annually in the United States (CDC, 2013). These figures account for reported and unreported cases, as many people do not seek medical attention and go unrecorded. Moreover, an assessment conducted in 2011 (Scallan et al., 2011) estimated that 31 significant pathogens are responsible for 9.4 million cases of foodborne illness, 55,961 hospitalizations, and 1,351 deaths each year in the USA. Regarding the number of illness

episodes, Norovirus is the primary cause, accounting for 58%, while *Salmonella* is the second most common cause at 11%. Moreover, *Salmonella* is the leading cause of hospitalizations at 35%, followed by Norovirus at 26%. Similarly, *Salmonella* is the primary cause of death at 28%, followed by *Toxoplasma gondii* at 24% (Scallan et al., 2011). Further estimations, such as those by Scallan et al. (2013), Majowicz et al. (2010), and Voetsch et al. (2004) arrive at similar conclusions considering different populations or estimation methods. This highlights the significant impact of *Salmonella* and the urgency of interventions to safeguard public health.

From an economic standpoint, the World Bank estimates that *Salmonella* causes US$110 billion per year in loss of productivity and medical expenses in low—and middle-income countries due to foodborne pathogens (World Bank, 2018). In the US, the Economic Research Service from the US Department of Agriculture (USDA-ERS) estimated that the cost of foodborne illness caused by *Salmonella* in 2018 was 4.1 billion USD (USDA-ERS, 2020). These estimates consider various factors such as the symptoms, severity, duration, outcomes, medical costs, productivity loss, and valuation of premature mortality (Hoffmann et al., 2012). Even though there has been an overall reduction in cases due to improvements in food safety practices (CDC, 2018; USDA-FSIS, 2015), salmonellosis remains a significant issue since the measures have not yet had an observable impact on human illness rates (USDA-FSIS, 2024c). To achieve the goals set by The Healthy People initiative by the US Department of Health and Human Services, salmonellosis rates must be reduced from 15.5 to 11.5 confirmed cases per 100,000 population by 2030 (HHS, 2022).

1.2. *Salmonella* in the poultry industry

1.2.1. *Salmonella* prevalence and outbreaks in poultry products

Salmonella is a significant pathogen of concern in the U.S. poultry industry. Poultry products (including chicken and turkey) have been known to be the primary food source of *Salmonella* contamination for human disease. In fact, The Interagency Food Safety Analytics Collaboration (IFSAC) attributes 18.6% of *Salmonella* illnesses to chicken and 5.5% to turkey products; together, poultry is responsible for almost a quarter of the total salmonellosis cases in the US (IFSAC, 2023). These numbers are reflected in the different outbreaks linked to specific food products through epidemiological investigation. According to The National Outbreak Reporting System (NORS), from 2010 to 2024, poultry accounted for 225 outbreaks, adding up to 5,670 illnesses, 910 hospitalizations, and six deaths. Out of these 225 outbreaks, 48 were traced to turkey products, seven specifically in ground turkey (CDC, 2024a).

Salmonella outbreaks occurring in the poultry industry are very diverse in terms of the cause, spread, and severity of the outcomes. For instance, in 2023, an outbreak investigation identified 1,072 illnesses and 247 hospitalizations in 48 states related to *Salmonella* Braenderup, Enteritidis, Indiana, Infantis, Mbandaka, and Typhimurium in backyard poultry (CDC, 2023). In 2018, 129 people got sick after consuming products associated with raw chicken (ground chicken, thighs, and whole chicken) in 32 states (CDC, 2019a). The outbreak serotype, *Salmonella* Infantis, was identified in routine inspections in 76 slaughterhouses, with isolates being genetically closely related between them; however, no single or common supplier of raw chicken products or live chicken was identified (CDC, 2019a).

Other major outbreaks have occurred in ground turkey particularly (CDC, 2011, 2021; Hassan et al., 2019). This product tends to have a higher risk of *Salmonella* contamination due to

the process of grinding, which mechanically separates bone and meat and then breaks down the muscle and blends it, creating a homogenous product exposed to the environment and microbial contamination. The 2021 *Salmonella* Hadar outbreak resulted in 33 sick people and four hospitalizations in 13 states; no product recall was necessary as authorities believed the product was no longer available (CDC, 2021). The 2011 *Salmonella* Heidelberg outbreak resulted in 136 infected people across 34 states (CDC, 2011). This incident had substantial repercussions for the company responsible, as 36 million pounds of ground turkey were recalled (CDC, 2011). Lastly, the 2018 *Salmonella* Reading outbreak accounted for 356 infected people, 132 hospitalizations, and one death in 42 states (Hassan et al., 2019).

Together, these public health events highlight the diversity of poultry products in which *Salmonella* contamination occurs, the different serotypes, the multistate spread, and the severity of the outcomes. Furthermore, it emphasizes the need for a more holistic approach to food safety in the poultry industry along the supply chain, particularly for ground turkey. For instance, the 2018 outbreak in ground turkey investigation traced *Salmonella* Reading to several establishments, the first industry-wide outbreak indicating a common source of contamination (Hassan et al., 2019). Therefore, interventions should be targeted along the supply chain, including processing plants and farm sources.

1.2.2. Transmission and *Salmonella* harborage

The intestinal tract of birds serves as the primary reservoir for *Salmonella* harborage; *Salmonella* is then excreted in feces, which then can be transmitted to other sources and vectors (e.g., insects, rodents, other birds, and the environment; Akpabio, 2015). *Salmonella* is a diverse pathogen that can rapidly adapt to various hosts and environments. This is partly due to the pathogenicity islands in its genome (Akpabio, 2015), which play a crucial role in *Salmonella's*

survival within the host, its ability to colonize and invade, and ultimately cause infection (Akpabio, 2015; Marcus et al., 2000). From a more practical standpoint, *Salmonella* can initially contaminate the poultry production systems from different potential points along the supply chain; two primary ways of transmission have been established: vertical transmission (e.g., from hens to eggs) and horizontal transmission (bird to bird by non-hereditary means) (Liljebjelke et al., 2005).

Vertical transmission in the poultry production context refers to the process by which *Salmonella* is passed from a parent (e.g., hens) to its offspring (e.g., eggs); it usually occurs from direct contamination of the yolk, shell membranes, or eggshell before spawning (Liu et al., 2023). Literature has shown evidence of how this transmission works by comparing specific bacteria populations (e.g., specific serotypes) between breeders, hatcheries, and production farms (Crabb et al., 2018; Isah et al., 2024; Liljebjelke et al., 2005). Understanding vertical transmission is extremely important as a big part of the poultry industry is fully integrated (NCC, 2023; Vukina, 2001), meaning a single company owns and controls multiple stages of production. For instance, in the 2018 *Salmonella* Reading outbreak, vertical transmission could have been a critical factor since the strain was linked to different establishments, suggesting systemic contamination rather than focal (Hassan et al., 2019).

On the other hand, horizontal transmission refers to the contamination between birds or the interaction of birds with the environment (Shaji et al., 2023); it commonly includes ingestion, inhalation, or other routes (Foley et al., 2011; Kallapura et al., 2014). On farms, birds can directly or indirectly come into contact with contaminated fecal matter, soil, bedding, litter, water, feed, wild birds, insects and rodents; all these sources represent potential contamination (Crump et al., 2002; Rimet et al., 2019; Wang et al., 2023). These factors have been documented

through a systematic review and meta-analysis of the sources of *Salmonella* in broilers (Wang et al., 2023). The study of Wang et al. (2023) evaluated 37 investigations to assess pre-harvest risk factors and identified and ranked sources of *Salmonella* in order—highest to lowest— as follows i. hatchery (48.5%), ii. litter (25.4%), iii. feces (16.3%), iv. poultry house internal environment (7.9%), v. poultry house external environment (4.7%), vi. feed (4.8%), and vii. chicks (4.7%) (Wang et al., 2023). Horizontal transmission of *Salmonella* in farms plays a crucial role and contributes significantly to the overall *Salmonella* dynamics in the poultry supply chain, which then influences the outcomes of final products. Furthermore, it highlights the need for biosecurity measures in place and correct management of the flocks to reduce contamination from farms (Logue et al., 2024).

Cross-contamination is another significant source of *Salmonella* contamination, especially in processing environments (Bailey et al., 1987). It involves the transfer, whether direct or indirect, of *Salmonella* from a contaminated product to an uncontaminated product (e.g., contaminated carcasses). Some studies have identified process stages more likely to contribute to cross-contamination in a processing plant, including scalding, de-feathering, evisceration, and carcass chilling (Bilgili et al., 2002; Clouser et al., 1995; Sukumaran et al., 2024; Xiao et al., 2019).

All these modes of *Salmonella* transmission interact in different ways throughout the poultry supply chain and require special programs to reduce the risk of contamination in each stage (e.g., biosecurity programs in farms). The study of these ways of transmission also highlights the different mechanisms and stages through which *Salmonella* can enter the supply chain and affect the safety of the final product. Therefore, evaluating the contamination status of

key steps in production process of a specific product (e.g., ground turkey) is essential to developing and implementing specific strategies to reduce the risk of salmonellosis.

1.3. Regulatory framework

The Food Safety and Inspection Service (FSIS) is a division of the United States Department of Agriculture (USDA) responsible for ensuring that meat, poultry, and eggs are safe, wholesome, and correctly labeled (USDA-FSIS, 2024a). The FSIS focuses on reducing the incidence of salmonellosis linked to poultry. Epidemiological data suggest significant challenges remain in the safety of the poultry industry, reflected primarily in the number of outbreaks and diseases associated with *Salmonella* and poultry products (CDC, 2013, 2019b, 2024a; USDA-FSIS, 2015).

Some FSIS programs and regulations established to accomplish this goal include the *Salmonella* performance standards (USDA-FSIS, 2021). These standards guide testing programs, including acceptable *Salmonella* contamination levels in different product categories (USDA-FSIS, 2021). For instance, the maximum permissible percentage in turkey carcasses is 7.1% of positives, meaning 4 of 56 carcasses tested positive for *Salmonella* in a 52-week window (USDA-FSIS, 2021). Another crucial strategy is the pathogen reduction, Hazard Analysis and Critical Control Point (HACCP) Systems' final rule (USDA-FSIS, 1996). This *Salmonella* rule applies to meat and poultry establishments. These regulations aim to reduce the incidence of pathogens in meat and poultry products and thus reduce the incidence of foodborne illness. The final rule includes developing and implementing written Sanitation Standard Operation Procedures (SSOPs), microbial testing to verify process controls, establishing pathogen reduction performance standards for *Salmonella*, and implementing HACCP programs for preventive controls (USDA-FSIS, 1996).

SSOPs are another crucial part of food safety systems (Schmidt & Pierce, 2016). The FSIS requires establishments to develop and implement SSOPs, which specify cleaning procedures, schedules, monitoring and verification, corrective actions, and recordkeeping according to the Code of Federal Regulations (CFR) detailed in Title 9, Part 416 (CFR, 1996). The FSIS is responsible for verifying the adequacy and effectiveness of the SSOPs and the procedures specified; it includes reviewing the SSOPs, reviewing the daily records that document the implementation of these programs, direct observation of the programs, performing testing procedures to assess the sanitary conditions in the establishments. The FSIS also has the authority to issue a product recall when there are reasons to believe products are unsafe for consumption. Federally inspected establishments must maintain a recall procedure; this recall plan must include specific actions to take if a recall is ever necessary. These written procedures must be available for the FSIS to review.

Recently, the FSIS has taken action to enhance regulations in the poultry industry; this is mainly since the reductions observed in *Salmonella* in inspected products have not led to an evident decrease in the number of reported salmonellosis cases (USDA-FSIS, 2015, 2024b, 2024c). In August 2024, the FSIS announced the new *Salmonella* Framework for Raw Poultry Products, enforcing the safety of poultry products through two main approaches, including process controls and final product standards (USDA-FSIS, 2024b). In turkey specifically, the announcement of final product standards for comminuted turkey will be *Salmonella* below ten colony forming units per gram (10 CFU/g) and detectable levels of at least one of the serotypes Hadar, Typhimurium, and Muenchen, as these have been the most frequent serotypes isolated from turkey products (USDA-FSIS, 2024b). In process control enhancement, establishments must develop, implement, and maintain written procedures to prevent cross-contamination throughout

slaughter and dressing operations and incorporate statistical process controls into their microbial monitoring programs (USDA-FSIS, 2024b). Although defining final product standards for ground turkey and strengthening process control requirements will be useful in controlling the risk associated with individual ground turkey production lots, there is a need to understand better how contamination occurs in ground turkey to implement targeted interventions.

1.4 Relevant studies in the poultry supply chain

A majority of the published research regarding *Salmonella* contamination in the poultry supply chain has been conducted in broiler production systems (Bailey et al., 2001; Evans et al., 2015; Obe et al., 2023). Although there are specific differences between broiler and turkey production, certain studies in broilers may still provide insights into specific contamination points applicable to both broilers and turkeys' production systems (Bailey et al., 2001; Evans et al., 2015; Obe et al., 2023). For example, a study by Bailey et al. (2001) investigated *Salmonella* transmission sources and dynamics throughout the broiler supply chain. Samples were collected from 26 sources, from hatcheries to carcasses, including pre-harvest samples (e.g., boot swabs, fan swabs, drag swabs, litter, and feces). Results from this study demonstrated that *Salmonella* was present in every sample type, but was most frequently found in samples collected from farms. Common sources of *Salmonella* in farms included paper pads at bird's placement (50.8%), fly strips (18.7%), drag swabs (14.2%), and boot swabs (12%). The results indicate that the farm serves as a key source of contamination within broilers (Bailey et al., 2001). Another study by Obe et al. (2023) evaluated the role of pre-harvest environments on the risk of *Salmonella* contamination by profiling broiler flocks based on *Salmonella* levels and serotypes. This study utilized boot swabs to sample broiler production farms. *Salmonella* profiles were used to develop a framework for processing approaches regarding *Salmonella* risk (Obe et al., 2023).

Finally, another study by Evans et al. (2015) was conducted to develop a pre-harvest diagnostic protocol for evaluating ground turkey contamination. To that end, drag samples were collected in live-haul trailers at receiving and compared with *Salmonella* enumeration in ground turkey. Results included a significant relationship between Salmonella-positive samples in the pre-harvest and final product, indicating that pre-harvest screening provides an adequate diagnosis for product contamination (Evans et al., 2015); however, in this study only *Salmonella* detection was evaluated in a single pre-harvest sample.

Expanding evaluations to more pre-harvest samples and integrating *Salmonella* detection and quantification could improve our understanding of contamination in ground turkey. A better understanding of *Salmonella* dynamics would allow the development of a holistic approach to reduce contamination along the ground turkey supply chain and, therefore, ensure a safe final product.

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Chapter 2 - Evaluation of *Salmonella* detection and enumeration throughout the ground turkey supply chain.

Marvin O. Tzirin[a], Ellen A. Mendez[a], Mariana B. Paredes[a], Anna V. Carlson[b], J A. Byrd[c], Savannah F. Applegate[d], Nora M. Bello[e], Morgan D. Zumbaugh[a], Travis G. O'Quinn[a], Jessie L. Vipham[a]

[a]Department of Animal Scienes and Industry, Kansas State University, Manhattan KS

[b]Global Food Safety Research & Scientific Services, Cargill Inc., Wichita KS

[c]Southern Plains Agricultural Center, USDA-ARS, College Station TX

[d]Market Development, Hygiena, Camarillo CA

[e]Agricultural Research Service Northeast Area, USDA-ARS, Beltsville MD

* Corresponding author: jessiev@ksu.edu, phone: +1-785-532-3486, 247 Weber Hall, Manhattan, KS, 66506

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Abstract

Salmonella is a foodborne pathogen of significant importance to public health. Poultry products have been known to be the primary reservoir, reflected in multiple outbreaks, some of which are particularly important in ground turkey. Recent efforts to minimize the risk of salmonellosis in poultry include the United States Department of Agriculture Food Safety and Inspection Services (USDA-FSIS) *Salmonella* Framework for Raw Poultry Products. Despite advancements in food safety regulations and controls, there is a need for a holistic approach to reduce *Salmonella* in the turkey industry. This study aims to assess the presence and level of *Salmonella* throughout the turkey supply chain in order to develop a more holistic understanding of *Salmonella* contamination within turkey production. Sixty-six commercial turkey barns were sampled across four levels of the supply chain (farm, early harvest, processing, and final product). Ten sample types were considered, including boot swabs, feathers, and fan dust swabs (farm); trailer swabs, paw swabs, and vent swabs (early harvest), livers, lungs, ceca and (processing); and ground turkey (final product). Samples were tested for *Salmonella* detection according to the BAX® System Real-Time PCR Assay. Quantification was performed on positive samples based on the BAX System SalQuant® assay. *Salmonella* was found in 21.6% of the samples (812/3,766). Empirical prevalence per sample type ranged from 2.9% (19/660) in ceca to 51.2% (66/129) in vent swabs; ground turkey was found at 28.2% (37/131). Significant differences were found in the estimated probability of *Salmonella* detection per sample type and location; early harvest had the highest probability estimated at 38.9% (95% confidence intervals, CI [28.3, 50.6]), and processing the lowest at 2.4% (95% CI [1.4, 4.1]). The quantification dataset in this study posed a challenge, as 44.74 % (357/798) of samples detected positive were below the limit of quantification (i.e., 1 CFU/unit), likely because of technical limitations of the

analytic assay. For quantifiable samples (n=441), the overall *Salmonella* mean was 2.19 log CFU/unit (SD 1.34), with levels per sample type ranging from 0.88 log CFU/mL (SD 0.59) in ceca to 2.56 log CFU/mL (SD 1.4) in trailer swabs; ground turkey was found at 1.12 log CFU/g (SD 0.81). Results showed that *Salmonella* prevalence fluctuates along the turkey supply chain, suggesting that supply chain practices and sampling points may influence the likelihood of identifying *Salmonella* within turkey flocks. Moreover, these findings provide insights into *Salmonella* dynamics along the supply chain, which can ultimately inform decision making for *Salmonella* monitoring and surveillance.

2.1 Introduction

Non-typhoidal *Salmonella enterica* (hereafter referred to as *Salmonella*) is a food-borne pathogen of major concern worldwide and represents one of the four most common causes of diarrheal diseases, among Norovirus, *Campylobacter spp*., and *Escherichia coli* (WHO, 2018). In the United States, The Centers for Disease Control and Prevention (CDC) estimates that 1.35 million infections, 16,500 hospitalizations, and 420 deaths are caused by *Salmonella* each year (CDC, 2013). Symptoms of salmonellosis range from mild to life-threatening, depending upon the serotype, infectious dose, and the host immune status (Langridge et al., 2012). *Salmonella* significantly affects consumer health and leads to losses in human and economic prosperity on a global level. For instance, Hoffmann et al. (2012) estimated that in the United States alone, salmonellosis leads to an annual cost of $3.3 billion USD and the loss of 17,000 quality-adjusted life years (i.e., a unit of measurement of quality of life; Salomon, 2017). Taken together, these estimates demonstrate the global impact of *Salmonella* and its influence on public health.

Salmonella can be found in various foods, ranging from animal source foods to fresh produce (Corredor-García et al., 2021; IFSAC, 2023). Though, poultry has been established as a

primary reservoir (Antunes et al., 2016; Foley et al., 2011). In 2023, the Interagency Food Safety Analytics Collaboration (IFSAC) attributed 18.6% of *Salmonella* foodborne cases (from 1998 to 2021) to chicken and 5.5% to turkey products (IFSAC, 2023). This implies that almost a quarter of all salmonellosis cases may be attributed to poultry products. Over the last 20 years, multiple *Salmonella* outbreaks have occurred in turkey (CDC, 2024a, 2024b). In fact, between 2007 and 2024 a total of 59 *Salmonella* outbreaks were associated with turkey products, with nine occurring in ground turkey (CDC, 2024a). Perhaps the most noteworthy being the 2011 outbreak attributed to *Salmonella* serotype Heidelberg, the 2019 outbreak caused by serotype Reading, and the 2021 outbreak caused by serotype Hadar (CDC, 2011, 2021; Hassan et al., 2019). These outbreaks underlined the public health risk associated with *Salmonella* in turkey products and demonstrated the need for more comprehensive controls for *Salmonella* within turkey production (CDC, 2011, 2021; Hassan et al., 2019). For example, the Reading outbreak investigation traced *Salmonella* contamination back to multiple slaughter facilities, indicating that the outbreak was not due to sanitation or process control failures, but likely occurred due to sourcing birds from a common supplier (Hassan et al., 2019). During a similar timeframe, multiple risk assessments also highlighted the impact of high *Salmonella* levels on the likelihood of a negative public health outcome (Cox et al., 2011; Sampedro et al., 2018; Straver et al., 2007; Teunis et al., 2010; Uyttendaele et al., 2009). Specifically, a dose-response model by Teunis et al. (2010) demonstrated that eliminating product with high levels of *Salmonella* was more effective at reducing the probability of illness than a reduction in prevalence alone. This public health data indicates a need for identifying sources of *Salmonella* contamination within turkey production systems, as well as a comprehensive assessment of the level of contamination within turkey products.

In recent years, the United States Department of Agriculture Food Safety and Inspection Services (USDA-FSIS) has prioritized identifying new approaches for the control of *Salmonella* in poultry. Thus, the *Salmonella* Framework for Raw Poultry Products enforces the safety of ground turkey through two main approaches, including process controls and final product standards (USDA-FSIS, 2024b). In August of 2024, USDA-FSIS announced that final product standards for ground turkey will be *Salmonella* below ten colony forming units per gram (10 CFU/g) and detectable levels of at least one of the serotypes Hadar, Typhimurium, and Muenchen (USDA-FSIS, 2024b). Additionally, establishments must develop, implement, and maintain written procedures to prevent contamination throughout slaughter and dressing operations and incorporate statistical process controls into their microbial monitoring programs (USDA-FSIS, 2024b). Although defining final product standards for ground turkey and strengthening process control requirements will be useful in controlling the risk associated with individual ground turkey production lots, there is a need for deeper understanding of *Salmonella* contamination throughout the supply chain, based upon the ubiquitous nature of *Salmonella* in the poultry industry (Bailey et al., 2001; Evans et al., 2015; Obe et al., 2023; Wang et al., 2023; Edrington & Brown, 2022; Nayak et al., 2003). Therefore, this study aims to evaluate *Salmonella* detection and quantification throughout the turkey supply chain to determine critical points of contamination and inform production practices and intervention decisions within the turkey industry.

2.2 Materials and Methods

2.2.1 Sample collection

From September 2022 to January 2024, samples were collected from 66 commercial turkey barns at pre-harvest and followed through processing to ground turkey. For each barn, 61

total samples were collected, including farm-level samples (boot swabs, feathers, and fan dust swabs), early-harvest-level samples (trailer swabs, paw swabs, and vent swabs), processing-level samples (livers, lungs, and ceca), and final product samples (ground turkey). At the end of the study, a total of 4,026 samples had been collected. Table 1 presents a detailed description of the samples collected per barn. All samples were kept on ice and transported to the meat microbiology lab at Kansas State University for analysis within 24 hours.

Farm-level samples were collected immediately after the birds were loaded and transported to the slaughterhouse. Two boot swab samples per barn were collected using cotton-polyester boot covers premoistened with Buffered Peptone Water (BPW) (Romer Labs, Union, MO). A pair of boot covers was worn to walk across one side of the barn between the feed and water line (where there was the most moisture). The process was repeated in the opposite direction. Feather samples (3 per barn) consisted of feathers shed on the litter along the load-out path. Fan dust swabs (3 per barn) were collected using a 10 mL BPW premoistened sponge and a 10x10 cm sterile template (Neogen, Lansing, MI). Sponges were used to sample the surface of three randomly selected individual fans.

Early harvest samples were taken at the slaughterhouse between live-hang and scalding. Trailer swabs were collected using a sterile Swiffer® wet mop (P&G, Cincinnati, OH) and a MicroTally® cloth (Fremonta, San Jose, CA) premoistened with 25 mL of BPW. The wet mop with the MicroTally® was used to swab the totality of each coop surface in the live haul trailer right after the birds were unloaded for live hang. Only the first three trucks arriving at the plant were sampled for every barn. Five out of 72 coops were selected for sampling within every truck, comprising 15 swabs per barn. Paws and vent swabs were collected at the same location post-exsanguination (pre-scald) using sponges with 10 mL of BPW (Neogen, Lansing, MI). A total of

20 paw (paw pad surface) and vent (feathers surrounding the cloaca) samples were collected throughout the processing lot.

Organs samples were collected during evisceration. A total of 30 birds per barn were randomly selected to collect ten ceca (one ceca per bird; including the tonsil), ten livers, and ten lungs. Finally, two grab samples of ground turkey, representing the final product, were collected from two combo bins per barn. Parts used for grind samples were scheduled for the first grind of the barn, to prevent that cross-contamination.

2.2.2 Sample enrichments

The BAX® real-time PCR system (Hygiena, Camarillo, CA) was used for detection, and the BAX system SalQuant® assay (Hygiena, Camarillo, CA) was used to enumerate *Salmonella*, following manufacturer instructions. Each sample type required a preparation step, including as weighing, smashing, or compositing. Once samples were prepared, a first enrichment was created for homogenization by adding media and stomaching for one minute. Buffered Peptone Water (BPW) or BAX® MP media (consisting of buffers and simple sugars) were used for the first enrichment; trailer swabs and ceca required the addition of novobiocin at 05 mL/L and 1 mL/L respectively. A second enrichment was created by transferring 30 mL of the first enrichment into a different bag and mixing it with an additional 30 mL of BAX® MP containing novobiocin or vancomycin (except for trailer swabs). Two incubation periods were followed before the creation of lysates; the first period corresponded to enumeration lysates at 37 °C and the second to detection lysates at 42 °C. Table 2.2 provides a detailed description of the protocol for every sample enrichment and incubation period.

2.2.3 Lysates for enumeration and detection

Lysates were created for both of the the first and second incubation periods for enumeration and detection. They only differed in the enrichment incubation time. For every sample type, 5 µL of the enrichment was transferred to 200 µL of lysis reagent in cluster tubes. This reagent consisted of 150 µL of protease for every 12 mL of lysis buffer (Hygiena, Camarillo, CA). Next, cluster tubes containing the samples were taken to thermocyclers to complete 20 min at 37 °C, 10 min at 95 °C, and finally cooled down to 4 °C for five min. Lysates were stored for up to 48 hours at 4 °C.

2.2.4 PCR analysis for detection and quantification

All samples were screened utilizing the detection incubation lysates. Samples that were positive for detection were then quantified. Briefly, PCR tubes were hydrated with 30 µL of lysates and allowed to sit in the cooling block between 10-30 min before loading into the BAX® PCR instrument. Results for *Salmonella* detection were recorded as negative or positive for every sample. A second PCR was run for positive samples using the SalQuant® assay, this time using the enumeration lysates following the procedure described for the first PCR. Results for the quantification assay consisted of a cycle threshold (CT) value that was then used to estimate the level of *Salmonella* through standard curves developed for every sample type (provided with SalQuant® assay). These estimations were reported in CFU/mL for fan dust swabs, paws swabs, vent swabs, ceca, liver, and trailer swabs; CFU/g for ground turkey and lungs; and CFU/sample for boot swabs. All values were then converted to a logarithm (i.e., base 10) scale. Samples with a negative result for enumeration assay were considered *Salmonella* detectable but not quantifiable (*Salmonella* below 1 CFU/unit).

2.2.7 Experimental design and statistical analysis

In this study, each barn served as the experimental unit. Feather samples were excluded from analyses as no *Salmonella*-positive samples were observed; additionally, 62 samples suffered disruption during transportation or sample processing and were reported as missing data points. The dataset used for *Salmonella* detection consisted of observations on 3,766 samples. *Salmonella* detection rate comparisons between sample types were completed by fitting a generalized linear mixed model (GLIMMIX) to the binomial response defined as number of samples detected positive. This model included the fixed effect of sample type and the random effects of visit and barn to identify blocking arrangements in the dataset. Additionally, the random effect of barns crossed with sample type was included as preliminary results showed moderate evidence of overdispersion. The final model was fitted using a Laplace approximation to maximum likelihood. The response variable was defined as the probability of *Salmonella* detection per sample type; comparisons were made through least square (LS) means estimations; pertinent pairwise comparisons between sample types were conducted using Tukey-Kramer adjustments. This analysis was performed using the Statistic Analysis System (SAS) Version 9.4 (SAS Institute, Cary, NC).

For *Salmonella* quantification, the dataset consisted of 798 of the 812 samples that tested positive for detection, as 14 observations were reported as missing data points. Observations below the limit of quantification of 1 CFU/unit assigned a value of 0.5 CFU/unit. Descriptive statistics are presented to characterize the raw data for *Salmonella* enumeration.

2.3 Results

2.3.1 *Salmonella* detection

Overall, *Salmonella* was found in 812/3,766 samples (21.6%), with empirical prevalence per sample type in descending order: vent swabs 51.2% (66/129), trailer swab 42.9% (418/975), boot swabs 34.9% (44/126), paw swabs 32.3% (84/260), ground turkey 28.2% (37/131), livers 14.6% (96/660), fan dust swabs 7.2% (14/195), lungs 5.4% (34/630), and ceca 2.9% (19/660). Table 2.3 shows descriptive summary statistics for detection grouped by visit. Table 2.4 shows model estimates for *Salmonella* prevalence per sample type.

Early harvest depicted the highest estimated prevalence with 38.9% (95% confidence interval, CI [28.3, 50.6]); Farm-level prevalence was estimated at 9.3% (95% CI [5.2, 16.0]), and processing at 2.4% (95% CI [1.4, 4.1]); the final product was at 20.4% (95% CI [11.4, 33.7]), prevalence between all supply chain stages were statistically different between each other (P<0.05) (Figure 2.1). By looking into specific sample types (sample types with different letters indicate significant differences between sample types, P<0.05), farm-level samples were estimated at 27.8% (95% CI [16.3, 43.3]) for boot swabs (ab), and 2.7% (95% CI [1.2, 6.0]) for fan dust swab (cd). For early-harvest samples were estimated at 40.9% (95% CI [29.15, 53.73]) for trailer swabs (ab), 53.1% (95% CI [37.0, 69.0]) for vent swabs (a), and 24.8% (95% CI [15.3, 37.6]) for paws swabs (b). For processing-levels samples were estimated at 7.7% (95% CI [4.4, 13.0]) for livers (c), 2.19% (95% CI [1.1, 4.3]) for lungs (d), and 0.80% (95% CI [0.4, 1.8]) for ceca (d). In ground turkey (b) the estimated prevalence was 20.4% (95% CI [11.4, 33.7]) (Figure 2.2).

2.3.2 *Salmonella* enumeration

Initially, comparisons between sample types and locations, based upon enumeration data were intended. However, 44.74% (357/798) of samples detected positive for *Salmonella* were below the detective limit of quantification, meaning that approximately half of the samples detected positive were non-quantifiable. Summary descriptive statistics were performed and are presented below.

Among the 441 samples enumerated for *Salmonella*, the mean concentration was 2.19 log CFU/unit (SD 1.34) (figure 2.3). Descriptive statistics for *Salmonella* concentrations per sample type are presented in descending order (mean log CFU/unit, SD and number of effectively quantified samples): 2.56 (1.4) in 240 trailer swabs, 2.03 (0.77) in 42 vent swabs, 1.96 (1.27) in 8 fan dust swabs, 1.95 (1.35) in 33 boot swabs, 1.87 (1.16) in 18 lungs, 1.75 (0.98) in 40 livers, 1.53 (1.24) in 37 paws swabs, 1.12 (0.81) in 17 ground turkey, and 0.88 (0.59) in 6 ceca. Figure 2.4 shows *Salmonella* levels on samples effectively quantified. The distribution of enumeration values per sample type shows high variability. For instance, trailer swabs had an average of 2.56 log CFU/mL (SD 1.4) with barns as low as 0.7 log CFU/mL and as high as 5.8 log CFU/mL. For ground turkey, 23 barns were *Salmonella* positive, of which 16 barns possessed levels below 10 CFU/g, and seven barns were at or above 10 CFU/g.

2.4 Discussion

The objective of this study was to evaluate the presence (detection) and level (quantification) of *Salmonella* throughout the turkey supply chain. Data collected from this study demonstrates that the prevalence of *Salmonella* differed depending upon sample type and the sampling location within the supply chain. Sampling locations presented a particular trend, whereby the probability of detection starts off low on farms at 9.3% (95% CI [5.2, 16.0]),

increasing to 38.9% (95% CI [28.3, 50.6]) at early harvest and decreasing to only 2.4% (95% CI [1.4, 4.1]) at processing. Finally, *Salmonella* prevalence rose again in ground turkey at 20.4% (95% CI [11.4, 33.7]). This data demonstrates that the detection of *Salmonella* is not uniform and is likely influenced by production practices and environments along the turkey supply chain.

Salmonella contamination on farm (i.e., pre-harvest) has been widely studied and documented (Bailey et al., 2001; Jibril et al., 2020; Obe, Boltz, et al., 2023; Velasquez et al., 2018). It has been hypothesized that *Salmonella* prevalence on-farm may be indicative of *Salmonella* carriage by birds to processing (Bailey et al., 2001; Velasquez et al., 2018). However, in this study, the increase in prevalence between farm and early harvest indicates that farm-level sampling may not always detect realistic *Salmonella* carriage within birds (i.e., feathers, boot swabs, and fan dust swabs). This may be due to the fact that there are differences between *Salmonella* carriage and shedding (Gopinath et al., 2012). The increase in detection in early-harvest samples could be attributed to increased *Salmonella* shedding through feces due to stress factors during load-out, transport, and lairage (Gopinath et al., 2012).

The effect of stress on the intestinal integrity of birds and how it can lead to changes in *Salmonella* prevalence has been previously documented (Alhenaky et al., 2017; Borsoi et al., 2015; Quinteiro-Filho et al., 2012). Although these studies focused on heat and cold stress in specific *Salmonella* serotypes (e.g., Heidelberg and Enteriditis), there is a lack of understanding of other stress factors, such as transportation and bird handling. Loading out birds, transportation conditions, and arrival at the slaughterhouse expose birds to high levels of stress, thereby contributing to fecal *Salmonella* shedding (Whyte et al., 2001). *Salmonella* is then detectable in the environment and exterior of the carcass in samples like trailer, vents, and paw swabs (Franz et al. 2012; Heyndrickx et al. 2002; Marin and Lainez 2009). A study in broilers assessed the

differences in *Salmonella* prevalence before loading and after transportation to the slaughterhouse and reported an increase from 15.4% at loading to 41.2% after transport (Marin & Lainez, 2009). The *Salmonella* shedding effect is also consistent with the low detection levels during evisceration (i.e., processing-level). *Salmonella* is primarily contained in the gastrointestinal track of birds and can be spread to liver, gallbladder and other organs (Cox et al., 2007; Wang et al., 2024). When *Salmonella* is shed through feces, gastrointestinal organs (e.g., ceca) may be emptied out and therefore presenting low levels of detection.

Contrasting with other bacteria like *Escherichia coli* and Enterobacteriaceae—used to monitor hygiene—*Salmonella* contamination in turkey carcasses does not only occur externally (e.g., fecal contamination of carcasses; (Adeyanju & Ishola, 2014; Belluco et al., 2016)). *Salmonella* detection levels throughout the ground turkey supply chain (specially between processing-level and final product) show evidence of internalization rather than strictly external contamination (Rimet et al., 2019); otherwise, adequate sanitation and acid bath interventions would significantly reduce the prevalence in ground turkey, as it does in post-chill carcasses (Berrang et al., 2009; Yang et al., 2001). Instead, populations of *Salmonella* can be internalized in turkey bones and muscles, then later exposed during the grinding process (Cui et al., 2015; Rimet et al., 2019). Differences in serotype populations in ground turkey *vs*. turkey parts further suggest differential sources (Cason et al., 2024; Erol et al., 2013). Furthermore, it emphasizes the urgency of a better understanding of *Salmonella* contamination in ground turkey and how the industry can manage the risk of salmonellosis in this product, as current process controls do not evaluate internalized loads of bacteria.

In this study, differences in sample type were averred. The probability of detection was not statistically different ($P>0.05$) between boot swabs (27.8% with 95% CI [16.3, 43.3]), trailer

swabs (40.9% with 95% CI [29.2, 53.7]), paw swabs (24.8% with 95% CI [15.3, 37.6]) and ground turkey (20.4% with 95% CI [11.4, 33.7]). Sample types are potential indicators for *Salmonella* carriage and shedding by live birds and ultimately in ground turkey. Similar findings have been noted in the literature. For instance, data from Evans et al. (2015) has previously shown no statistical difference in the prevalence of *Salmonella* between trailers drag samples and ground turkey (Evans et al., 2015). Moreover, a study evaluating *Salmonella* prevalence between boot swabs and pre-scald samples (i.e., vent and paws swabs) also demonstrated no statistical differences when compared with ground turkey (Cason et al., 2024).

Feather samples collected from farms were the least informative samples in this study, as no feather samples tested positive for *Salmonella*. Some authors have suggested using feathers as a surveillance analysis of antimicrobials in the poultry industry (Cornejo et al., 2023; Gajda et al., 2019). Feathers may contain antimicrobial compounds that inhibit the growth of *Salmonella* and, therefore, not be an informative sample to evaluate *Salmonella* prevalence. Similarly, ceca, livers, lungs, and fan dust swabs had noticeably low detection rates, which conflicts with previous research indicating that ceca be used to evaluate *Salmonella* status in the poultry industry (Kallapura et al., 2014; Ramirez et al., 1997; Rostagno et al., 2006).

Unfortunately, enumeration results reported in this study pose a big challenge for interpretation, as 44.7% of the samples detected positive for *Salmonella* showed enumeration below threshold lever for assay (i.e., 1 CFU/unit). Indeed, a histogram for the log CFU/unit distribution (figure 2.3 A) shows a peak at the detection threshold. As other authors have reported, the issue could be directly related to the enumeration methodology used (Cason et al., 2024; Chavez-Velado et al., 2024). As an alternative, future studies may consider implementing real-time PCR methodologies based on DNA extraction rather than lysates; However, it is

important to acknowledge the differences in time and labor allocation of these alternatives compared to lysates-based PCR but not informative, especially with a large number of samples.

In terms of variability, the random effect of barns could be attributed to the different management and biosecurity practices each farm implements. All barns were processed at the same slaughterhouse, but turkeys came from multiple farms in Arkansas and Missouri; these differences would likely contribute to the variability in *Salmonella* detection, as different management systems impact the *Salmonella* prevalence in farms (Alali et al., 2010; Obe et al., 2023).

2.5 Conclusions

The probability of detecting *Salmonella* in the turkey supply chain differed between the sample type and the sampling location along the ground turkey supply chain. Detection rates increased from farm to early harvest, decreased during processing, and reemerged at the final product, reflecting *Salmonella* populations fluctuating throughout the supply chain. *Salmonella* enumeration poses a big challenge for interpretation as almost half of the samples detected positive were not quantified by the method utilized. Therefore, although this study serves as a promising preliminary assessment of *Salmonella* dynamics throughout the turkey supply chain, further investigation is warranted. For example, future studies should focus on 1. Understanding the factors that drive fluctuations in *Salmonella* detection throughout the supply chain; 2. identifying what is a “representative sample”; and 3. Establishing methods to more accurately quantify *Salmonella* in turkey samples.

2.6 Figures and tables

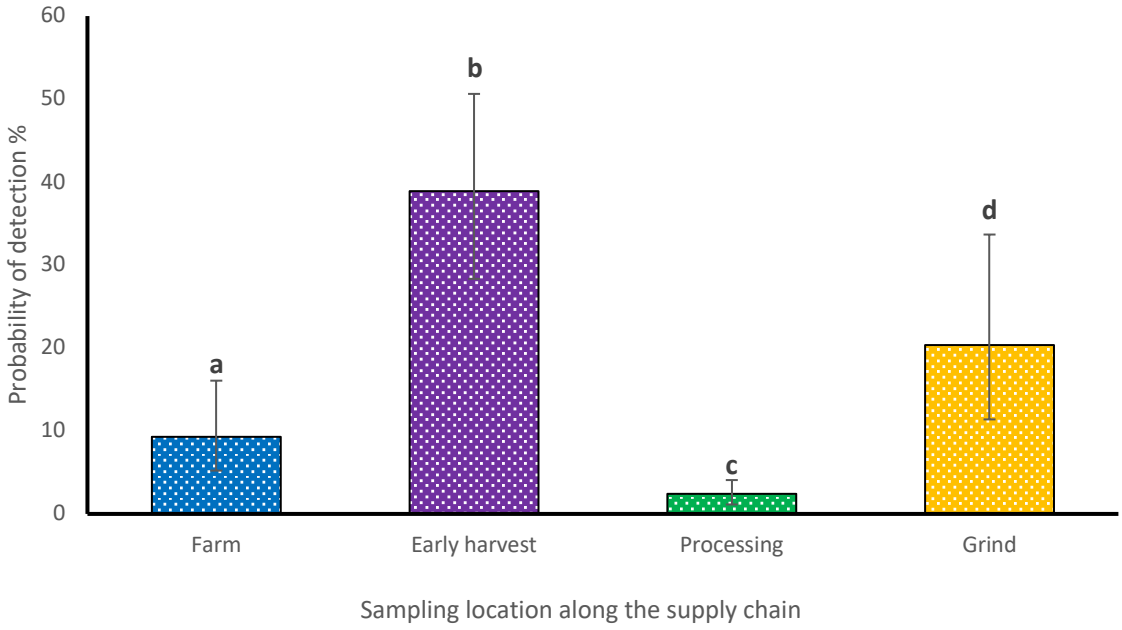

Figure 2.1. Estimated *Salmonella* detection per sampling location.

Values are presented as estimated means with a 95% confidence interval.
Different letters indicate significant differences between sample categories (P<0.05).

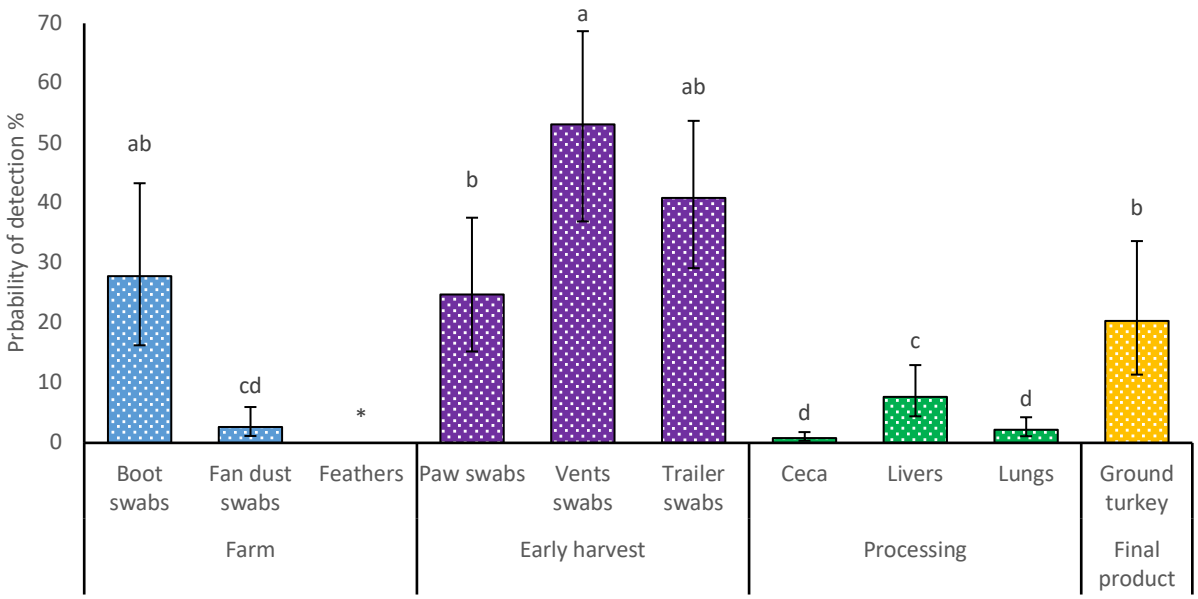

Figure 2.2. Estimated *Salmonella* detection within each sample type.

Values are presented as estimated mean with a 95% confidence interval.

Different letters indicate significant differences between sample types ($P<0.05$).

* Indicates no *Salmonella* detection.

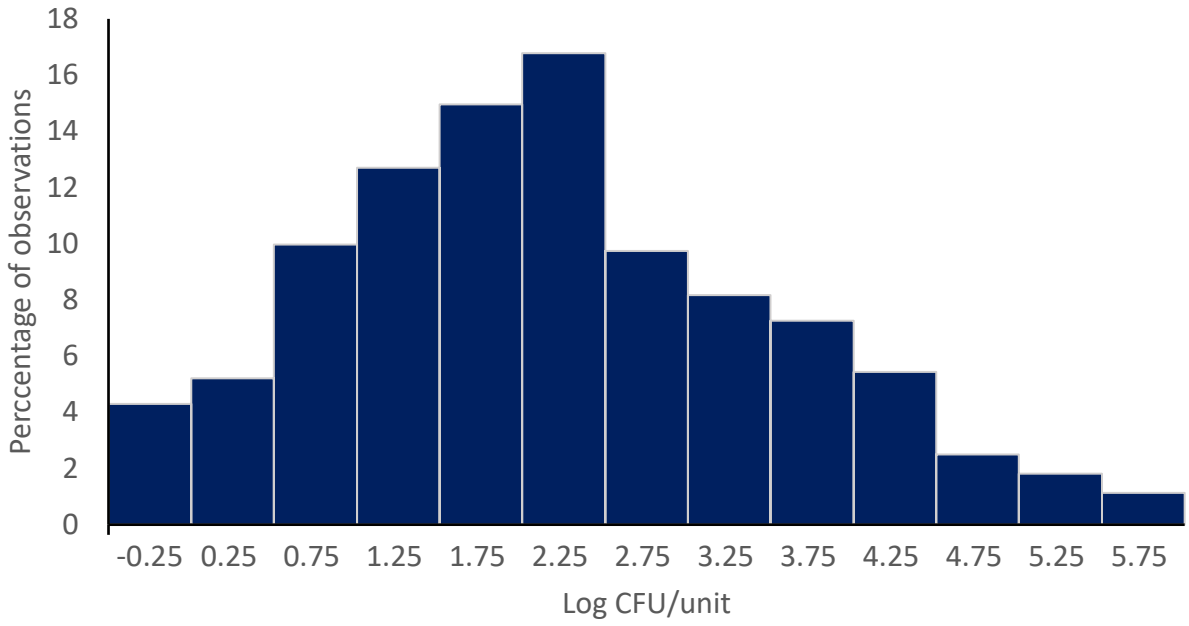

Figure 2.3. Empirical distribution of Log CFU/unit across all sample types. Histogram corresponds to samples quantified above the threshold of detection (n=441).

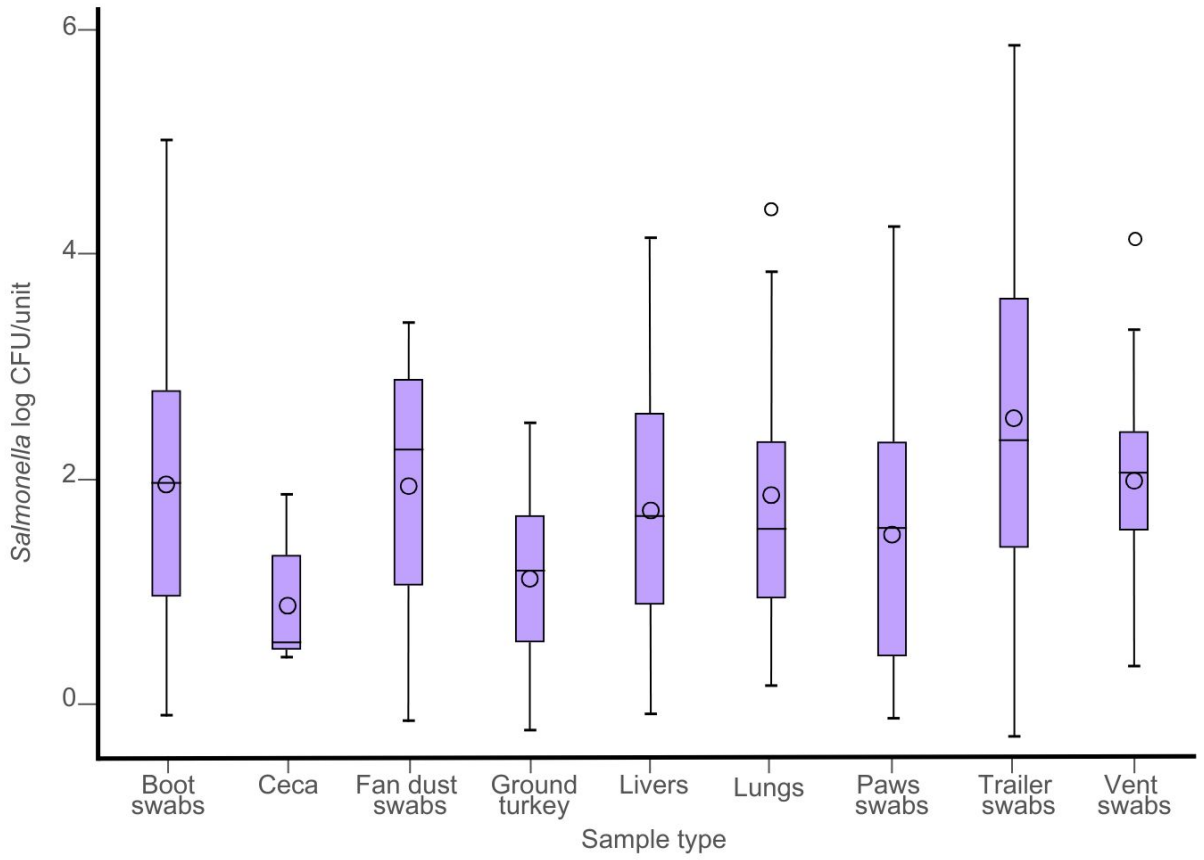

Figure 2.4. Distribution of *Salmonella* enumeration per sample type for samples quantified above threshold of detection.

Table 2.1 Description of sample collection

Sample location	Sample type	# of collected samples per barn
Farm	Boot swabs	2
	Feathers	3
	Fan dust swab	3
Early harvest	Trailer swab	15
	Paws swab	4*
	Vent swab	2**
Processing	Livers	10
	Lungs	10
	Ceca	10
Final product	Ground turkey	2
Total		61

* Indicates four composites of five swabs, comprising 20 swabs per barn.

** Indicates two composites of ten swabs, comprising 20 swabs per barn.

Table 2.2. Description of enrichment methods for each sample type

Sample type	Sample preparation	First enrichment (FE)	Second enrichment	First incubation at 42 °C	Second incubation at 37 °C
Boot swabs	N/A	200 mL BPW	30 mL of FE + 30 mL BAX MP at 42 °C (QS 1 mL/L)	10 h	10-14 h
Feathers	Weight 10g of the sample	100 mL BPW at 42 °C	30 mL of FE + 30 mL BAX MP at 42 °C + 0.24 g vancomycin	10 h	10-14 h
Fan dust swabs	N/A	100 mL BPW	30 mL of FE + 30 mL BAX MP at 42 °C (QS 1 mL/L)	12 h	8-12 h
Trailer swabs	Remove the wet mop and keep the MicroTally swab	200 mL BAX MP at 42 °C (QS 1 mL/L)	N/A	8 h	12-16 h
Paws swabs	Combine 5 sponges	100 mL BPW at 42 ° C	30 mL of FE + 30 mL BAX MP at 42 °C (QS 1 mL/L)	10 h	10-14 h
Vent swabs	Combine 10 sponges	100 mL BPW at 42 °C	30 mL of FE + 30 mL BAX MP at 42 °C (QS 1 mL/L)	10 h	10-14 h
Livers	Smash liver	200 mL BPW	30 mL of FE + 30 mL BAX MP at 42 °C (QS 1 mL/L)	6 h	14-18 h
Lungs	Smash lung	300 mL BPW	30 mL of FE + 30 mL BAX MP at 42 °C (QS 1 mL/L)	6 h	14-18 h
Ceca	Smash ceca	100 mL BAX MP at 42 °C (QS 0.5 mL/L)	30 mL of FE + 30 mL BAX MP at 42 °C (QS 0.5 mL/L)	10 h	10-14 h
Ground turkey	Weight 325 g of the sample	325 mL BPW	30 mL of FE + 30 mL BAX MP at 42 °C (QS 1 mL/L)	6 h	14-18 h

Table 2.3. *Salmonella* detection per visit

Visit date	Number of barns	*Salmonella* detection		
		Negative count (%)	Positive count (%)	Total
9/29/2022	4	197 (81.74)	44 (18.26)	241
10/14/2022	4	203 (83.88)	39 (16.12)	242
11/8/2022	6	205 (62.31)	124 (37.69)	329
2/28/2023	5	258 (86.87)	39 (13.13)	297
3/21/2023	5	265 (86.89)	40 (13.11)	305
4/18/2023	5	189 (62.58)	113 (37.42)	302
5/22/2023	7	379 (88.76)	48 (11.24)	427
6/19/2023	5	232 (76.82)	70 (23.18)	302
7/23/2023	9	415 (75.73)	133 (24.27)	548
8/6/2023	4	178 (72.95)	66 (27.05)	244
10/17/2023	3	152 (83.06)	31 (16.94)	183
11/27/2023	5	272 (89.18)	33 (10.82)	305
1/18/2024	4	201 (86.27)	32 (13.73)	233
Total	66	3146	812	3958

Table 2.4. Model estimates for *Salmonella* prevalence per sample type

Sample type	Probability of detection %			
	Mean	Lower mean 95% CI	Upper mean 95% CI	Standard error Mean
Bootie Pair	27.2	16.3	43.2	7.0
Ceca	0.8	0.3	1.8	0.3
Fan Dust	2.7	1.2	6.0	1.1
Grind	20.4	11.4	33.7	5.7
Liver	7.7	4.4	13.0	2.1
Lungs	2.2	1.1	4.3	0.8
Paws Swab	24.8	15.3	37.6	5.7
Trailer Swab	40.9	29.2	53.7	6.4
Vent Swab	53.1	37.0	68.7	8.4

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Chapter 3 - Future direction

The above study presented results on the detection and quantification of *Salmonella* throughout the ground turkey supply chain. However, the overall project included two additional objectives: To utilize the *Salmonella* detection and quantification data to identify a predictive sample for *Salmonella* contamination in ground turkey; and to utilize data collected through grower surveys and bird health date to identify potential risk factors and.

To identify a predictive sample for *Salmonella* contamination in ground turkey a preliminary predictive analysis was performed to identify explanatory variables for *Salmonella* contamination in ground turkey. This analysis included characterizing the response variable based on *Salmonella* detection and quantification in ground turkey to classify barns in risk categories (i.e., no risk, low and high risk). Then, a screening variable procedure on the detection and quantification was assessed to identify those variables associated with ground turkey; it considered the proportion of *Salmonella*-positive, the median, and the maximum enumeration value in each sample type per barn. A final model was fitted to characterize the type and strength of associations. The median value of paw swabs and the proportion of *Salmonella*-positive samples in trailer swabs were identified to be significantly associated with the risk of *Salmonella* in ground turkey.

To identify potential risk factors, this study included the collection of on-farm production practice data from grower surveys and health data collected at processing, reflecting bird health status and yield at processing. The following is a description of the methods used for this data collection, as well as a description of future analysis still needing to be conducted. This data analysis may provide a deeper understanding of factors—such as production practices and bird health—that may influence *Salmonella* contamination throughout the supply chain.

3.1 Grower survey data collection

A grower survey was developed to understand specific production practices and bird management practices; this survey included 15 multi-part questions. Some questions required simple choice answers, while others allowed multiple-choice answers. Survey questions covered stress factors, infrastructure, supplements, and biosecurity practices, and was intended to identify risk factors associated with *Salmonella* status in the barn. Questionnaires were completed using online services (KoboToolbox, Cambridge, MA) or by information provided directly to the researchers. Responses were converted into a binary (Yes/No) format for analysis.

3.2 Health data collection

Health data was collected during the birds' processing. These metrics provide information on each barn's health status of birds at processing. This was done through a third-party service operating in the processing plant and reporting to a national surveillance system. Health data was intended to be used as potential risk factors to explain *Salmonella* status at the barn level. Variables considered in this dataset include footpad score, head condemned, parts condemned, dead on arrival, age, weight, scab damage, scratch on the wing, breast, and back, leg synovitis, leg edema, breast button, drop crop, airsacculitis, wing synovitis, osteomyelitis, fecal contamination, and fecal salvage.

3.3 Grower survey data results

The survey described the environmental conditions for the turkeys, infrastructure, use of additives, biosecurity, and other management practices in 66 barns. Table 3.1 describes the frequencies of answers to each question in a binary format (i.e., yes or no). Extreme weather (e.g., heat, cold, rain) was reported for 17 barns. Regarding infrastructure, the surface around the barn was commonly dirt, vegetation, or gravel (33, 32, and 11 out of 66 barns, respectively),

while the surface near the access doors most often consisted of concrete, gravel, and dirt (46, 13, 7 out of 66 barns, respectively). Most common drinkers used were bell drinkers (62/66 barns), Most barns reported moving those drinkers weekly, less, or more than weekly.

Additives or feed treatments were used in all barns; specifically, 45/66 received antibiotic treatments with penicillin, lincomycin, or tetracyclines. Barns administering no antibiotic treatments were given other water/feed additives or alternative treatments, including prebiotics, acidifiers, essential oils, or medicated feed.

From a biosecurity perspective, the most common routine practices used in turkey barns included the use of footbaths (64/66) and dedicated shoes (56/66), followed by disposable boots (6/66), and washing and disinfecting of boots (8/66). Approximately half of the barns reported having other animals on the farms besides turkeys, mostly cattle and pets.

For other related management practices, half of the farms reported carry over feed, meaning they use feed from the previous barn to provide it in the next barn; all these farms carry the feed blended with new feed only. Five farms reported cleaning out the barn on the same day as the birds were shipped to slaughterhouse. Meanwhile, windrowing, the practice of piling up the litter in rows, was performed on 31 farms. Most barns remained empty 5 to 10 days after the birds were harvested (48). These results are merely descriptive of the barns surveyed.

3.4 Health data results

Information regarding birds' performance and health status at processing was collected on the 64 barns surveyed; unfortunately, data recovery from two barns was problematic as information could not be retrieved. Health occurrence in airsacculitis, wing synovitis, and fecal contamination were none. Table 3.2 contains descriptive statistics for the 19 indicators evaluated.

On average, birds were processed at 139.7 (SD 6.3) days of age with a commercial weight of 18.7 (SD 1.4) Kg per turkey.

Relevant indicators of performance and yield are as follows: On average, 0.4% (SD 0.4) of birds in each barn died during transportation, categorized as dead-on-arrival (DOA). During processing, "heads condemned" refers to carcasses deemed unfit for consumption due to damage; this occurred in 0.2% (SD 0.2) of birds from each barn. "Parts condemned" (such as thighs, wings, and breasts) accounted for 1.1% (SD 0.5) of the birds. No fecal contamination was observed on any bird during visual inspection. However, "fecal salvage," which involves cleaning and conditioning birds after fecal contamination is detected during evisceration, was recorded at 0.1% (SD 0.4) of the birds.

Additionally, health measurements were collected for individual birds, with the footpad score assessed on a scale from 1 to 5, where 5 represents the most severe damage due to lacerations on the bird's feet. The median footpad score was 1.4 (1st quartile 1.3, 3rd quartile 1.9). Observations for other health indicators, including scab damage, wing scratches, breast and back scratches, leg synovitis, leg edema, breast button, drop crop, airsacculitis, wing synovitis, and osteomyelitis, were limited to a few barns. On average, these conditions affected no more than 1.4% of birds per barn. However, some barns had higher prevalence, including one barn with 11% of birds affected by leg synovitis and another with 8% affected by breast button.

3.5 Future analyses

This data is intended to be used as explanatory variables of the risk of *Salmonella* contamination in ground turkey at the barn level. Similarly to the preliminary results presented at the begging off this chapter, a screening variable will be performed using a stepwise approach to evaluate individual associations between potential risk factors (i.e., growers and health data) and

the risk level in ground turkey. A generalized linear mixed model will be fitted to the binomial response defined as *Salmonella* risk in ground turkey samples, assuming a binomial distribution. This model will describe the type and strength of associations between significantly associated explanatory and the response variable. Finally, the accuracy of predictions of such explanatory variables will be assessed using receiving operating characteristic (ROC) curve analysis. ROC analysis will allow to estimate the sensitivity and specificity of the risk of *Salmonella* in ground turkey prediction based on thresholds established on the significantly associated explanatory variables.

If such analyses successfully identify effective predictors of *Salmonella* risk in ground turkey, the industry could use these findings to assess the risk of *Salmonella* in individual turkey barns based on specific risk factors. This would enable a proactive evaluation of *Salmonella* contamination levels in ground turkey, allowing for timely adjustments to interventions. As a result, the risk of salmonellosis associated with ground turkey could be significantly reduced.

3.6 Tables

Table 3.1 Frequencies of answers to survey questions asked of growers

Question	Answer	Yes	No	Total
Q1: Have there been any excessive weather-related stressors in the last 4 weeks of life? (i.e. excessive weather such as lightening, hail, rain over 2 inches, intense heat/cold, below freezing temps)	Yes/No	17	49	66
Q1.1: If Q1 is "Yes", please specify	Heat	4	13	17
	Rain	10	7	17
	Cold	11	6	17
Q2: Specify the surface around the barn that is adjacent to the footing.	Gravel	11	55	66
	Concrete	0	66	66
	Dirt	33	33	66
	Vegetation	32	34	66
Q3: Specify the surface near the most frequently used door	Gravel	13	53	66
	Concrete	46	20	66
	Dirt	7	59	66
	Vegetation	0	66	66
Q4: Were antibiotics administered to the birds in this barn?	Yes/No	45	21	66
Q4.1: If Q4 is "Yes", what antibiotic was given to the birds in this barn?	Penicillin	35	10	45
	Lincomycin	18	27	45
	Oxytetracycline	14	31	45
	Chlortetracycline	3	42	45
Q5: Were any additional water or feed treatments/additives given to the birds in this barn?	Yes/No	22	44	66
Q5.1: If Q5 is "Yes", what was the water or feed additive used?	Activo	5	17	22
	Prosper	8	14	22
	Kem san	6	16	22
	Medicated feed	5	17	22
	Essential oils	5	17	22
	Acidifiers	5	17	22

	Pro Oxine	0	22	22
	LpH	0	22	22
Q6: What are the routine practices for people before entering the turkey barns? (Please select all that apply)	Footbaths	64	2	66
	Hand washing	1	65	66
	Dedicated shoes	56	10	66
	Dedicated clothing	3	63	66
	Dedicated overshoes	2	64	66
	Dedicated overclothes	0	66	66
	Disposable boots	6	60	66
	Wash and disinfected boots	8	58	66
Q7: Are there other livestock and/or pets besides turkeys on the same farm?	Yes/No	32	34	66
Q7.1: If Q7 is "Yes", please specify species of other animals or birds on the farm. (Please select all that apply)	Pigs	0	32	32
	Cattle	29	3	32
	Sheep	1	31	32
	Goats	0	32	32
	Game chickens	0	32	32
	Other chickens	0	32	32
	Other turkeys	0	32	32
	Cats	1	31	32
	Dogs	8	24	32
	Horses	0	32	32
Q8: How many other commercial poultry facilities (other farms) are within 1/4 mile radius of the farm?	None	36	30	66
	1	22	44	66
	2	5	61	66
	>2	3	63	66
	Total	66		
Q9: What type of drinkers are in the barn?	Nipple	4	62	66
	Nipple and cup	0	66	66
	Bell	62	4	66
	Cup	0	66	66
	Trough	0	66	66

	Total	66		
Q9.1: If Q9 is "Bell", are bell drinkers moved?	Yes/No	52	10	62
Q9.2: If Q9.1 is "Yes", how often are bell drinkers moved?	Daily	0	52	52
	Multiple times per week	15	37	52
	Weekly	31	21	52
	Less than weekly	6	46	52
	Total	52		
Q10: Do you carryover feed fed to this barn?	Yes/No	33	33	66
Q10.1: If Q10 is "Yes", how do you carryover feed?	Blended	33	0	33
	Fed straight	0	33	33
	Total	33		
Q11: Was this grow barn cleaned out?	Yes/No	5	61	66
Q12: Was windrowing performed on this grow barn?	Yes/No	31	35	66
Q13: Who removes the litter from the farm when the litter is completely changed?	Contract	19	47	66
	Self	47	19	66
	Shared equipment	0	66	66
	Total	66		
Q14: How long did the grow barn remain empty between the sampled flock and the previous one?	<5 days	1	65	66
	5-7 days	37	29	66
	8-10 days	11	55	66
	> 10 days	17	49	66
	Total	66		
Q15: How far away is used or caked litter stock piled or spread from the sampled barn? (Please respond in yards)	0-200 yards	28	38	66
	201-400 yards	12	54	66
	>400 yards	26	40	66
	Total	66		

Single choice questions include a total at the end of the choices while multiple-choice questions do not.
Shaded regions with the same color match the total of the previous question in a logical order.

Table 3.2. Descriptive statistics for barn-level health indicators.

Indicator	Scale/values	N	Mean	Std Dev	Min	1st Quartile	Median	3rd Quartile	Max
Foot pad score	1-5	63	-	-	1	1.3	1.4	1.9	3.9
Head condemned	%	64	0.2	0.2	0	0.1	0.1	0.2	1.5
Parts condemned	%	62	1.1	0.5	0.4	0.8	1	1.3	2.8
Dead on Arrival	%	63	0.4	0.4	0	0.2	0.3	0.4	2.6
Age	days	63	139.7	6.3	120	138	140	144	151
Average weight	Kg	60	18.8	1.4	14.3	17.9	19.0	19.6	21.3
Scab damage	%	64	-	-	0	0	0	0	2
Scratch on wing	%	64	-	-	0	0	0	0	5
Scratch on breast	%	64	-	-	0	0	0	1	3
Scratch on back	%	64	-	-	0	0	0	0	3
Leg synovitis	%	64	-	-	0	0	0	0	11
Leg edema	%	64	-	-	0	0	0	0	4
Breast button	%	64	-	-	0	0	0	1.5	8
Drop crop	%	64	-	-	0	0	0	0	3
Airsacculitis	%	64	-	-	0	0	0	0	0
Wing synovitis	%	64	-	-	0	0	0	0	0
Osteomyelitis	%	64	-	-	0	0	1	2	9
Fecal contamination	%	64	-	-	0	0	0	0	0
Fecal salvage	%	64	-	-	0	0	0	0	3