INVESTIGATING BIOAEROSOL TRANSFER OF *SALMONELLA* AND *ESCHERICHIA COLI* FROM COMMERCIAL POULTRY OPERATIONS IN THE SOUTHEASTERN UNITED STATES

by

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(Under the Direction of Manpreet Singh and Faith Critzer)

ABSTRACT

This work investigated bioaerosol transfer from poultry farms in the Southeastern U.S. using passive and active methods. Proximity to the exhaust fan corresponded with high *E. coli* in passive samples (5.88 ± 0.51 log CFU/sampler), and a statistically significant reduction in *E. coli* was found at 100m (3.97 log CFU/sampler) ($p = 0.0037$), with a significant correlation with PM_{10} at 50 m. Active sampling confirmed elevated *E. coli* concentrations near poultry exhaust fans, with peak fecal indicator concentrations measuring 2.45 ± 0.70 log CFU/m³ (total coliforms) and 2.42 ± 0.73 log CFU/m³ (*E. coli*). Significant *E. coli* reductions were observed at 50 m ($p < 0.0001$) and 100 m ($p = 0.0011$), and coliforms/*E. coli* strongly correlated ($p < 0.0001$) with wind speed, gust speed, $PM_{2.5}$ and PM_{10}.

INDEX WORDS: Bioaerosol, Particulate matter, Passive sampling, Active Sampling, Poultry operations

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DEDICATION

To my parents for their unwavering support. To Uncle Barry. To Grandpa Sam. To Frankie.

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TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

Estimates indicate that over 9.4 million cases of foodborne illness occur annually in the U.S. from 31 known pathogens. When factoring in unidentified agents, this estimate is approximately 38.4 million cases annually (Scallan et al., 2011). *Salmonella* spp. is the predominant cause of bacterial infections and *Salmonella* is associated with the highest number of fatalities among all foodborne illnesses, surpassing both viral and parasitic agents (Scallan et al., 2011). Nearly half of all foodborne illnesses in the U.S. are linked to fresh produce, largely due to the lack of a kill step in their processing (Painter et al., 2013). Additionally, approximately 25% of foodborne illnesses can be traced back to poultry, primarily caused by *Salmonella* and *Campylobacter*, which are commonly found in these animals (Chai et al., 2017). In a recent Center for Disease Control and Prevention report of foodborne illness source attribution estimates over the period 1998-2022, chicken could be attributed to 20% of *Salmonella* illnesses, with fruits and seeded vegetables attributed to about 27% of *Salmonella* illnesses (CDC, 2024). These statistics highlight the need for improved food safety, particularly *Salmonella* in produce and poultry.

In recent years, *Salmonella* has been linked to numerous foodborne illness outbreaks in fresh produce, including cantaloupes, red onions, peaches, and leafy greens. (U.S. Food and Drug Administration [FDA], 2021a; FDA, 2021b; FDA, 2022; FDA, 2023). Traceback investigations have revealed possible contamination pathways such as canal water, packing house equipment, and fugitive dust from adjacent poultry or cattle operations (FDA, 2021a).

While research on many of these contamination routes has been conducted, the relationship between animal operations and produce deserves more attention.

Animal feeding operations (AFOs) have become increasingly prevalent in the U.S. as an efficient method for producing meat and eggs, supplying approximately 99% of the animal products consumed nationwide (Walton and Jaiven, 2020). Most research on produce safety concerning AFOs has primarily focused on water quality, with runoff from nearby animal operations posing a significant risk for microbial contamination, particularly in waters used for irrigation. (Suslow et al., 2003). While studies have highlighted potential solutions like buffer zones and vegetation barriers, there is uncertainty about whether these measures may inadvertently trap contaminated dust particles, potentially exacerbating issues for nearby produce operations (Gutierrez-Rodriguez and Adhikari, 2018). Unlike the well-studied pathways of waterborne transmission, there remains a significant gap in understanding the quantification of pathogens transmitted through bioaerosols from animal operations to adjacent fresh produce.

While research has been conducted on the microbiome of air inside of poultry houses, identifying a variety of microorganisms including *Campylobacter* and *Salmonella* (Andersen et al., 2022; Adell et al., 2014), less has been explored regarding the fate of microbes transported outside through ventilation. A few studies have investigated the presence of *Salmonella* and *E. coli* inside and outside of poultry houses, isolating positive samples from inside all houses, as well as outside up to 10 m from the ventilation fans (Davis and Morishita, 2005; Chinivasagam et al., 2009). The recovery of pathogens as bioaerosols outside animal operations can present a food safety risk, necessitating further research to determine their concentrations and the extent of their dispersion. Additionally, type and size of animal operation (i.e., broiler vs. layer chickens vs. cattle) impacts management practices which can in turn affect bioaerosol dispersion.

A major challenge associated with bioaerosol studies is the effect of extraneous environmental variables such as temperature, wind speed, and relative humidity. Since different regions of the U.S. and the world have different weather patterns and topography, meteorological impacts on bioaerosol transmission are likely to vary. Despite the large agricultural presence in the southeast U.S., there has yet to be a comprehensive study of bioaerosol spread from animal operations conducted in this region.

This study was designed to investigate the spread of bioaerosol pathogens like *Salmonella* and indicator organisms from commercial broiler operations in the southeast U.S. By measuring microorganism concentration at numerous set back distances and corresponding environmental parameters, these data can be used to help model bioaerosol pathogen spread while accounting for environmental conditions, and providing a comprehensive understanding of how bacterial laden bioaerosols behave in this region. Ultimately, this research will help inform guidance and risk assessments regarding set-back distances and potential mitigation strategies to safeguard nearby produce.

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CHAPTER 2

LITERATURE REVIEW

Fresh Produce Production in the United States

Overview

Specialty crops, defined as "fruits and vegetables, tree nuts, dried fruits and horticulture and nursery crops, including floriculture" (Agricultural Marketing Service, n.d.), play a vital role in U.S. agriculture. According to the latest agricultural census of 2022, more than 200,000 farms span over 70 million acres dedicated to cultivating these crops, contributing a market value exceeding $115 billion (National Agricultural Statistics Service [NASS], 2024b). In comparison, the poultry broiler and egg sectors were valued at over $75 billion (NASS, 2024c). This highlights the substantial contribution of specialty crops to the agricultural sector and their crucial role in sustaining and feeding our growing population.

California leads the U.S. in specialty crop production, due to its temperate climate and abundant sunlight. Based on the 2022 Sensus of Agriculture, California achieved a market value of $40.5 billion - in specialty crops sold. The southern region of the country, with its favorable weather conditions, is particularly well-suited for cultivating fruits and vegetables, where Florida, North Carolina, Texas, Arizona, and Georgia - make substantial contributions to the national market value of specialty crops, with respective market values of $7.2 billion, $2.3 billion, $2.3 billion, $2.2 billion, and $2.1 billion (NASS, 2024b). These regions are known for producing high-value agricultural commodities, including grapes, lettuce, tomatoes, apples, oranges, and almonds (California Department of Food and Agriculture, 2024; Wechsler, 2024).

Such crops play a crucial role in sustaining not only the U.S. but also international markets, underscoring the importance of these states in the broader agricultural landscape.

To promote a healthy diet, the World Health Organization (WHO) advises consuming a minimum of five servings of fruits and vegetables each day (Food and Agricultural Organization, n.d.). Numerous studies have demonstrated that a diet rich in fruits and vegetables supports immune function and helps prevent obesity, type 2 diabetes, and certain cancers (Boeing et al., 2012; Slavin et al., 2012). Despite these benefits, fruit and vegetable consumption in the U.S. remains alarmingly low. According to the 2019 Behavioral Risk Factor Surveillance System (BRFSS) data, only one in ten adults meets the recommended daily intake of fruits and vegetables (Lee et al., 2022). Over the past two decades, total fruit and vegetable availability estimates have demonstrated a declining trend. Annual vegetable availability was highest in 2004 at 415.4 pounds per capita and has since decreased to 365.9 pounds per capita as of 2022, decreasing 12 percent (Kantor and Blazejczyk, 2024). Similarly, per capita loss adjusted fruit availability has declined 14 percent from 2003 to 2021 (Young et al., 2025). To address this public health issue, various initiatives have been launched, employing a range of strategies including school-based programs, community interventions, food pricing strategies, and mass media campaigns (Wolfenden et al., 2021). As stakeholders continue their efforts to promote a healthier America, the goal is to significantly increase fruit and vegetable consumption across the population.

Associated Pathogens

As the distance between farms and consumers has increased, so too has the potential for foodborne contamination to occur. During the period of 1998-2013, 972 raw produce outbreaks were reported resulting in over 34,000 illnesses and 72 deaths. The most common etiological

agents were norovirus, causing over half of the infections, followed by *Salmonella enterica* and Shiga toxin-producing *E. coli* (Bennett et al., 2018). In more recent years, these pathogens have remained a significant threat to the produce industry, causing numerous outbreaks across a variety of produce commodities (Table 2.1). A recent CDC report on foodborne illness source attribution from 1998 to 2022 indicated that there were 1,010 reported outbreaks of *Salmonella*, with nearly 27% attributed to fruits and seeded vegetables. During the same period, there were 281 outbreaks of *E. coli* O157, of which 63.7% were linked to vegetable row crops such as leafy greens (CDC, 2024c). These findings highlight the ongoing risk *Salmonella* and *E. coli* pose to the produce industry and public health.

Table 2.1. Selected multistate foodborne outbreaks associated with *Salmonella* and Shiga toxin-producing *E. coli* in fresh produce in the United States over the past five years

Year	Pathogen	Produce Commodity	Reported # of Illnesses
2020	*E. coli* O103	Clover Sprouts[1]	51
2020	*Salmonella* Newport	Onions[2]	1127
2020	*Salmonella* Enteritidis	Peaches[3]	101
2020	*E. coli* O157:H7	Leafy Greens[4]	40
2021	*Salmonella* Oranienburg	Onions[5]	1040
2021	*E. coli* O157:H7	Baby Spinach[6]	15
2021	*E. coli* O157:H7	Packaged Salads[7]	10
2022	*Salmonella* Typhimurium	Alfalfa Sprouts[8]	63
2023	*Salmonella* Thompson	Fresh Diced Onions[9]	80
2023	*Salmonella* Sundsvall	Cut Cantaloupe[10]	407
2024	*Salmonella* Typhimurium	Basil[11]	36
2024	*E. coli* O157	Walnuts[12]	13

2024	*Salmonella* Africana	Cucumbers[13]	551
2024	*E. coli* O129:H19	Carrots[14]	48
2024	*Salmonella* Typhimurium	Cucumbers[15]	113

[1](CDC, 2020a) [2](CDC,2020c) [3](CDC, 2020d) [4](CDC, 2020b) [5](CDC, 2022c) [6](CDC, 2022b) [7](CDC, 2022a) [8](CDC, 2023d) [9](CDC, 2023c) [10](CDC, 2023b) [11](U.S. Food and Drug Administration [FDA], 2024b) [12](CDC, 2024c) [13](CDC, 2024f) [14](FDA, 2024a) [15](CDC, 2025)

Produce contamination has been found to occur both pre- and post-harvest. Major sources of pre-harvest contamination include manure, soil, irrigation water, atmospheric deposition, and contact with wildlife and small vectors such as insects (Alegbeleye et al., 2018; Beuchat, 2006). Once produce is contaminated, various factors determine the survival, growth, and potential of a pathogen to cause disease. Environmental conditions such as temperature, relative humidity, and sunlight play a critical role, along with ecological factors such as the presence of background microflora on the surface of the produce (Beuchat, 2002). Additional factors include the type of produce, the genotypes of cultivars within a commodity group, the specific zone of contamination, and any mechanical damage sustained (Critzer and Doyle, 2010). A study by Stine et al. (2005) revealed that relative humidity impacts pathogen survival differently on cantaloupe, bell peppers, and lettuce surfaces, depending on the pathogen and produce type. Notably, *E. coli* O157:H7 had a significantly higher inactivation rate in lettuce over a two-week period compared to cantaloupe and bell peppers, regardless of humidity levels. In contrast, on cantaloupe, both *E. coli* O157:H7 and *Salmonella enterica* subsp. *enterica* exhibited a considerably lower inactivation rate under humid conditions compared to dry conditions. These findings underscore the complexity of foodborne disease development during preharvest conditions and the varying interactions between pathogens and produce.

Moreover, contamination from the pre-harvest environment can persist and be spread during post-harvest activities, such as through equipment or handlers (Pérez-Rodríguez et al., 2014; Smolinski et al., 2018, Zhao et al., 2021). Post-harvest contamination is another major pathway through which foodborne diseases can be introduced to produce. After harvesting, produce undergoes various processes, that may include washing, cutting, grading, and packaging, each of which can introduce pathogens (Gil et al., 2015). The conditions present during processing and subsequent transportation further contribute to the survival and proliferation of unwanted microorganisms.

To mitigate the growth of pathogens once produce reaches consumers, maintaining appropriate storage conditions is essential. A study by Zeng et al. (2014) examined the effects of temperature fluctuations during the transport, storage, and display of bagged romaine lettuce, finding that populations of both *E. coli* O157:H7 and *Listeria monocytogenes* increased by approximately 2 log before reaching consumers. Similarly, research by Luo, He, and McEvoy (2010) showed that while *E. coli* O157:H7 survived without growing on lettuce stored at 5°C, it proliferated rapidly at 12°C, significantly increasing in number without compromising the lettuce's visual quality.

Given the complexities of the farm-to-fork continuum, a sustained emphasis on food safety across all stages is essential to control the burden of foodborne diseases. Despite the availability of training resources and stricter regulations, pathogens such as *Salmonella* and *E. coli* continue to affect our food system and pose a significant risk to public health globally. Understanding the specific challenges and mechanisms associated with these pathogens is vital in devising effective strategies for prevention and response.

Salmonella

General Characteristics

Bacteria of the genus *Salmonella* are Gram-negative, rod-shaped, non-sporulating, facultative anaerobes. They are distinguished by their peritrichous flagella, which aid in motility, adhesion, and biofilm formation (Crump and Wain, 2017; Yamaguchi et al., 2020; Horstmann et al., 2020). As mesophiles, *Salmonella* species are well-suited for life in the animal gut, thriving optimally at 37°C. *Salmonella* have been shown to grow within the range 4°C - 46°C, and at water activities above 0.94 (Billah and Rahman, 2024). Members of this genus are oxidase negative, catalase positive, hydrogen sulfide producing, and primarily utilize glucose as their carbon source. They are typically unable to ferment lactose or sucrose, however, they can efficiently utilize other carbon sources, including secondary metabolites produced by gut microflora (Fàbrega and Vila, 2013; Han et al., 2023). Furthermore, *Salmonella* spp. are particularly resistant to many stresses including desiccation, sanitizers, and physical stressors such as UV treatment (Morasi et al., 2022). This adaptability contributes to *Salmonella*'s ability to persist in a wide range of environments, making it a continued concern for public health.

Taxonomy

The *Salmonella* genus belongs to the family *Enterobacteriaceae* and was first isolated in 1855 by Theobald Smith and Dr. Daniel Elmer Salmon from the intestines of pigs exhibiting symptoms of swine fever (Eng et al., 2015). The genus consists of two species: *Salmonella bongori* and *Salmonella enterica.* While *S. bongori* is mostly found in cold blooded animals and is rarely associated with human infection, *S. enterica* is a much more complex and diverse species with greater clinical importance (Chattaway, Langridge, and Wain, 2021). The species can be divided into six subspecies: *enterica* (I), *salamae* (II), *arizonae* (IIIa), *diarizonae* (IIIb),

houtenae (IV), and *indica* (VI). The White- Kauffmann-Le Minor scheme designates a further subclassification, serotypes, which distinguish members of the same subspecies based on somatic (O), flagellar (H), and K (capsular) antigens on the surface of the bacteria. Over 2500 *S. enterica* serotypes have been discovered to date (Deng et al., 2014; Liu, 2017). Of the *Salmonella* subspecies, *S. enterica* subsp. *enterica* contains the most serotypes (1765) and is the most significant to human pathology (Issenhuth-Jeanjean et al., 2014; Lamas et al., 2018).

Epidemiology and Disease

Salmonella has a diverse host range, from insects and reptiles to birds and mammals. *Salmonella* serotypes vary not only in physiology, but also in host specificity and adaptability. Some serotypes, like *S.* Typhimurium and *S.* Enteritidis, are considered generalists because they can infect a broad array of hosts including humans, rodents, poultry, cattle, equine, and swine. These serotypes typically cause self-limiting gastroenteric disturbances and rank among the top five *Salmonella* serotypes responsible for foodborne illness in humans (CDC, 2023; Cheng et al., 2019; Ferrari et al., 2019; Silva et al., 2014). Other *Salmonella* serotypes are classified as host-restricted, affecting a limited range of hosts. Examples include *S.* Typhi, which is specific to humans; *S.* Gallinarum, associated with poultry; and *S.* Dublin, which primarily affects cattle. However, microorganisms are constantly adapting, and their host specificity can shift over time. Although *S.* Dublin is primarily cattle-adapted, there have been rare instances where it has infected humans. In these cases, the symptoms were significantly more severe, including bloodstream infections and fatalities (Harvey et al., 2017). In many animals, including cattle, poultry, and rodents, *Salmonella* often exists as a commensal organism, capable of colonizing the gut without causing any symptoms of disease (FDA, 2023). These asymptomatic carriers can act as reservoirs of infection, posing a risk of transmission to humans through fecal shedding.

Based on clinical manifestations of human salmonellosis, strains may either be classified as typhoidal *Salmonella* or non-typhoidal *Salmonella* (NTS). While both can be obtained through foodborne transmission, their expression differs significantly. Typhoidal *Salmonella* consists of host-restricted strains *S.* Typhi, and *S.* Paratyphi A, B, and C, resulting in a combination of distinct symptoms known as enteric fever (Eng et al., 2015). These typically include an incubation period of around 14 days followed by gradual onset of sustained fever lasting three or more weeks. Other symptoms include abdominal pain, constipation and diarrhea, enlarged spleen and liver, and a rosy rash on the chest or abdomen (Kuvandik et al., 2009; Gal-Mor, Boyle and Grassl, 2014). While enteric fever is endemic in the developing world, primarily due to inadequate access to sanitary water, it remains rare in the U.S.. Within the U.S. the CDC estimates that approximately 5,700 individuals are infected with enteric fever annually, with most cases occurring in people returning from international travel (CDC, 2024g).

In contrast, NTS is the most common bacterial foodborne disease in the U.S., infecting approximately 1.35 million Americans annually (Francois Watkins et al., 2024). Most NTS infections are self-limiting, featuring a brief incubation period of less than a day. In healthy adults, the infectious dose is approximately 1 million cells, with symptoms including gastroenteritis, fever, and abdominal pain that last two to seven days. However, in young children, the elderly, pregnant women, and immunocompromised individuals, the infectious dose is significantly lower, and infections may progress to systemic conditions that can be potentially life-threatening. (WHO, 2018). Based on data for 1998-2008 provided by the CDC's Foodborne Disease Outbreak Surveillance System, a majority of NTS infections in the U.S. were caused by *S.* Enteritidis (36%), followed by *S.* Typhimurium (14%) and *S.* Newport (10%). These outbreaks were most commonly implicated with eggs, chicken, and fruit (Jackson et al., 2013). Today, the

most frequently reported *Salmonella* serotypes are *S.* Enteritidis and *S.* Newport, followed by *S.* Typhimurium, *S.* Javiana, and *S.* I 4,[5],12:i: (Shah et al., 2024). More than 75% of these illnesses could be attributed to seven food categories: chicken, fruits, seeded vegetables, pork, other produce (i.e. nuts), beef, and turkey (CDC, 2024d).

Several well-known virulence factors help make *Salmonella* a successful pathogen. Most *S.* Typhi strains express a capsular polysaccharide antigen (Vi) which helps prevent identification and phagocytosis from immune cells (Wain et al., 2005). While NTS strains do not express the Vi antigen, other mechanisms assist in their pathogenesis. *Salmonella* are equipped with an acid tolerance response which can be induced when the extracellular pH is unfavorable, such as in the highly acidic environment of the stomach. Modulation of certain cellular proton pumps allow *Salmonella* to maintain intracellular homeostasis to survive before crossing the mucus layer and attaching to the epithelium wall (Foster and Hall, 1991; dos Santos, Ferrari, and Conte-Junior, 2019). After adhesion, *Salmonella* releases effector proteins with the help of Type III Secretion System 1 (T3SS-1) which cause cytoskeletal rearrangements. This results in membrane ruffles which engulf the bacteria into *Salmonella*-containing vesicles (SCVs) where they can grow and multiply (Finlay, Ruschkowski, and Dedhar, 1991; dos Santos, Ferrari, and Conte-Junior, 2019).

Salmonella pathogenicity islands (SPIs) encode many of the virulence factors involved in infection. While many SPIs exist in the genome, SPI-1 and SPI-2 are the most widely studied, encoding for proteins assisting in invasion, colonization, and evasion of host defenses (Luo et al., 2019; Pico-Rodríguez et al., 2024) *Salmonella* serovars may also contain *Salmonella* virulence plasmids, collectively referred to as pSV plasmids. These extrachromosomal regions encode genes which play a critical role in host infection (Khajanchi and Foley, 2022; Silva et al., 2017).

As a consequence of invasion, the immune system activates a pro-inflammatory cell-death response known as pyroptosis to break down SCVs and eliminate the intracellular niche of the pathogen. Through this process, the epithelial inflammation helps to promote diarrhea and ultimately flush the pathogen from the system (Broz, Ohlson, and Monack, 2012).

Escherichia coli

General Characteristics

E. coli are Gram-negative, rod-shaped, non-spore-forming facultative anaerobes. Typically motile, they possess peritrichous flagella and are equipped with fimbriae, small hair-like protein structures that play a crucial role in cell adhesion. This is particularly important in the gastrointestinal tract, where *E. coli* is ubiquitous (McClure, 2005; Percival and Williams, 2014; Aijuka and Buys, 2019). As mesophiles, *E. coli* thrive in the gut at an optimal temperature of 37°C. *E. coli* typically survive well at refrigeration temps, but do not exhibit measurable growth until 7.5°C, with some strains growing at temperatures up to 53°C (Tuttle et al., 2021). While they prefer neutral conditions (pH 6.5-7.5), *E. coli* can grow in the pH range 4.4-9.0 in high moisture environments (Suehr et al., 2020). Their preferential carbon source is glucose, but *E. coli* will ferment lactose and sorbitol in some cases and will also produce indole during metabolism (Basavaraju and Gunashree, 2022). Many biological functions of indole have been discovered in *E. coli* including intracellular signaling, drug resistance, plasmid stability, virulence control, and biofilm formation (Han et al., 2011). Furthermore, *E. coli* is recognized for its genetic diversity, thriving in various niches as both a commensal and pathogenic organism.

Taxonomy

Like *Salmonella*, *E. coli* belongs to the family *Enterobacteriaceae.* Although these genera share significant similarities in their housekeeping genes, the differential regulation of certain orthologous genes has facilitated their divergence (Winfield and Groisman, 2004; Meysman et al., 2013). *E. coli* was first identified in 1884 when Theodor Escherich isolated it as a common commensal bacterium in the intestinal tract of neonates. Since then, advancements in molecular techniques have resulted in fluctuations in the number of species within the genus, with some being reclassified into entirely new genera, such as *Leclercia adecarboxylata* (formerly *Escherichia adecarboxylata*). Today, the genus *Escherichia* comprises four recognized species: *E. albertii, E. marmotae*, *E. fergusonii,* and most importantly *E. coli* (Yu, Banting and Neumann, 2021). *E. coli* can further be classified into eight major phylogroups: A, B1, B2, C, D, E, F, G (Lagerstrom and Hadley, 2023) These groups differ in lifestyles, niches, phenotypic characteristics, and propensity to cause disease, accounting for the immense diversity within the species.

Epidemiology and Disease

A majority of *E. coli* strains are commensal, inhabiting the guts of humans, food-animals, and environmental niches such as soil and groundwater. Intestinal *E. coli* are found in a thin layer of mucus that lines the gut, where they aid in digestion, vitamin production, and maintaining homeostasis within the gut microflora (Blount, 2015). Their ubiquity makes them a great indicator for fecal contamination.

Indicators of fecal contamination have been utilized for over a century, tracing back to 1897 when the American Public Health Association established standard procedures for the coliform test in drinking water (National Research Council (US) Committee on Indicators for

Waterborne Pathogens [NRCCIWP], 2004). Coliforms are a group of Gram-negative, rod-shaped, non-spore-forming bacteria, including *E. coli*, which can ferment lactose, leading to the production of acid and gas at temperatures between 35-37°C (Martin et al., 2016). Other genera within this group, such as *Erwinia*, *Enterobacter*, and *Klebsiella*, may not originate from fecal matter. Consequently, testing methods evolved to specifically target fecal coliforms, which require a higher incubation temperature for more accurate detection (Brackett, 1993) However, numerous false positives continued to be an issue with fecal coliform tests, prompting a shift toward the specific identification of *E. coli* as the definitive fecal indicator. This was achieved using the substrate 4-methylumbelliferyl-β-D-glucuronide (MUG), which reacts with β-glucuronidase, an enzyme uniquely present in *E. coli* (NRCCIWP, 2004).

A smaller portion of the *E. coli* species are pathogenic; however, they represent a major cause of diarrheal disease globally. Pathogenic *E. coli* strains can be differentiated into pathotypes depending on their genotypes, pathologies and hosts (Kaper, Nataro, and Mobley, 2004). Six different categories of diarrheagenic *E. coli* are recognized and differ in their host colonization sites, virulence mechanisms, and clinical symptoms (Table 2.2).

Table 2.2 Diarrheagenic *E. coli* pathotypes and their characteristics[1]

Pathotype	Clinical Presentation	Groups Most Affected	Typical Genetic Markers
Enteropathogenic *E. coli* (EPEC)	Watery diarrhea, fever, vomiting and dehydration	Children less than 1 year	LEE, *eae, bfp*
Enterohemorrhagic *E. coli* (EHEC)	Bloody diarrhea, fever, abdominal cramps, and vomiting	Children under 5; adults aged 65 and older	*stx1* or *stx2*, LEE
Enterotoxigenic *E. coli* (ETEC)	Watery diarrhea, stomach cramps, vomiting	Younger adults (18-35 years) and international travelers	heat labile enterotoxin (LT)

Enteroinvasive *E. coli* (EIEC)	Watery diarrhea that is sometimes bloody, fever, and potential damage to intestine walls	Children, international travelers	invasion plasmid pINV, lack of flagella
Enteroaggregative *E. coli* (EAEC)	Watery diarrhea that has mucus, sometimes vomiting	Children, people living with HIV, international travelers	pAA virulence plasmids containing genes for aggregative adherence fimbriae
Diffuse-adhering *E. coli* (DAEC)	Persistent watery diarrhea	Children (3-5 years)	Afa/Dr adhesins

[1]Adapted from Geurtsen et al., 2022 and CDC, 2024a

EHEC is the pathotype most commonly associated with multistate foodborne outbreaks in the U.S.. This group is commonly referred to as Shiga toxin-producing *E. coli* (STEC) due to their ability to excrete the Shiga toxin. Compared to other STEC infections, EHEC are most severe, with the ability to cause hemorrhagic colitis (Bertoldi et al., 2018; Taylor, 2008) In the U.S. STEC results in over 97,000 illnesses per year, primarily attributed to the serotype *E. coli* O157:H7 (CDC, 2024b). However, other non-O157 serotypes, such as O121:H19 and O103, have also demonstrated the capacity to cause a similar progression of disease and have been implicated in multistate outbreaks linked to produce over the past five years (CDC, 2020a; FDA, 2024a).

The infectious dose for STEC is estimated to be less than 100 organisms. Once ingested, *E. coli* use their innate acid-resistance mechanisms to survive the unfavorable conditions in the stomach (Nguyen and Sperandio, 2012). *E. coli* then move to the large intestine, forming attaching and effacing lesions on the mucosal epithelium. Formation of these lesions is controlled by genes encoded on the pathogenicity island called locus for enterocyte effacement

(LEE). Also encoded on the LEE is the T3SS used to inject effector proteins into the host cell (Martín-Rodríguez, Joffré, and Sjöling, 2022).

STEC are characterized by their ability to form Shiga-toxins which induce apoptosis in affected cells. After attachment, expression of *stx* genes leads to the release of toxin into the intestinal lumen and onto enterocytes. The toxins bind to cell-surface globotriaosylceramide (GB_3) receptors, removing a specific adenine residue from RNA, thereby inhibiting protein synthesis and ultimately resulting in cell death. Considerable damage to the endothelium through this mechanism contributes to the bloody stools, characteristic of this disease (Karpman and Ståhl, 2014).

In most cases, STEC infections are self-limiting, and patients typically recover with supportive fluid therapy (WHO, 2018) However, in rare instances, sequelae may follow, with infections progressing to life-threatening conditions. One of these conditions, hemolytic uremic syndrome (HUS), can lead to renal failure and anemia. It is estimated that 5-15% of individuals infected with STEC, primarily children, will develop HUS (National Organization for Rare Disorders, 2016). The onset of HUS is believed to be mediated by stx-induced damage to renal endothelial cells (Melton-Celsa et al., 2011). Surface lipopolysaccharides (LPS) may also enter systemic circulation, triggering an inflammatory response that upregulates the expression of renal GB_3 receptors, further contributing to the potential onset of HUS (Zoja, Buelli, and Morigi, 2010).

Typically, the use of antibiotics is not recommended for STEC infections, as they can exacerbate the release of Stx and increase the likelihood of HUS (Wong et al., 2012). Another rare but potentially life-threatening condition associated with STEC infection is Thrombotic Thrombocytopenic Purpura (TTP), which affects approximately 1 to 2 cases per million people

(Trachtman, 2013). Although TTP shares similar symptoms with HUS, the two conditions differ in terms of pathophysiology and demographics, with TTP being more commonly observed in adults (Tarr et al., 2009). Understanding the mechanisms of toxicity and the risks associated with treatment options is vital for effectively managing these infections and preventing severe outcomes. Since these pathogens have natural reservoirs in many common feed animals in our food system, the likelihood of outbreaks continuing to occur remains high.

Animal Feeding Operations

Overview

To meet the growing food demands of the past century, large-scale animal operations have become increasingly common across the U.S., moving away from the traditional small farm model. According to federal regulations, animal feeding operations (AFOs) are defined as agricultural operations where animals are confined and fed for at least 45 days within a 12-month period. Additionally, these operations must not support crop growth regularly (EPA, 2024) Depending on the type of animal operation, certain size thresholds established by the EPA can designate an AFO as a concentrated animal feeding operation (CAFO). These facilities are regulated under the National Pollutant Discharge Elimination System (NPDES) permit system, which aims to protect the nation's water resources (EPA, 2015). Currently, about 99% of meat and animal products consumed in the U.S. originate from AFOs (Walton and Jaiven, 2020).

In the Southeastern U.S., broiler production is a pivotal segment of the animal agriculture industry. U.S. poultry products lead both domestic and international markets, providing nearly all of the nation’s chicken meat (Economic Research Service, 2025). In 2023, the U.S. produced approximately 9.16 billion broilers, with Georgia, Alabama, and North Carolina accounting for around 38% of this total, housing billions of broilers in confinement until they were ready for

slaughter (NASS, 2024a). While these operations are vital for sustaining the population, a growing body of research has highlighted their negative impacts on both the environment and human health.

AFOs and Human Health

An estimated 133 million tons of manure are produced annually in the U.S. from AFOs (on a dry weight basis), containing contaminants such as pathogens, pharmaceuticals, and heavy metals. These contaminants can enter the environment through soil, water, or air, resulting in adverse health effects for both wildlife and humans (Burkholder et al., 2006). Due to certain animal husbandry practices, antibiotic resistance genes (ARGs) can be found in livestock waste, even following proper treatment. Antibiotics may be administered to entire flocks to treat and prevent disease, as well as to promote growth. Since 2017, the FDA has introduced regulations through the Veterinary Feed Directive, requiring veterinary oversight for the use of medically important antibiotics (Roth et al., 2018; Wallinga et al., 2022). Despite this, studies examining the persistence of ARGs after these and other similar regulations have indicated an increase in ARG detection in the years following the reduction in antibiotic use (Lynch et al., 2020; Wen et al., 2022).

Water quality is another concern for locations near AFOs. During adverse weather events, such as flooding, animal waste and wastewater can introduce pollutants into surface water and the surrounding environment, negatively impacting recreational water sources. This contaminated water may also infiltrate groundwater, impacting drinking water systems as well CDC, 2024e; Meyer et al., 2024). Additionally, water contamination can extend to nearby produce operations, potentially leading to multistate outbreaks, as evidenced by the 2019 E. coli O157:H7 outbreak linked to romaine lettuce (FDA, 2020).

The mechanisms by which AFOs impact nearby soil and water microbial communities are not fully understood and likely depend on various factors, including the type of animal operation, the robustness of the ecosystem, and meteorological conditions. Nevertheless, AFOs serve as an important reservoir for many foodborne pathogens, and the mechanisms through which contamination occur warrant further research.

There is broad research consensus that AFOs adversely affect air quality. The confinement of large numbers of animals generates emissions such as ammonia, volatile organic compounds, and bioaerosols, which consist of airborne microorganisms and their byproducts, including endotoxins (Hornbuckle, 2002). Considerable research indicates that consistent exposure to air in and around AFOs increases the likelihood of respiratory illnesses. Schultz et al. (2019) found that living within three miles of AFOs was linked to reduced lung function, asthma, and allergies. Workers inside AFOs face heightened risks, with one in three hog confinement workers experiencing chronic or intermittent lower respiratory tract symptoms (Von Essen and Auvermann, 2005). Additionally, a study in swine AFOs revealed that 98% of airborne isolates of *Enterococcus, Staphylococcus*, and *Streptococcus* exhibited high-level multi-drug resistance, suggesting that inhalation could be a pathway for the transmission of antibiotic resistance genes (Chapin et al., 2004).

While less research has focused on the effects of air pollution from AFOs on food safety, this area is deserving of attention. Studies examining the airborne microbiome of various AFOs reveal the presence of foodborne pathogens, including *Salmonella* and *Campylobacter* (Gast, Mitchell, and Holt, 2004; Smith and King, 2023). Moreover, recent foodborne outbreaks, such as the 2020 *Salmonell*a Enteritidis outbreak linked to peaches, have been potentially associated with contamination from fugitive dust originating from nearby animal operations (Center for Food

Safety and Applied Nutrition, 2021). This highlights the urgent need for further research on bioaerosols to assess their risks to food safety.

Measuring Bioaerosol Transmission

Sampling Methods

While there is no standard protocol for the sampling of bioaerosols, there are two overarching approaches: active and passive sampling. Both include a variety of methods which alter the final metric obtained, often making it difficult to compare the results of different studies. Types of active sampling include impingement, impaction, filtration, and cyclone separation (Gollakota et al., 2021). Active sampling allows for the quantification of microbes per unit volume of air (i.e. CFU/m^3), allowing one to sample a large volume of air in a short amount of time. There are, however, several downfalls, including physical stress and desiccation (Ghosh, Lal, & Srivastava, 2015). Types of passive sampling include gravity sampling and electrostatic precipitation methods. Unlike active sampling, passive techniques can only enumerate bacteria per unit area per unit of time (i.e. CFU/m^2s), and sample much less air over a given timeframe. Some advantages of passive sampling include the reduced physical stress on microorganisms and low cost (Manibusan & Mainelis, 2022). In their study involving experimentally infected laying hens, Gast et al. (2004) assessed the effectiveness of three different sampling methods for recovering *Salmonella*. The results indicated that the electrostatic precipitator outperformed the settle plate method significantly, while no notable differences were found between the electrostatic precipitation method and the impaction method. Conversely, in a comparative study conducted by Karigoudar et al. (2020), the Air Petri sampling system (impaction) was evaluated alongside the settle plate method in operating theaters, with both methods being run concurrently. The findings revealed that the passive settle plate method significantly

outperformed the active impaction method ($p = 0.0336$) in terms of the total colony-forming units (CFU) of bacteria and fungi recovered per sampler. These studies illustrate that the choice of sampling technique can lead to differing results.

Bioaerosol Transmission from AFOs

Several studies have investigated the transfer of bioaerosols from animal operations using a combination of methods. Berry et al. (2015) explored the impact of proximity to a cattle CAFO on airborne *E. coli* transmission. Using Mas-100 Eco samplers, a type of impaction method, researchers collected samples at distances of 0, 60, 120, and 180 meters from the feedlot. While they were unable to isolate *E. coli* O157:H7, the target pathogen, they did recover generic *E. coli* at all distances. Notably, concentrations of *E. coli* were significantly higher at the edge of the feedlot (0m) compared to further distances. However, there was no significant decrease in *E. coli* concentrations with increasing distance, which ranged from below the limit of detection to 5.3 CFU/m^3 of air at 180 meters. Additionally, the study revealed the presence of STEC on leafy green surfaces at both 60 and 180 meters from the source. While air deposition was not confirmed as the mechanism of transfer, the lower quantities of STEC compared to indicator organisms in the feedlot manure may help to explain its absence in the air samples.

The spread of bioaerosols is not limited to cattle. Davis and Morishita (2004) measured ammonia and dust concentrations, as well as the presence of aerosolized *Salmonella* and *E. coli* inside and outside five commercial poultry layer facilities. Notably, ammonia concentrations measured outside the facilities decreased with increasing setback distance. However, at all five farms, ammonia levels were higher at 3 m away from the facility than those recorded inside the houses. Microbial samples were obtained 3, 6, and 12 m away from the ventilation fan using high-purity glass microfiber filters, an active sampling method. Both *Salmonella* and *E. coli* were

isolated from the air filters at all five farms, inside the house as well as outside up to 12 m. While the researchers confirmed the presence of these microorganisms, they did not report their concentrations, indicating a need for further research to quantify these pathogens at greater distances.

Source identification is an important aspect of bioaerosol studies. Duan et al. (2009) investigated airborne *E. coli* in swine houses and their surrounding areas using Andersen 2-stage impact samplers for indoor sampling and Reuter-centrifugal samplers for outdoor sampling. Samples were collected at various locations, including 10 and 50 m upwind from the swine houses, as well as 10, 50, 100, 200, and 400 m downwind of the facilities. Additionally, fecal samples were collected for comparison with airborne samples to facilitate source identification. A total of 19 *E. coli* isolates were recovered from downwind air at distances of 10, 50, and 100 m. Notably, 36.8% of the recovered bacteria displayed 100% genetic similarity with corresponding indoor air and fecal samples. In contrast, upwind samples showed low genetic similarity, indicating a direct transmission of bioaerosols from the swine houses in a downwind direction.

Extraneous variables, including meteorological factors, time of day, vegetation barriers, and dust-generating activities, influence the transmission of bioaerosols from animal operations. Wei et al. (2023) examined the impact of distance from beef cattle feedlots and various environmental factors on the presence of foodborne pathogens and bacterial indicators in the Imperial Valley, California. Using Mas-100 Eco Samplers, researchers collected samples at distances ranging from 15 -1609m from the feedlot. While no pathogens were recovered, indicator *E. coli* was isolated at all distances. Enriched samples exhibited no significant difference in prevalence beyond 122m. Logistic regression models indicated that morning air

samples, characterized by low relative humidity and low wind speed, a function of the arid desert location, along with dust-generating activities, were associated with higher odds of airborne *E. coli*. This underscores the intricate dynamics of bioaerosol transmission and emphasizes the need for further studies that consider factors beyond just setback distances.

Factors Influencing Bioaerosols Transmission

Type of Particles

Bioaerosols from animal operations are typically thought to be dry particles. Fugitive dust from barns and/or poultry houses can carry bacterial particles through the air (Nguyen et al., 2022). Particulate matter plays an important role in the characterization of dust, most often distinguished by its size. Fine particulate matter, $PM_{2.5}$, consists of air particles less than 2.5 μm in diameter. Many bacteria, including *E. coli* and *Salmonella* fall into this size range, and can contribute the particulate matter composition. Estimates indicate that bioaerosols account for 13.7 ± 12.5% of $PM_{2.5}$ mass, though this can change significantly depending on location, season, and anthropogenic factors (Zheng et al., 2022). On the other hand, coarse particulate matter, PM_{10}, consists of air particles less than 10 μm in diameter. While these particles are larger, primarily consisting of dust and pollen, fungal spores and plant debris, they are still small enough to be inhalable into the lungs and induce adverse health effects (Hyde and Mahalov, 2020). Wei et al., (2020) demonstrated that composition of particulate matter has direct effects on airborne microbial growth. Their findings revealed that bacterial concentrations peaked during moderate pollution levels but decreased during heavy or severe pollution events. Moreover, they found that microbial diversity increased significantly with rising air pollution.

Wet aerosolization is an aspect of agricultural bioaerosols that receives less attention, yet it is important. Water droplets carrying microorganisms likely behave differently from dry

particles and warrant further study. Hutchison et al. (2008) investigated pathogen transmission following the application of pig slurry and discovered that marked pathogens could spread up to 125 meters from the spray site. Similarly, Cevallos-Cevallos et al. (2012) examined the potential for contaminated irrigation puddles to aerosolize after a brief rain event and found that *Salmonella* could be recovered from air samples at least 85.5 cm from the source, posing a risk to low-hanging produce like tomatoes and peppers. These findings suggest that bioaerosols can significantly impact produce safety, making this an important area for further research.

Meteorological Conditions

Studies analyzing bioaerosols in urban environments have sought to understand the relationship between meteorological parameters and bioaerosol concentrations. Many studies have found that seasonality significantly affects recoverable airborne microorganisms, with counts typically decreasing during the summer months when UV radiation is more extreme and unfavorable for bacterial survival (Bai et al., 2021; Balyan et al., 2019; Gao et al., 2015). Relative humidity also plays a critical role in bioaerosol survival. In a study by Stiller et al. (2024) examining aerosolized *S. aureus* and *G. stearothermophilus* spores at varying relative humidities, bacterial mortality was significantly linked to increases in relative humidity above 60%. Additionally, relative humidity impacts the aerodynamics of particulate matter. High relative humidity can inhibit dust lifting and alter diffusion behavior, but it may also facilitate dry deposition (Huang et al., 2024). Wind speed also affects microbial survival and aerodynamics. High winds can raise dust from various surfaces and facilitate further transmission of bioaerosols into the surroundings. However, strong winds may also bring in exogenous bacteria, diluting the concentration of local bacteria (Zhen et al., 2017). The effects of wind are variable and likely depend on a multitude of environmental factors.

While bioaerosols may behave differently in agricultural environments, the fundamental principles of meteorological effects remain applicable. In their study of bioaerosol concentrations near a cattle feedlot in Neuquén, Argentina, Cogliati et al. (2022) found the highest airborne *E. coli* concentrations in the summer, when average ground temperatures reached 39.5°C, accompanied by low relative humidity, minimal rainfall, and a light breeze. Similarly, Sanz et al. (2015) observed significantly elevated *E. coli* concentrations during the summer months when temperatures were high and relative humidity was low. This study also highlighted the crucial role of wind direction in the recovery of airborne microorganisms. In contrast, Wei et al. (2023) identified no significant association between the detection of airborne *E. coli* and relative humidity or temperature. However, they found detection rates were significantly linked to low wind conditions, with the likelihood of detection decreasing by 0.52 times for every additional meter of wind speed. Given the complexity of the relationship between bioaerosols and the environment, studies should incorporate *in-situ* weather stations to obtain the most accurate and up-to-date data.

Conclusion

The critical intersection between animal feeding operations (AFOs), particularly poultry production, and the safety of fresh produce highlights significant challenges within the agricultural landscape. As nearly 99% of meat and animal products consumed in the U.S. originate from these large-scale operations, their influence on public health and food safety cannot be overstated. Poultry AFOs generate substantial quantities of manure, which, when mishandled, contribute not only to nutrient runoff and water contamination but also create bioaerosols rich in pathogens, including *Salmonella* and *E. coli.* These airborne microorganisms

can disperse beyond the confines of AFOs, posing significant risks to nearby crops and enhancing the potential for foodborne outbreaks.

The complexities surrounding bioaerosol transmission from poultry AFOs to produce underscore the urgent need for comprehensive research into their environmental impacts and the mechanisms influencing pathogen dispersal. As studies have demonstrated, various factors, including meteorological conditions and particulate matter composition, significantly affect the viability and spread of these bioaerosols. Understanding these dynamics is essential for devising effective strategies that ensure food safety, protect public health, and safeguard the integrity of the fresh produce supply. As the agricultural landscape continues to evolve, ongoing efforts to monitor and mitigate the impact of bioaerosols generated from livestock operations, particularly in relation to produce, will play a vital role in fostering safer and more sustainable farming practices.

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CHAPTER 3

INVESTIGATING BIOAEROSOL TRANSFER OF AND *ESCHERICHIA COLI* AND OTHER COLIFORMS FROM CONCENTRATED POULTRY OPERATIONS IN THE SOUTHEASTERN UNITED STATES USING A PASSIVE SAMPLING APPROACH[1]

[1] Greenbaum, H., Chévez, Z., Tobar Sandoval, E., Cruz, V. H., Rodrigues, C., Mishra, A., Kapoor, H., Thippareddi, H., Den Bakker, H., Mann, A., Singh, M., and Critzer, F. To be submitted to *Journal of Food Protection*.

Abstract

Recent outbreaks of fresh produce have raised concerns about bioaerosol contamination from nearby animal operations, underscoring the need for further research. This study investigated the spread of bioaerosol fecal indicator organisms from commercial broiler farms in the Southeastern U.S. using passive sampling. Four farms in Georgia and Alabama were sampled three times each over one flock cycle (n=12 visits). Cheesecloths (91.44 x 45.72 cm, grade 90 mesh) were tautly positioned on wooden stakes (0.4–0.9 m height), and at distances of 0, 50, 100, 400, and 1,000 meters from exhaust fans. Cheesecloths at 0, 50, and 100 m were paired with weather stations measuring temperature, humidity, wind speed, gusts, and wind direction, alongside particulate matter sensors. Samples were stomached with buffered peptone water and Tween 80, then plated on Brilliance™ coliform/*E. coli* agar, incubated at 37°C for 48 h for microbial enumeration. Proximity to the exhaust fan corresponded with the highest *E. coli* concentrations (5.88 ± 0.51 log CFU/sampler). A statistically significant reduction in *E. coli* populations was detected only at a setback distance of 100 m (3.97 log CFU per sampler) ($p = 0.0037$). No significant correlations were observed between *E. coli* and air temperature, relative humidity, or wind parameters, however there was a significant correlation between *E. coli* and PM_{10} at 50 m ($r = 0.7292$; $p = 0.0167$). These data can inform models of bioaerosol spread considering environmental factors, enhancing understanding of bacterial bioaerosol behavior in this region.

Keywords: Bioaerosol, particulate matter, passive sampling, poultry operations

Highlights:

- *E. coli* bioaerosols significantly decreased at setback distances beyond 100 meters.

- No significant correlations were detected between *E. coli* concentrations and relative humidity, air temperature, or wind parameters.
- Coarse particulate matter (PM_{10}) and *E. coli* were significantly correlated at the 50 meter setback distance.

Introduction

In recent years, the U.S. has experienced numerous multistate outbreaks of foodborne *Salmonella* associated with fresh produce. Investigations conducted by regulatory agencies have highlighted concerns regarding adjacent land use to animal operations, emphasizing the potential for pathogens to be transferred through the air in the form of bioaerosol particles (U.S. Food and Drug Administration [FDA], 2024). In the 2020 *Salmonella* Enteritidis outbreak linked to peaches, several *Salmonella* isolates from samples of peaches and peach tree leaves demonstrated genetic similarities to historical isolates from chickens and cattle. FDA traceback investigations did not identify the outbreak strain through whole genome sequencing (WGS) analysis; however, four samples positive for *Salmonella* Alachua were collected from an orchard adjacent to a poultry operation. Geospatial analysis of the affected orchards combined with WGS suggested that contamination could have occurred through fugitive dust from nearby animal operations (FDA, 2021a). That same year, a notable outbreak linked to *Salmonella* Newport in red onions resulted in over a thousand reported illnesses; this was particularly striking as whole red onions had not previously been associated with foodborne illness. Investigators identified sheep grazing on adjacent lands as a potential contributing factor to this contamination, although a definitive root cause was not established (FDA, 2021b). Most recently, in 2022, cantaloupes were contaminated with *Salmonella* Typhimurium, resulting in 87 reported illnesses across 11 states. On-farm sampling detected genetically similar *Salmonella* isolates to the outbreak strain, as well as other previous outbreak strains and poultry isolates. The traceback investigation also revealed several poultry feeding operations in the affected area, though their specific contributions to the outbreak remain undetermined (FDA, 2023).

Poultry are a well-documented reservoir for *Salmonella* (Foley et al., 2011; Nair and Kollanoor Johny, 2019; Naumovska et al., 2025; Park et al., 2008; Siceloff et al., 2022; Velasquez et al., 2018). Studies examining the microbiome of the air within poultry houses have identified a range of microorganisms, including *E. coli* and *Salmonella,* which are present as bioaerosol particles in recoverable concentrations. (Andersen et al., 2022; Adell et al., 2014; Gast et al., 2004; Smith and King, 2023). However, less research has been focused on the fate of microbes transported outside through ventilation.

A few studies have examined the presence of *Salmonella* and *E. coli* both inside and outside broiler houses using various active sampling methods, successfully isolating positive samples from within all houses and from locations up to 155 meters away from the ventilation fans (Chinivasagam et al., 2009; Davis and Morishita, 2005; Theofel et al., 2020). Nonetheless, the restricted sampling distances may not fully capture the potential impact of poultry operations on the contamination of nearby produce fields. Furthermore, the enrichment methods employed in these studies do not yield definitive information on the quantities of specific microorganisms transported outdoors; they merely confirm their presence. Active sampling methods also introduce several challenges for pathogen recovery, such as physical stress and desiccation (Ghosh et al., 2015). In contrast, studies utilizing passive sampling approaches from animal operations have demonstrated quantifiable recovery of airborne microorganisms (Cogliati et al., 2022; Jahne et al., 2016; Kostadinova et al., 2014; Rittscher et al., 2023). In addition to minimizing physical stress, passive methods are often cost-effective, low-maintenance, and highly flexible (Manibusan & Mainelis, 2022).

Hence, this study was designed to investigate the spread of fecal indicator organisms from commercial broiler operations in the southeast U.S. using a passive sampling approach. By

measuring microorganism concentration at numerous set back distances and corresponding environmental parameters, these data can be used to help model fecal indicator organism spread while accounting for environmental conditions, providing a comprehensive understanding of how bacterial laden bioaerosols behave in this region. Ultimately, this work can serve as the basis for more complex work that is *Salmonella*-specific, helping inform guidance and risk assessments regarding set-back distances and potential mitigation strategies to safeguard nearby produce.

Materials and Methods

Poultry Farm Sampling Sites. Commercial poultry farms were located in the Southeastern U.S. in Alabama (N=3) and Georgia (N=2). The farms each contained between four and eight broiler houses (18 x 183 m) and varied in land size and use. Each house contained approximately 30,000 broilers. Houses were equipped with ventilation systems to maintain temperature, moisture, and dust conditions for the confined animals, with 8-12 exhaust fans (1.5 m) placed on one end of the houses, expelling air at an average rate of 708 cubic meters per minute when fans were on. Due to extreme weather events, one farm was inaccessible for the majority of sampling visits and therefore excluded from further analysis. Identities of the farms were kept confidential.

Air Sampling Methodology. Each farm was visited three times over the course of one flock cycle, approximately three, five, and seven weeks after hatching (n = 12 visits). Microbial populations deposited by air were determined using a passive sampling approach. Cheesecloths (91.44 cm x 45.72 cm) (Sceng™, Shenzhen, Guangdong, China), grade 90 mesh, were positioned tautly on wooden stakes, with a vertical height of 0.4–0.9 m above the ground at one of five setback distances: 0, 50, 100, 400 or 1000 m away from one of the poultry house exhaust

fans. Cheesecloths at 0, 50 and 100 m were accompanied by a weather station (Hobo™, Onset, Bourne, MA) measuring air temperature, relative humidity, wind speed, gust speed, wind direction. Separate sensors were deployed to measure particulate matter concentration (GatorGasp, UF, Gainesville, FL) at these distances. Cheesecloths and weather stations were set for 12 h (overnight) to collect samples. Cheesecloths were placed in Whirl-Pak™ bags (Whirl-Pak™, Pleasant Prairie, WI) and combined with 100 ml of buffered peptone water (BPW; Difco, Becton, Dickinson, Sparks, MD) with 0.2% (v/v) Tween 80 (TCI America, Portland, OR) and stomached for 1 min at 240 rpm. Rinsate was plated Brilliance™ coliforms/*E. coli* agar (Oxoid, Hampshire, England) in duplicate and incubated at 37 °C for 48 h prior to enumerating the microbial populations.

Quantification of Bioaerosols. Bacterial populations were determined according to the manufacturer's instructions. For Brilliance™ coliforms/*E. coli* agar plates, dark purple colonies characteristic of *E. coli* were quantified in addition to mauve colonies, characterized as other coliforms. Both concentrations were summed to provide a total coliform metric.

Statistical Analysis. Data was analyzed using JMP® Pro 18.0.2 (SAS Institute Inc, Cary, NC, USA). Concentrations were transformed to $\log_{10}$ CFU per sample and were analyzed for normality. The population of total coliforms and *E. coli* were considered normal and analyzed using parametric methods. Mixed model analyses with Tukey HSD post hoc analysis when necessary were performed to understand the relationships between microorganism concentrations with distance, farm and visit. Pearson correlation analysis was utilized to ascertain any relationships between microorganisms and meteorological parameters.

Results and Discussion

Fecal indicator bioaerosol concentrations in commercial poultry farms. For all sampling visits, total coliform concentrations ranged from 2.65 - 9.75 log CFU/sampler with a mean concentration of 5.57 log CFU/sampler. *E. coli* concentrations ranged from 2.65 – 7.62 log CFU/sampler with a mean concentration of 4.10 log CFU/sampler. The results did not significantly differ by farm or visit ($p \geq 0.05$) and were therefore reported as overall concentrations. The limit of detection for this passive sampling method was 2.65 log CFU/sampler

Effect of setback distance on indicator concentrations. Distance did not have a significant impact on total coliform concentrations ($p \geq 0.05$) However, an increase in sampling distance from the poultry exhaust fan significantly influenced the concentrations of *E. coli*. Figure 3.1 provides a visualization of the mean bioaerosol concentrations aggregated across all farms and sampling events for each specified setback distance. The proximity to the exhaust fan corresponded with the highest *E. coli* concentrations, measured at 5.88 ± 0.51 log CFU per sample. A statistically significant reduction in *E. coli* populations was detected only at a setback distance of 100 meters, where concentrations declined to 3.97 log CFU per sample ($p = 0.0037$). No further significant reductions in bacterial concentrations were observed beyond the 100-meter mark.

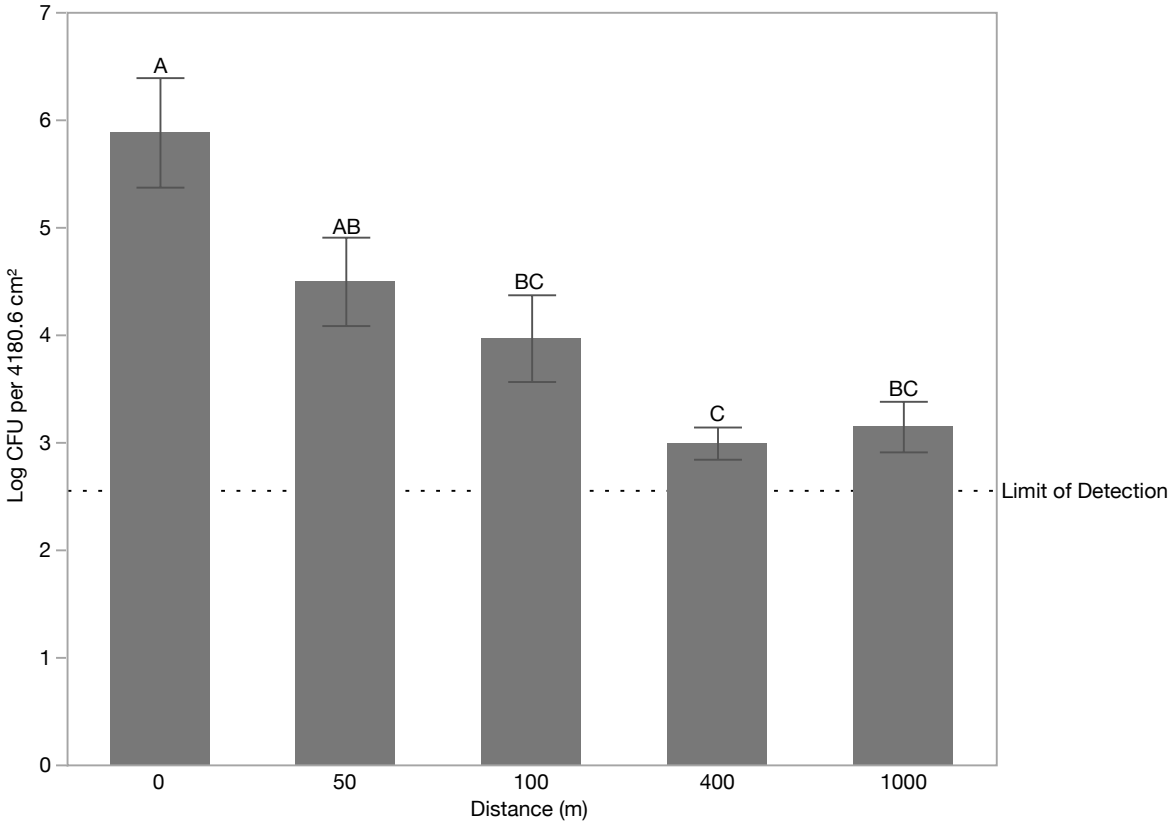

Figure 3.1 Mean *E. coli* concentrations in passive air samples collected at various setback distances from commercial poultry operations (N = 4 farms; n = 12 visits). Each error bar is constructed using 1 standard error from the mean. Letters above bars denote statistical significance when varied.

The elevated concentrations of *E. coli* detected immediately adjacent to the exhaust fan demonstrate a significant dispersion of fecal bacteria into the surrounding environment. The heightened levels observed within the first 50 meters emphasize the role of poultry operations as substantial sources of airborne bacteria at close range. While this study did not conduct assessments within the poultry houses, findings from a related investigation in five broiler houses in China provide relevant insights. Utilizing the Andersen six-stage impactor for bioaerosol quantification up to 400 meters from the houses, researchers reported no significant differences in *E. coli* bioaerosol concentrations within three houses up to 50 meters downwind. Furthermore, utilizing ERIC-PCR for source identification, 54.5% of the *E. coli* strains collected downwind

exhibited 100% genetic similarity with strains isolated from indoor air or feces (Duan, 2008). These results reinforce the assertion that *E. coli* bioaerosols are reliable indicators of fecal contamination originating within the poultry houses.

This pattern of elevated concentrations near emission sources is consistent with findings by Berry et al. (2015), who noted higher *E. coli* levels at the edge of a commercial cattle feedlot compared to plots at further distances, albeit with no significant differences observed beyond 180 meters. In a related study, Sanz et al. (2015) documented a similar trend around a small dairy cattle farm, recording 25 CFU at 0 meters, which decreased to 3 CFU at 50 meters and 5 CFU at 100 meters. In contrast, the data from the current study indicate a significant reduction in airborne *E. coli* concentrations beyond 100 meters coupled with significantly higher concentrations at proximity compared to the passive sampling results from the aforementioned studies, suggesting that dispersal patterns may vary according to the type and scale of animal operation. The design of poultry house exhaust systems may inadvertently promote more efficient aerosolization and dispersion of bacteria compared to cattle operations, which generally operate in more open-air environments.

Other investigations have yielded variable results concerning *E. coli* concentrations in relation to setback distance. In a study conducted on commercial dairy farms in California, researchers found that the prevalence of indicator *E. coli* in all enriched samples ranged from 13% to 32% within 366 meters of the feedlot edge (Wei et al., 2023b). Notably, this prevalence decreased to 8–9% at distances exceeding 366 meters, which generally aligns with the findings of the current study. However, when analyzing three Leafy Greens Management Association (LGMA)

guidance distances, no significant differences were observed ($p = 0.51$) in the prevalence of *E. coli* in enriched air samples collected at 122 meters (8.3% positive), 366 meters (15.0% positive), and 1609 meters (10% positive). These results emphasize the complexity of microbial dispersion dynamics in agricultural environments, particularly across different geographical contexts.

Other studies have reported an increase in *E. coli* concentrations with increasing setback distance. In a study carried out on a research farm housing over 15,000 commercial layers and 8000 broiler breeders, researchers analyzed the concentration of *E. coli* in air at various distances from a vegetation barrier placed directly at the edge of the houses (Glaize et al., 2022). They reported no detection of *E. coli* 10 meters from the vegetation barrier, with greater detection as sampling distance increased (61 meters: 1 CFU, 122m: 3 CFU). Similarly, a study conducted on a cattle feedlot in Argentina during the autumn sampling revealed the highest concentration of *E. coli* was detected at 160 meters from the pen, measuring 11 CFU/m³ (Cogliati et al., 2022). However, during the summer sampling, *E. coli* exhibited a similar trend to the present study, presenting an 82% decrease in *E. coli* presence as the distance from the source of emissions increased. This variability suggests that setback distance alone does not solely dictate the dissemination of bioaerosols; rather, it emphasizes the influence of other environmental factors on microbial concentrations in the air.

Effect of meteorological parameters on indicator concentrations. Figure 3.2 illustrates the fluctuations in air temperature and relative humidity recorded by *in situ* sensors at distances of 0, 50, and 100 meters during the 12-hour overnight period across each farm and visit.

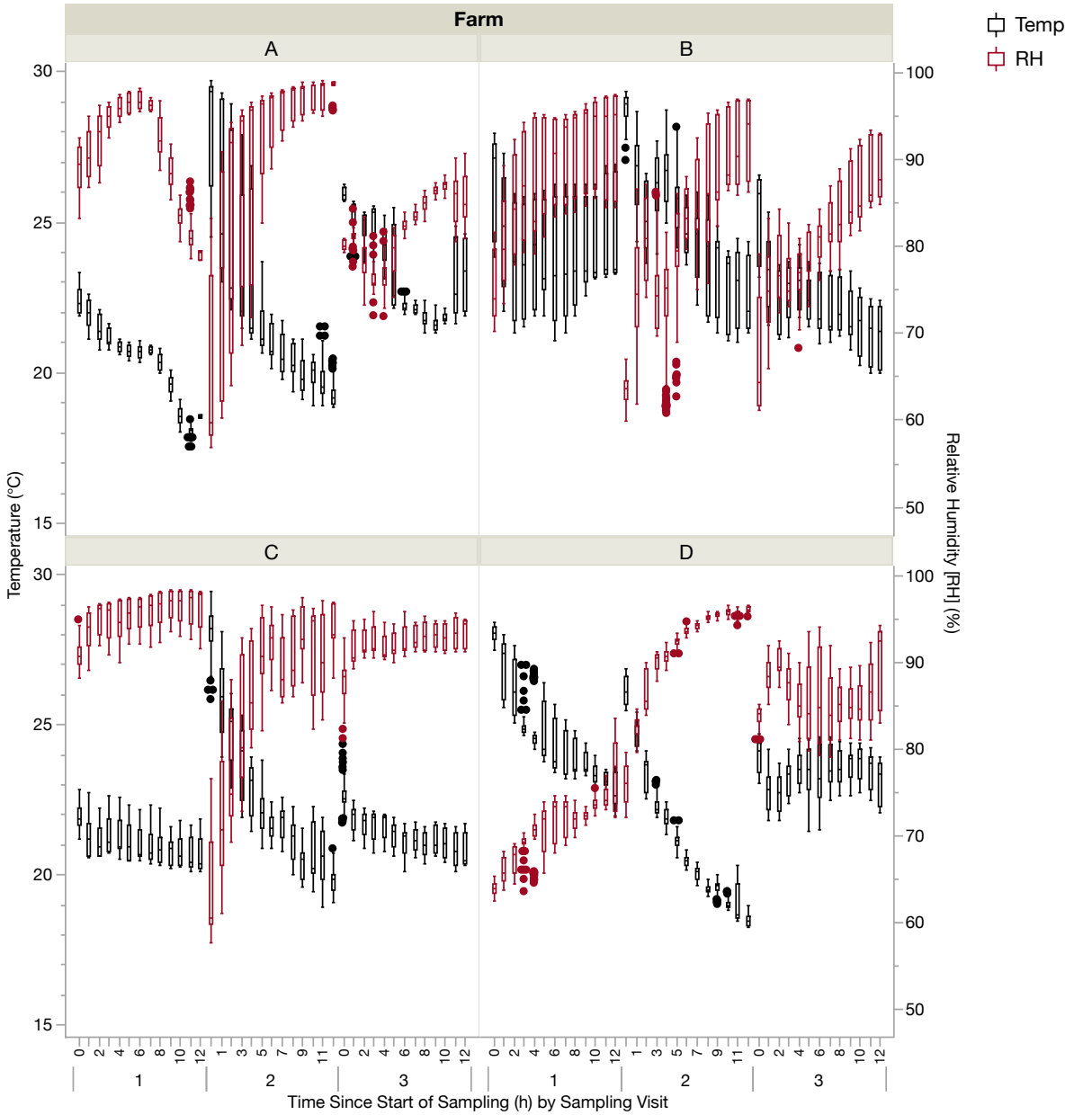

Figure 3.2 Variability in air temperature and relative humidity during sampling visits (N = 4 farms; n = 12 visits). The box bounds the interquartile range (IQR) divided by the median, and Tukey-style whiskers extend to a maximum of 1.5 × IQR beyond the box. Solid dots represent outliers.

During the sampling visits, temperatures ranged from 17.5°C to 29.7°C, with an average of 22.5°C. Relative humidity levels ranged from 56.9% to 99.3%, yielding a mean of 87.1%. These values are characteristic of the Southeastern U.S. during the late summer and early autumn

months when sampling occurred (National Weather Service, n.d.; Weather Underground, n.d.). Statistical analyses revealed significant differences in temperature among the farms ($p = <0.0001$), with Farm B exhibiting the highest mean temperatures, followed by Farm D. Farms A and C exhibited temperatures that did not significantly differ from one another. In terms of relative humidity, significant differences were also observed across farms ($p = <0.0001$). Visits at Farms A and C recorded the highest relative humidity, which was significantly greater than those of Farm B, while Farm D visits exhibited the lowest levels. Despite these variations by farm, no statistically significant relationships were found between air temperature or relative humidity and the concentrations of total coliforms or *E. coli* recovered.

This is consistent with findings from a similar study conducted on a commercial dairy operation in Idaho, where researchers using liquid impingers found no significant relationship between heterotrophic plate counts and temperature or relative humidity (Dungan et al., 2010). Similarly, researchers investigating bioaerosols on commercial dairy farms in California observed no significant associations between the detection odds of airborne *E. coli* and relative humidity ($p = 0.92$) or air temperature ($p = 0.97$) (Wei et al., 2023a). Conversely, other investigators have elucidated significant relationships between these meteorological parameters and microorganism counts (Adell et al., 2014; Cogliati et al., 2022; Wei et al., 2023b). Given this variability, it is essential to consider other meteorological parameters which may impact bioaerosol transfer.

Figure 3.3 illustrates the variability in wind speed, gust speed, and wind direction recorded by sensors on each farm during the sampling visits. Wind speeds remained relatively low (< 8 m/s), a common characteristic of the region (Weather Underground, n.d.). Wind direction exhibited

considerable variability, with instances where minimal wind prevented accurate directional readings. The sampling headings for each farm were as follows: Farm A (Visit 1) at 246.6°, Farm A (Visits 2 and 3) at 22.75°, Farm B at 305.6°, Farm C at 180.4°, and Farm D (Visits 1 and 2) at 153.58°, with Visit 3 at 339.3°, with sampling paths adjusted as necessary due to ongoing farm activities. The prevalence of median values generally aligning with these headings suggests that the passive samplers effectively captured downwind microorganisms from the point source.

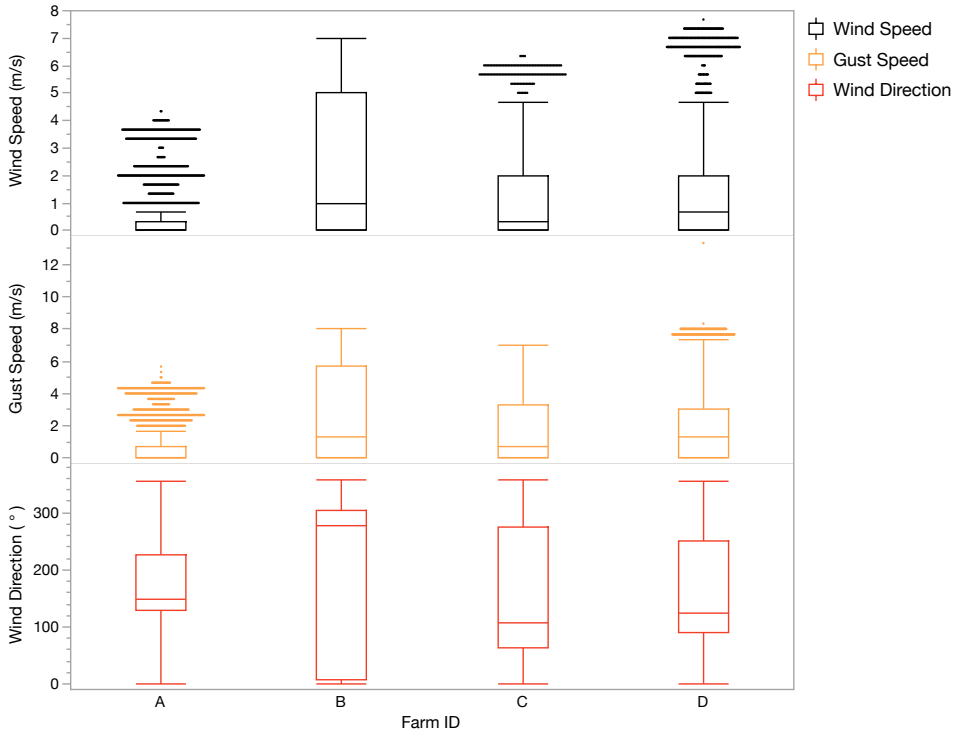

Figure 3.3 Variability in wind speed, gust speed, and wind direction during sampling visits (N = 4 farms; n = 12 visits). The box bounds the interquartile range (IQR) divided by the median, and Tukey-style whiskers extend to a maximum of 1.5 × IQR beyond the box. Solid dots represent outliers.

The variability observed in all parameters, in part, stems from the force exerted by the exhaust fans at the 0-meter sensor. Factors such as temperature and relative humidity and bird age can influence the operation of mechanical ventilation exhaust fans, further complicating the

relationship (Ferriera et al., 2024). Despite these dynamics, no significant relationships were found between measured wind parameters and microbial counts.

In addition to data collected by the weather station, separate particulate matter sensors were deployed at the same distances to measure particulate matter. For each 12-hour sampling period, frequencies of particulate matter fractioned by size were also compared across sampling distances in Figure 3.4. Concentrations of $PM_{1.0}$, $PM_{2.5}$, and PM_{10} were averaged then compared across sampling distances and presented in Figure 3.5.

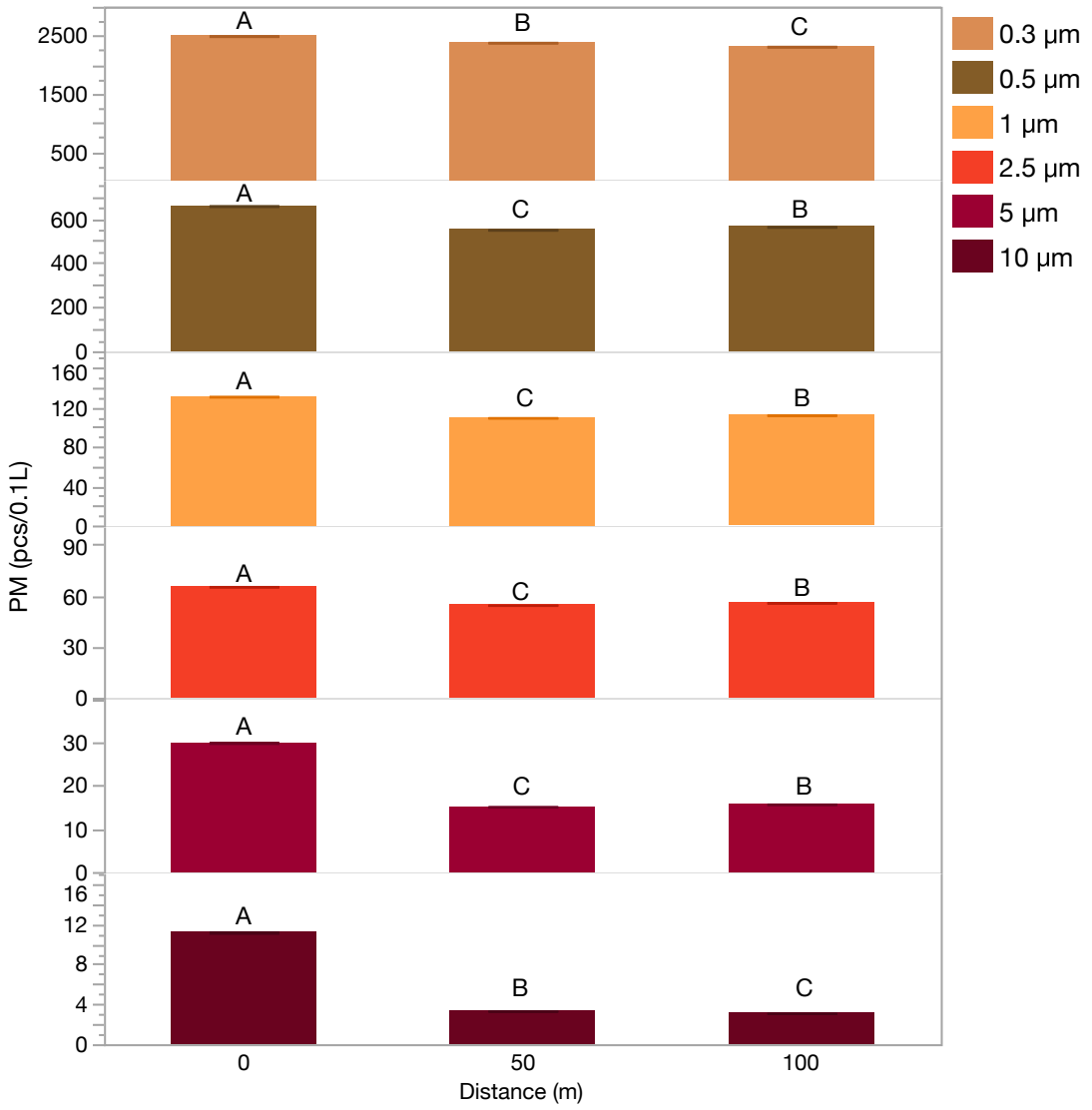

Figure 3.4 Mean particulate matter frequencies in passive air samples collected at various setback distances from commercial poultry operations (N = 4 farms; n = 12 visits). Each error bar is constructed using 1 standard error from the mean. Letters above bars denote statistical significance when varied.

Particulate matter frequency for all particles is significantly higher at 0 meters compared to other distances ($p < 0.0001$), indicating that proximity to the source contributes to elevated levels. At the exhaust fan, fine particulate matter is emitted at the greatest frequency at a mean rate of 2504.3 particles per 0.1 L air. The rate of decrease in particulate matter concentration is inversely

related to particle size: smaller particles disperse more slowly and remain airborne longer, while larger particles settle out more quickly, consistent with aerosol physics. (Morawska & Salthammer, 2003).

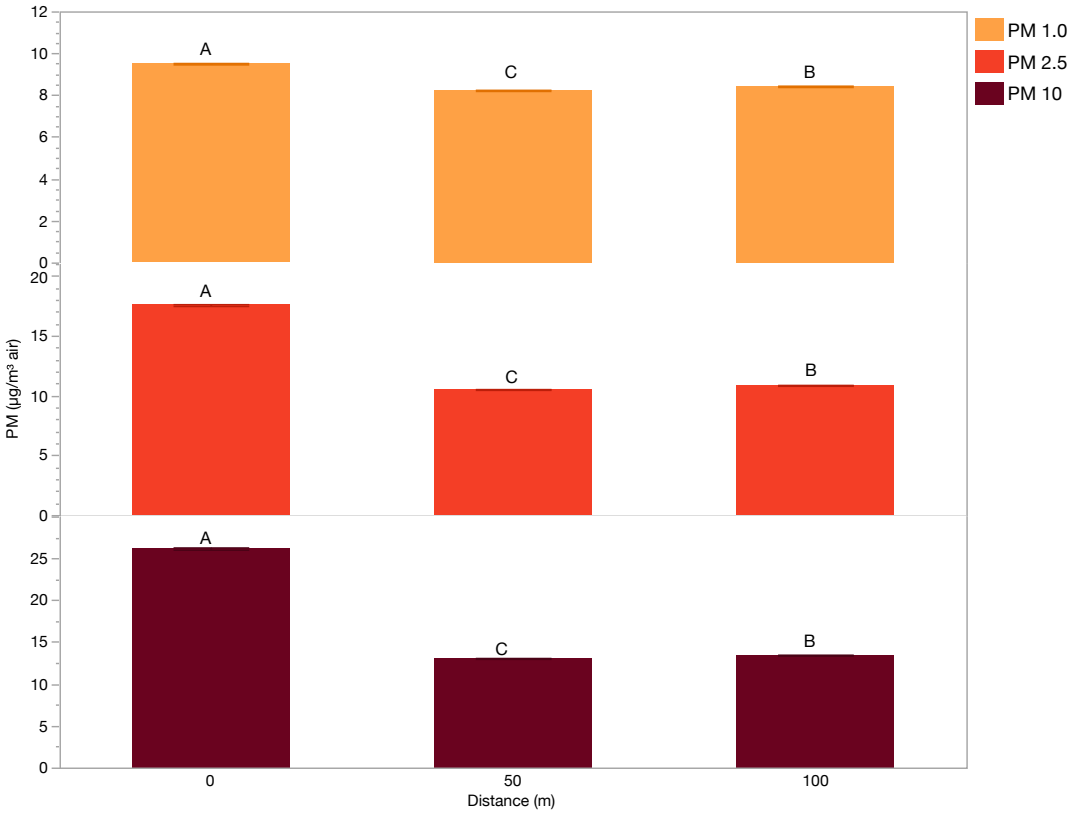

Figure 3.5 Mean particulate matter concentrations in passive air samples collected at various setback distances from commercial poultry operations (N = 4 farms; n = 12 visits). Each error bar is constructed using 1 standard error from the mean. Letters above bars denote statistical significance when varied.

As distance increased, concentrations of $PM_{1.0}$, $PM_{2.5}$ and PM_{10} exhibited a significant decline ($p = <0.0001$), particularly observed at the 50-meter mark. This decline, however, was most pronounced for the intermediate and coarse particles. Given that the trends in concentrations of coarser particles closely mirror those of *E. coli*, a Pearson's correlation analysis was conducted

to further explore the relationship between mean particle size and *E. coli* concentrations. The results of this analysis are presented in Table 3.1.

Table 3.1 Pearson correlation analysis for particulate matter and microbial concentrations collected on commercial poultry farms (N = 4 farms; n = 12 visits).

Variable	Distance (m)	Total Coliforms		*E. coli*	
		r	***P* value**	**r**	***P* value**
$PM_{1.0}$ ($\mu g/m^3$ air)	0	-0.1470	0.6852	0.2250	0.5319
	50	-0.1599	0.6590	0.2395	0.5051
	100	-0.3013	0.4308	0.4140	0.2680
$PM_{2.5}$ ($\mu g/m^3$ air)	0	-0.3301	0.3516	-0.0172	0.9625
	50	-0.0214	0.9532	0.5379	0.1088
	100	-0.3391	0.3719	0.5411	0.1325
PM_{10} ($\mu g/m^3$ air)	0	-0.3493	0.3225	-0.0954	0.7931
	50	0.1149	0.7520	0.7292	**0.0167**
	100	-0.3086	0.4191	0.5735	0.1064

While no significant relationships were identified between total coliforms and particulate matter, a significant correlation (r = 0.7292; p = 0.0167) was observed between PM_{10} concentrations and *E. coli* levels at the 50-meter setback distance. This finding aligns with a study conducted by Nguyen et al. (2022), which documented that a substantial proportion of *E. coli* (47.58%) adhered to particles exceeding 7 µm in diameter following the aerosolization of experimentally inoculated poultry litter. In contrast, particles smaller than 2 µm represented a negligible proportion (1.11%) of the total culturable *E. coli.*

Evidence suggests that microorganisms are capable of associating with substantially larger particles when aerosolized, such as through biological (extracellular polymeric substances) or physical interactions (aggregation or electrostatic forces) (Lighthart, 1997; Tarnowski et al., 2007). These findings imply that larger particles may serve as carriers for *E. coli* and other microorganisms, facilitating their transportation. Moreover, it is plausible that particle size influences not only the dispersion pattern but also the viability of microorganisms. In support of this hypothesis, Zuo et al. (2013) demonstrated that the size of the carrier particle has a pronounced effect on the transmission and longevity of airborne viruses, noting increased survivability on larger particles relative to their smaller counterparts.

The findings of this study underscore the complex interplay between particulate matter and bioaerosol concentrations in commercial poultry farm environments. Acquiring comprehensive data on the distribution of viable bacteria across distinct particle size ranges, in conjunction with meteorological parameters, is essential for developing robust models of microbial aerosol deposition and elucidating the mechanisms of long-range microbial air transport. Thus, it is imperative to systematically expand data collection efforts across a diverse array of sampling sites throughout the U.S. to enhance our understanding of these processes.

Limitations of this study include the fixed positions of the air samplers, which were selected based on accessibility and operational considerations, potentially limiting their ability to consistently capture the directionally relevant downwind airflow. Future research should include sampling in multiple direction, if possible, as well as within the broiler houses (e.g. litter or feces), to provide more definitive source confirmation of airborne microorganisms.

Conclusion

The observed decline in *E. coli* concentrations beyond 100 meters underscores the importance of setback distance as a key factor in limiting bioaerosol dispersion from poultry exhaust sources. Investigating this relationship further, alongside particulate matter concentrations, is essential for developing targeted mitigation strategies. A more comprehensive understanding of these dynamics is vital for designing effective control measures, such as implementing vegetative barriers or establishing mandated setback distances, to protect adjacent agricultural lands and ensure the safety of produce cultivated near animal operations. Future research should also focus on advancing pathogen detection methods, particularly since pathogens are present in much lower quantities than fecal indicators. Additionally, expanding data collection across diverse regions of the U.S. and among different types and scales of commercial animal operations will facilitate the modeling and prediction of bioaerosol transmission under various environmental conditions, ultimately supporting strategies to improve produce safety.

CRediT Authorship Contribution Statement

Halle Greenbaum: Writing – original draft, Methodology, Investigation, Formal analysis. **Zoila Chévez**: Writing – review & editing, Investigation. **Elisa Tobar Sandoval:** Writing – review & editing, Investigation. **Victor Hugo Cruz:** Writing – review & editing, Investigation. **Camila Rodrigues:** Writing – review & editing, Methodology, Investigation, Supervision. **Abhinav Mishra:** Writing – review & editing, Methodology. **Harsimran Kapoor:** Writing – review & editing. **Harshavardhan Thippareddi:** Writing – review & editing. **Hendrik Den Bakker:** Writing – review & editing, Investigation. **Amy Mann:** Writing – review & editing,

Investigation. **Manpreet Singh:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Faith Critzer:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

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CHAPTER 4

INVESTIGATING BIOAEROSOL TRANSFER OF *SALMONELLA* AND *ESCHERICHIA COLI* FROM COMMERCIAL POULTRY OPERATIONS IN THE SOUTHEASTERN UNITED STATES USING AN ACTIVE SAMPLING APPROACH[2]

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Investigating bioaerosol transfer of *Salmonella* and *Escherichia coli* from commercial poultry operations in the Southeastern United States using an active sampling approach

Short title: Bioaerosol transfer from commercial poultry operations

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Abstract

Recent outbreaks involving fresh produce have heightened concerns about bioaerosol contamination from nearby animal operations, underscoring the need for further research. This study examined the dispersal of aerosolized *Salmonella* and fecal indicator organisms from commercial broiler farms in the Southeastern U.S., utilizing active sampling methods. Sampling took place at five farms in Georgia and Alabama, with each farm visited up to three times across a single flock cycle (n=11 visits). Andersen 2-stage impactors, positioned at heights of 0.1 m, 1 m, and 5 m, were paired with weather stations recording air temperature, relative humidity, wind speed, gust speed, wind direction, and particulate matter (PM) concentrations. Samplers operated for 10 minutes at a flow rate of 28.3 L/min, impacting onto CHROMagar™ *Salmonella* and Brilliance™ coliforms/*E. coli* agar plates. Samples were collected at five distances (0, 50, 100, 400, and 1000 meters) from the poultry exhaust fan. Incubation at 37°C for 48 hours allowed for microbial enumeration. No *Salmonella* bioaerosols were recovered during sampling. However, proximity to the exhaust fan was associated with the highest *E. coli* concentrations (2.42 ± 0.73 log CFU/m³) Significant reductions in *E. coli* populations were observed at 50 meters ($p < 0.0001$) and 100 meters ($p = 0.0011$), with concentrations plateauing beyond this point. Additionally, total coliform and *E. coli* populations were strongly correlated with wind speed, gust speed, and $PM_{2.5}$ and PM_{10} levels ($p < 0.0001$). These findings offer valuable data for developing models that incorporate environmental factors, thereby improving understanding of bacterial bioaerosol transport and dispersion in this region.

Introduction

In recent years, the U.S. has experienced several multistate foodborne outbreaks linked to fresh produce, with potential connections to adjacent animal operations[1] Traceback investigations for leafy greens[2], onions[3], and cantaloupe[4] outbreaks have pointed to possible contamination from nearby cattle, sheep, and poultry farms, though the exact mechanisms of transmission remain unclear. Notably, during the 2020 *Salmonella* Enteritidis outbreak associated with peaches[5], multiple isolates from peach samples and peach tree leaves showed genetic similarities to historic strains from poultry and cattle. Integrating geospatial analysis with whole genome sequencing (WGS) data suggested that airborne dust from nearby poultry or cattle operations could have contributed to the contamination.

Given that the Southeastern U.S., particularly Georgia and Alabama, leads the nation in broiler production[6], these outbreaks raise critical questions among stakeholders about whether airborne transmission of pathogens necessitates mitigation strategies. Investigations into the poultry house microbiome have identified various pathogens, including *Salmonella* and *Campylobacter*, present as bioaerosol particles at detectable levels under both natural conditions and controlled experimental inoculations[7,8,9,10]. Nonetheless, studies focusing on the fate of microbes as they are transported beyond the confines of poultry houses through ventilation are limited.

A few investigations have explored the presence of *Salmonella* and *E. coli* both inside and outside broiler houses, using active sampling techniques. These studies have successfully isolated these organisms within the poultry houses and at distances up to 155 meters from ventilation outlets[11,12,13]. However, the relatively short sampling distances may not adequately represent the potential for these microorganisms to impact nearby agricultural fields.

Additionally, the enrichment procedures used in these studies primarily confirm microbial presence rather than providing quantitative data on the specific levels transported outdoors.

Building upon our initial research on cattle and poultry farms aimed at identifying optimal sampling techniques[14], this study employs Andersen 2-stage impactors without mineral oil to examine the dispersal of bioaerosol pathogens such as *Salmonella* and indicator organisms from commercial broiler farms in the Southeastern U.S.. By collecting data on microbial concentrations at multiple setback distances alongside environmental variables, this work aims to support the development of models of bioaerosol transmission that incorporate environmental conditions. This comprehensive approach will enhance our understanding of how bacteria-containing bioaerosols move and persist in this region. The insights gained will support the development of evidence-based guidelines and risk assessments, particularly related to setback distances and potential mitigation strategies, to better protect neighboring produce from microbial contamination.

Materials and Methods

Poultry Farm Sampling Sites

Commercial poultry farms in the Southeastern U.S. were located in Alabama (N=3) and Georgia (N=2), each with 4–8 broiler houses (18 x 183 m). Each house contained around 30,000 broilers and was equipped with ventilation systems comprising 8–12 exhaust fans (1.5 m), which maintained environmental conditions by expelling air at approximately 708 m^3/min when operating.

Air Sampling Methodology

Each farm was sampled up to three times throughout a single flock cycle (n = 11 visits), utilizing an active air sampling method to assess bioaerosol levels. The sampling setup included Andersen 2-stage impactors (Tisch Environmental, Cleves, OH), positioned at heights of 0.1 m, 1 m, and 5 m, designed to collect bioaerosols sorted by size (stage 1: >7 μm, stage 2: 0.65–7 μm). At all three heights, these impactors were paired with weather stations (Hobo™, Onset, Bourne, MA) recording air temperature, relative humidity, wind speed, gust speed, and wind direction, along with separate sensors for particulate matter concentration (GatorGasp, UF, Gainesville, FL).

For each stage of the Andersen sampler, CHROMagar™ *Salmonella* (CHROMagar™, Paris, France) or Brilliance™ coliforms/*E. coli* (Oxoid, Hampshire, England) agar plates were employed to quantify *Salmonella* and coliform/*E. coli* colonies, respectively. Each stage housed a sterile 100 mm × 15 mm Petri dish with 30 mL of the specified agar. The samplers were operated for 10 minutes adjacent to the poultry exhaust fan, with impactors linked to a vacuum pump (Gast Manufacturing, Inc., Benton Harbor, MI) running at 28.3 L/min, collecting 283 L of air in total. This procedure was repeated for both agar types before moving the sampling apparatus to increased setback distances from the fan (50 m, 100 m, 400 m, 1000 m). Plates were subsequently incubated at 37°C for 48 hours before microbial populations were counted.

Quantification of Bioaerosols

Bacterial counts were obtained following the protocols provided by the manufacturer. On Brilliance™ coliforms/*E. coli* agar, dark purple colonies, indicative of *E. coli*, were counted alongside mauve colonies, which represented other coliform bacteria. The sum of these counts was used to calculate the total coliform concentration. On CHROMagar™ *Salmonella* plates,

mauve colonies were enumerated as presumptive *Salmonella* isolates and subsequently confirmed through molecular methods.

Confirmation of Salmonella Isolates

Presumptive *Salmonella* colonies were transferred to TSA plates (Difco, Becton Dickinson, Sparks, MD) and incubated at 37°C for 16–24 hours for subculture. The isolates were then prepared for whole genome sequencing following the protocol described by Pal et al. (2024), with some modifications. DNA was extracted from the isolates using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany), with DNA concentrations measured via Qubit 3.0 Fluorometer and the Qubit dsDNA HS Assay Kit (Invitrogen, Waltham, MA). The purified DNA was subsequently sequenced on an Illumina MiSeq platform, utilizing the Nextera XT Library Prep Kit (Illumina, San Diego, CA) for library construction and subsequent isolate identification.

Statistical Analysis

Data was analyzed using JMP® Pro 18.0.2 (SAS Institute Inc, Cary, NC, USA). Concentrations were transformed to $\log_{10}$ CFU per m^3 and were analyzed for normality. The population of total coliforms and *E. coli* were considered normal and analyzed using parametric methods. Mixed model analyses with Tukey HSD post hoc analysis when necessary were performed to understand the relationships between microorganism concentrations with distance, height, farm and visit. Pearson correlation analysis was utilized to ascertain any relationships between microorganisms and meteorological parameters.

Results and Discussion

Effect of Setback Distance on Indicator Concentrations

Figure 4.1 displays the bioaerosol concentration means in log CFU per m^3 for the sum of both stages for total coliforms and *E. coli* by distance. The results did not significantly differ by stage, visit or sampling height ($p \geq 0.05$) and were therefore reported as overall concentrations. For all sampling visits, total coliform concentrations ranged from 0.84 – 3.39 log CFU/m^3 with a mean concentration of 1.54 log CFU/m^3. *E. coli* concentrations ranged from 0.54 – 3.39 log CFU/m^3 with a mean concentration of 1.22 log CFU/m^3. The limit of detection for the sampling method was 0.55 log CFU/m^3.

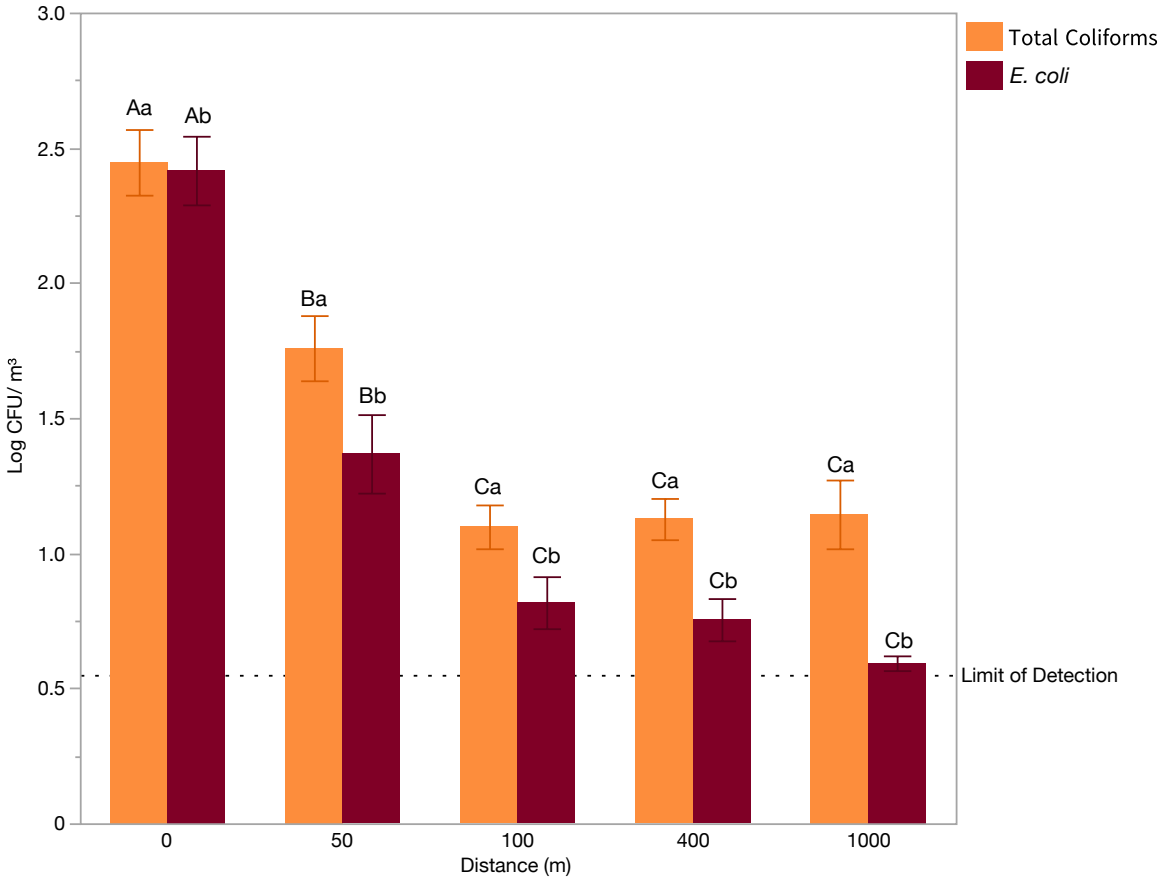

Figure 4.1 Mean fecal indicator concentrations in active air samples collected at various setback distances from commercial poultry operations (N = 5 farms; n = 11 visits). Each error bar is constructed using 1 standard error from the mean. Capital letters above bars denote statistical significance between distance when varied. Lower case letters above bars denote statistical significance within distances when varied.

Peak fecal indicator concentrations were observed closest to the exhaust fan, measuring 2.45 ± 0.70 log CFU/m³ for total coliforms and 2.42 ± 0.73 log CFU/m³ for *E. coli*. Both organisms exhibited similar distance-dependent trends, showing significant declines at 50 m (1.76 ± 0.69 log CFU/m³, p = < 0.0001; 1.37 ± 0.83 log CFU/m³; p = < 0.0001) and further reductions at 100 m (1.10 ± 0.44 log CFU/m³, p = 0.0002; 0.82 ± 0.53 log CFU/m³, p = 0.0011), with concentrations stabilizing beyond this point. *Salmonella* bioaerosols were not detected on any of the farms as confirmed by WGS. This aligns with findings from several studies that have also reported non-detection of targeted pathogens[13, 15, 16, 17, 18, 19, 20], or the requirement for enrichment procedures to identify these bioaerosols[11, 12, 21, 22, 23]. The lack of recovery of these bioaerosols may be due to loss of cultivability resulting from stress incurred during aerosolization, the choice of sampling method, or issues in subsequent handling processes[24, 25]. Furthermore, the concentrations of these microorganisms might have been below the detection threshold or altogether absent.

The *E. coli* concentrations measured at the exhaust fan via impaction sampling are consistent with results from Chinivisigam et al.[11], who examined four commercial broiler farms in Australia. Researchers reported *E. coli* levels at 10 meters from the exhaust fan ranging from below detection to 2–4 log CFU/m³. Similarly, Ruiz-Llacsahuanga et al.[14] employed Andersen two-stage impactors to measure total coliform and *E. coli* bioaerosols, finding concentrations between 2.76 and 5 log CFU/m³ at 1 meter from the exhaust fan of a broiler house housing approximately 28,000 chickens aged 4–6 weeks, comparable to the conditions in this study.

Conversely, Duan et al.[26], investigating five broiler houses in China using Reuter centrifugal samplers, reported significantly lower airborne *E. coli* concentrations at 10 meters downwind, ranging from 0.5 to 1.4 log CFU/m³. Notably, these houses contained between 2,000 and 6,000 birds, suggesting that operation size may influence the emission levels of fecal bioaerosols.

This pattern of decreasing indicator organism concentrations with increasing distance from animal operations observed in this study aligns with the findings of Berry et al.[27], who observed higher *E. coli* bioaerosol levels at the periphery of a commercial cattle feedlot (0.25 – 2.9 log CFU/m³), compared to more distant leafy green plots (60m; -0.15 – 1.22 log CFU/m³), with a plateau in low concentrations at measured distances beyond this (120m, 180m). Similarly, Sanz et al.[28] documented a decreasing trend in *E. coli* bioaerosols around a small dairy farm, with counts of 25 CFU at 0 meters, dropping to 3 CFU at 50 meters before leveling out.

In contrast, several studies have documented an apparent increase in *E. coli* concentrations with greater setback distances. For example, Glaize et al.[16] examined air samples collected around a research facility housing over 15,000 commercial layers and 8,000 broiler breeders. Their investigation focused on *E. coli* levels at various distances from a vegetation barrier positioned directly at the edge of the poultry houses. They found no detectable *E. coli* at 10 meters from the barrier, with detectable levels rising at greater distances. Similarly, research conducted on a cattle feedlot in Argentina during autumn revealed the highest *E. coli* concentration (11 CFU/m³) at 160 meters from the pens[29]. Conversely, in summer sampling, *E. coli* concentrations decreased markedly with increasing distance, showing an approximately 82% reduction relative to concentrations near the emission source.

Other studies regarding the relationship between setback distance and *E. coli* concentrations have led to inconsistent findings. On commercial dairy farms in California, Wei

et al.[19] reported that the presence of *E. coli* in enriched air samples ranged from 13% to 32% within a 366-meter radius from the feedlot boundary. Beyond this distance, the prevalence declined to 8–9%. Conversely, analysis only factoring in three distances recommended by the Leafy Greens Marketing Agreement (LGMA) revealed no statistically significant differences ($p = 0.51$) in *E. coli* prevalence among samples collected at 122 meters (8.3% positive), 366 meters (15.0% positive), and 1,609 meters (10% positive). These contrasting results underscore the complex nature of microbial dispersion in agricultural settings, highlighting that setback distance alone does not fully explain the dispersion of bioaerosols; environmental factors such as seasonality, wind patterns, and atmospheric conditions likely play significant roles in influencing airborne microbial concentration.

Effect of Meteorological Parameters on Indicator Concentrations

Table 4.1 presents the mean ± standard deviation and range of meteorological parameters observed across all sampling sites and visits. The recorded temperature, relative humidity, and low wind speed values are representative of the typical environmental conditions in the sampling region during late summer to early fall, aligning with the seasonal context of the study[30, 31].

Table 4.1 Mean, standard deviation (SD), and range of meteorological parameters determined throughout the eleven sampling days

Meteorological Parameter	Mean ± SD	Range
Temperature (°C)	29.44 ± 2.95	22.73 - 38.68
Relative Humidity (%)	67.66 ± 13.37	33.57 - 93.59
Wind Speed (m/s)	1.09 ± 1.18	0 - 6.3
Gust Speed (m/s)	1.71 ± 1.48	0 - 7
Wind Direction (°)	185.9 ± 95.7	0 - 358

Wind direction showed significant fluctuation throughout the sampling period, with periods of low wind speed limiting the precision of directional measurements. The observed variability in wind parameters is partly attributable to the influence of the exhaust fans at the 0-meter sensor, which can alter local airflow patterns when operating. Additionally, environmental factors such as temperature, relative humidity, and bird age may affect the operation of mechanical ventilation exhaust systems, further complicating the relationship between wind dynamics and bioaerosol dispersion[32].

Table 4.2 presents the Pearson correlation analysis for meteorological parameters and indicator organism concentrations across all sampling sites and visits. Strong significant correlations ($p < 0.0001$) were observed between wind speed, gust speed, $PM_{2.5}$ and PM_{10} with both total coliform and *E. coli* concentrations. Additionally, relative humidity, temperature, and wind direction were significantly correlated ($p = 0.0206$, $p = 0.0285$, $p = 0.0014$) with total coliform concentrations only.

Table 4.2 Pearson correlation analysis for meteorological parameters and microbial concentrations collected on commercial poultry farms (N = 5 farms; n = 11 visits).

	Total Coliforms		***E. coli***	
Variable	**r**	***P* value**	**r**	***P* value**
Temperature (°C)	-0.4353	**0.0206**	-0.1948	0.3205
Relative Humidity (%)	0.4001	**0.0285**	0.1596	0.3995
Wind Speed (m/s)	0.6966	**< 0.0001**	0.6551	**< 0.0001**
Gust Speed (m/s)	0.6530	**< 0.0001**	0.5837	**< 0.0001**
Wind Direction (°)	0.4772	**0.0014**	0.5126	0.0005
$PM_{1.0}$ (µg/m^3 air)	0.1453	0.3041	0.2136	0.1283
$PM_{2.5}$ (µg/m^3 air)	0.5620	**< 0.0001**	0.6723	**< 0.0001**
PM_{10} (µg/m^3 air)	0.6027	**< 0.0001**	0.7083	**< 0.0001**

These results align with a study conducted on seven beef cattle feedlots in the Imperial Valley, California, where no significant correlations were found between the detection probability of airborne *E. coli* and either relative humidity ($p = 0.92$) or air temperature ($p = 0.97$)[33]. However, contrasting findings from other research, both within this region and internationally, have demonstrated significant associations between temperature, relative humidity, and *E. coli* concentrations[8, 19, 29]. Given this variability, it is crucial to consider regional environmental conditions and meteorological factors when evaluating bioaerosol dispersion, as local climate and geographic characteristics can significantly influence microbial transfer and distribution patterns.

Regarding wind parameters, these findings differ from previous research conducted in the Imperial Valley, California, on commercial cattle farms. In two studies by Wei et al.[19, 33], wind speed was found to have a significant negative correlation with the odds of airborne *E. coli* detection, with wind speeds ranging from 0.6 to 7.5 m/s and an average of approximately 3.3 m/s, values slightly higher than those observed in the current study. In contrast, the present research revealed a strong positive correlation between wind speed, gust speed, and the concentrations of both *E. coli* and total coliforms. This contrasting relationship suggests that other factors, such as the variability introduced by the operation of exhaust fans or site-specific environmental conditions, may play a more prominent role in influencing bioaerosol dynamics in this context.

Across all sampled farms and distances, both intermediate ($PM_{2.5}$) and coarse (PM_{10}) particulate matter showed significant correlations with total coliform and *E. coli* concentrations. Existing evidence indicates that microorganisms can attach to larger aerosol particles through various mechanisms, such as incorporation of extracellular polymeric substances or physical

interactions like aggregation and electrostatic forces[34, 35]. These results suggest that larger particles may act as carriers for *E. coli* and other microbial entities, potentially enhancing their dispersal and transport within the environment.

To support this, Nguyen et al.[36] investigated the aerosolization of experimentally inoculated poultry litter and observed that a significant proportion of *E. coli* (47.58%) were associated with particles larger than 7 μm in diameter. Conversely, particles smaller than 2 μm accounted for only approximately 1.11% of culturable *E. coli*, indicating a predominant association of the bacteria with larger aerosol particles post-aerosolization. Further research which larger sample sizes is needed to elucidate the specific role of particle size in facilitating bioaerosol dispersion and bacterial transport.

Previous research indicates that the size of carrier particles markedly impacts microbial viability, with larger particles generally promoting greater microbial survival compared to smaller ones[37]. However, aerosol physics also significantly influence dispersion and transmission, as heavier particles tend to remain suspended for shorter durations[38]. Figure 4.2 displays the distribution frequencies of particulate matter segmented by size and compared across various sampling distances. Additionally, Figure 4.3 presents the concentrations of different particulate matter fractions by distance, providing a visual representation of the long-range transport of particles emanating from the poultry exhaust fan. Together, these findings underscore the complex interplay between particle size, aerosol dynamics, and microbial dispersal within the environment.

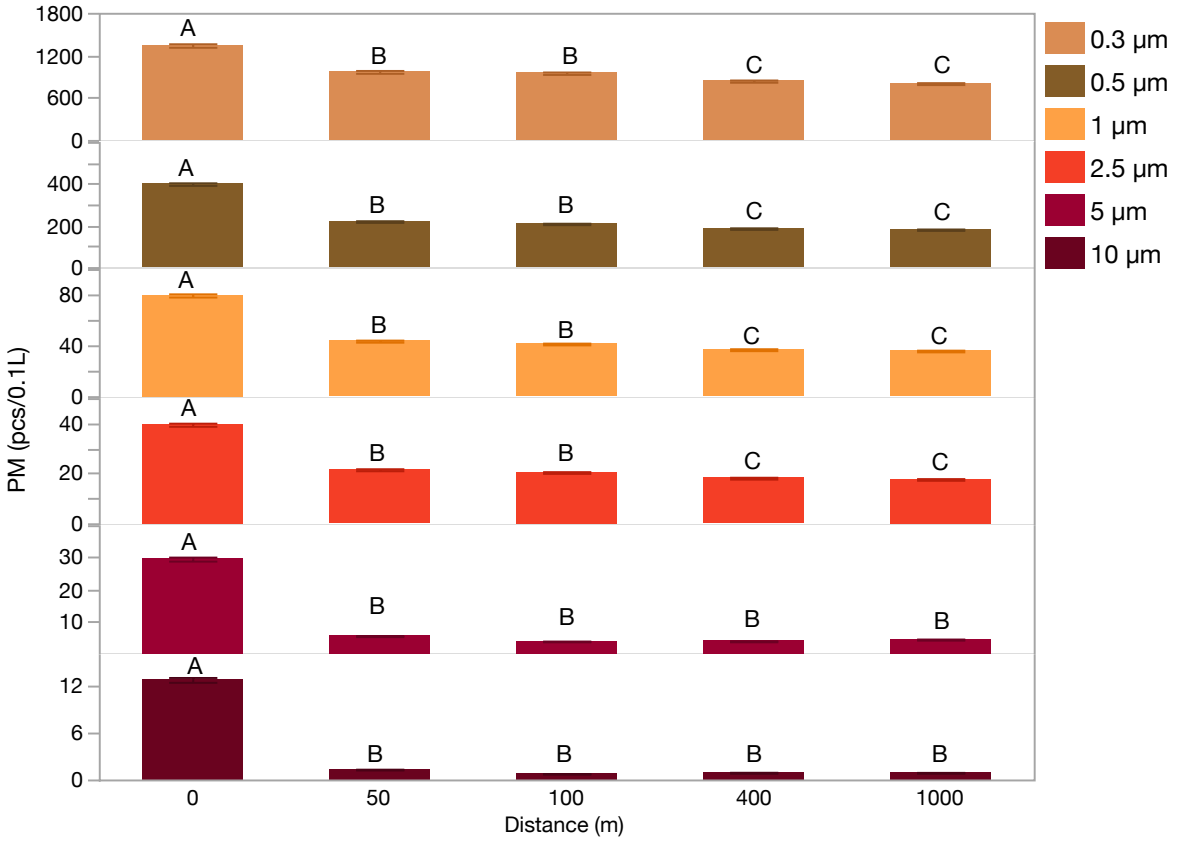

Figure 4.2 Mean particulate matter frequencies in active air samples collected at various setback distances from commercial poultry operations (N = 5 farms; n = 11 visits). Each error bar is constructed using 1 standard error from the mean. Letters above bars denote statistical significance when varied.

The frequency of particulate matter for all diameters measured was significantly greater at 0 meters compared to subsequent distances ($p < 0.0001$), suggesting that proximity to the emission source plays a key role in elevated particle levels. At the exhaust fan, the highest frequency was observed for fine particulate matter with a diameter of 0.3 µm, averaging 1,343.5 particles per 0.1 L of air sampled. The decline in particulate matter concentration with increasing distance appears to follow an inverse relationship with particle size: smaller particles tend to disperse more slowly and remain airborne for extended periods, whereas larger particles tend to settle out more rapidly.

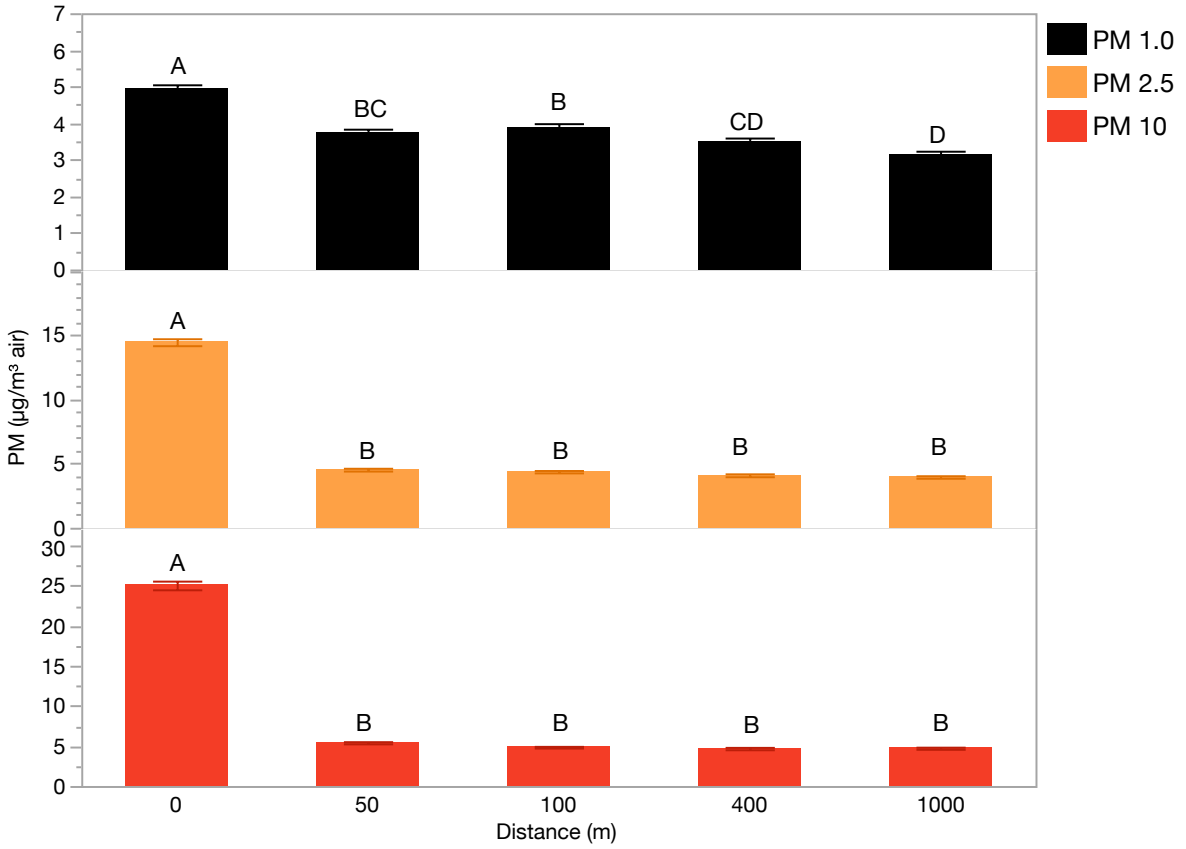

Figure 4.3 Mean particulate matter concentrations in active air samples collected at various setback distances from commercial poultry operations (N = 5 farms; n = 11 visits). Each error bar is constructed using 1 standard error from the mean. Letters above bars denote statistical significance when varied.

As the distance from the source increased, concentrations of $PM_{1.0}$, $PM_{2.5}$, and PM_{10} declined significantly ($p < 0.0001$), with the most substantial reduction observed at 50 meters. This decrease was especially prominent for the intermediate and coarse particle fractions, which also demonstrated strong correlations with *E. coli* concentrations. While individual *E. coli* cells are classified within the fine particulate matter group[39], their tendency to exist as aggregates can influence their settling behavior, often causing them to deposit more rapidly out of the air than if present as single cells. Understanding this interaction between particle size, aggregation, and sedimentation is crucial for elucidating microbial dispersion, particularly in the context of how

poultry operations may influence the contamination of surrounding environments and fresh produce.

The results of this investigation highlight the intricate relationship between particulate matter and bioaerosol concentrations within commercial poultry farm settings. Obtaining detailed data on the distribution of viable microorganisms across varying particle size fractions, combined with meteorological information, is crucial for constructing accurate models of microbial aerosol deposition and understanding the mechanisms driving long-distance microbial transport. Therefore, expanding systematic sampling efforts across a broader range of locations throughout the U.S. is essential to deepen our insights into these processes.

Limitations of this study include the fixed placement of air samplers, which were chosen primarily based on accessibility and operational constraints. This approach may have restricted the ability to consistently capture the predominant downwind airflow, potentially affecting the representativeness of the samples. Future studies should consider multi-directional sampling, where feasible, along with in-house collection, such as from litter or fecal matter, to better identify and confirm the primary sources of airborne microorganisms.

Conclusions

This research highlights the significant role of particulate matter in the transport and dispersal of microbial bioaerosols from poultry farms, with microbial concentrations highest near the exhaust source and decreasing with distance. The study emphasizes that intermediate and coarse particles, which are strongly correlated with *E. coli* concentrations, are key carriers facilitating microbial movement over long distances. The findings also demonstrate a clear inverse relationship between particle size and dispersion, where smaller particles remain airborne

longer, whereas larger particles settle more quickly. Moreover, correlations with meteorological parameters such as wind speed, gust speed, and particulate matter fractions underscore the influence of environmental conditions on microbial transport, providing a more comprehensive understanding of bioaerosol dynamics in farm environments.

Building on these key findings, efforts should be directed toward expanding sampling across multiple regions, employing multi-directional and in-house approaches to better capture sources and pathways of microbial dispersion. Enhancing pathogen detection methods, including more sensitive molecular techniques, will be essential for accurately quantifying low-level or viable pathogens transported beyond poultry facilities. Improved detection will support the development of predictive models of bioaerosol movement and inform mitigation strategies aimed at reducing the risk of microbial contamination. Addressing current limitations in sampling design and increasing spatial and methodological resolution will be critical for refining risk assessments, ultimately aiding in the formulation of effective evidence-based guidelines to reduce microbial contamination risks to surrounding environments, agricultural fields, and fresh produce.

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CHAPTER 5

CONCLUSIONS

This thesis provides a comprehensive exploration of bioaerosol dynamics in the context of commercial poultry operations, responding to mounting concerns regarding foodborne disease outbreaks linked to fresh produce and the potential role of animal operations in the contamination pathway. Employing both passive and active air sampling methodologies, this research seeks to quantify the dissemination of fecal indicator organisms and foodborne pathogens and assess the influence of environmental factors on their transport from poultry houses to surrounding environments. The findings offer valuable insights into the complex interplay between microbial sources, atmospheric conditions, and the potential for produce contamination in agricultural landscapes.

The initial literature review established the framework for this investigation, highlighting the significant public health burden associated with foodborne illnesses, particularly those linked to *Salmonella* and Shiga toxin-producing *E. coli* (Bennett et al., 2018; Scallan et al., 2011). Furthermore, it outlined the increasing prevalence of animal feeding operations (AFOs) and the growing concerns regarding their potential impacts on environmental quality and food safety (Walton & Jaiven, 2020). Given the limited understanding of bioaerosol transmission pathways, a critical gap in knowledge was identified, emphasizing the need for a comprehensive evaluation of microbial dispersion from poultry operations.

The passive air sampling study provided an initial assessment of bioaerosol concentrations across commercial broiler farms in the Southeastern U.S. While demonstrating a

statistically significant reduction in *E. coli* concentrations at greater setback distances, such as a detectable reduction in concentrations beyond 100 meters, the passive sampling approach revealed nuanced relationships with meteorological parameters. For instance, a significant correlation between *E. coli* and PM_{10} at the 50-meter mark ($r = 0.7292$; $p = 0.0167$) suggests that complex interactions among environmental variables and microbial characteristics influence bioaerosol transport.

To complement the passive sampling results and leverage well-established methodologies for bioaerosol quantification (Adell et al., 2014; Berry et al., 2015), the investigation employed active air sampling using Andersen two-stage impactors. This method allowed for the capture of microorganisms across distinct particle size ranges, enabling a finer-grained analysis of bioaerosol composition and transport dynamics. These efforts confirmed elevated concentrations of *E. coli* near poultry exhaust fans, affirming their role as a significant source of bioaerosol emissions. Specifically, peak fecal indicator concentrations were observed closest to the exhaust fan, measuring 2.45 ± 0.70 log CFU/m³ for total coliforms and 2.42 ± 0.73 log CFU/m³ for *E. coli*. A statistically significant decline in *E. coli* populations was observed at 50 meters ($p < 0.0001$) and 100 meters ($p = 0.0011$), underscoring the potential for proximity to influence microbial exposure in surrounding areas.

Moreover, the strong correlations found between total coliform and *E. coli* concentrations with wind speed, gust speed, and particulate matter fractions shed light on the mechanisms driving bioaerosol transport. For example, both total coliform and *E. coli* concentrations were strongly positively correlated with wind speed and gust speed ($p < 0.0001$), indicating that meteorological conditions play a crucial role in the dissemination process. Interestingly, the absence of *Salmonella* detection in the active air samples, despite its presumed presence in

poultry houses, highlighted the need for more sensitive and targeted pathogen detection methods to accurately assess potential risks associated with low-abundance microorganisms, especially given that related research did identify their prevalence in experimental conditions or in litter samples, indicating that it can be present at different times (Chinivasagam et al., 2009; Nguyen et al., 2022).

Building upon these findings, the thesis explored the interplay between particulate matter and bioaerosol concentrations. The significant correlations between intermediate ($PM_{2.5}$) and coarse (PM_{10}) particulate matter with total coliform and *E. coli* concentrations suggest that larger particles serve as carriers for microorganisms, enhancing their dispersal (Lighthart, 1997; Tarnowski et al., 2007). These observations underscore the complex relationship between particle size, aerosol dynamics, and microbial survival during atmospheric transport.

However, it is essential to acknowledge the limitations of this research, particularly the use of fixed-position air samplers, which may have introduced directional biases in the collection of bioaerosols. Additional limitations include the limited sampling duration of air in the active sampling study, and the unknown shedding status of pathogens and fecal indicators in the poultry facilities. Future studies should seek to incorporate more comprehensive sampling strategies, including multi-directional sampling approaches and source tracking methodologies, to address these limitations and provide a more robust assessment of bioaerosol dynamics (Lindsley et al., 2017; Smith et al., 2025).

In conclusion, this thesis underscores the need for integrated, interdisciplinary approaches to understanding and mitigating the risks associated with bioaerosol transmission from poultry operations to surrounding environments. By combining quantitative microbial data with meteorological information and comprehensive analytical techniques, this research provides a

foundation for developing more effective risk assessments, management practices, and public health interventions aimed at safeguarding the integrity of the fresh produce. The pursuit of more sensitive pathogen detection methods, coupled with the expansion of data collection efforts across diverse geographic regions and farm types, will be essential for refining understanding of these complex processes and informing the development of evidence-based strategies for reducing microbial contamination in agricultural landscapes.

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CHAPTER 6

FUTURE WORK

To build upon the findings presented in this thesis, several avenues for future research warrant exploration. First, broadening the geographic scope of data collection across a more diverse array of regions within the U.S., and potentially internationally, is crucial. This expanded sampling should include a variety of animal operation types and sizes to account for variations in management practices and emission profiles. Such data is essential for validating and refining the bioaerosol transmission trends presented herein under a wider range of climatic and environmental conditions, potentially leading to more data-driven conclusions regarding the impact of regionality on bioaerosols.

Additionally, future studies should incorporate multi-directional sampling approaches, where feasible, along with in-house air sampling to accurately assess the dispersion patterns while accounting for wind direction and establishing a clear relationship between indoor poultry-house bioaerosols and their impact on outdoor environments. Another high-priority area involves advancing pathogen detection methodologies to enhance the sensitivity and specificity of detection, particularly for low-abundance pathogens like *Salmonella*, to improve the accuracy of risk assessments. Finally, integrating source tracking methodologies, such as comparative genomics and spatial analysis, could help provide a definitive source confirmation of airborne microorganisms. More comprehensive data can contribute to models driving regionally tailored risk assessments and mitigation strategies to safeguard nearby produce.