# Airborne transmission of pathogens emerging in the poultry industry

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# ABSTRACT

Poultry houses might have high levels of airborne pathogens, including *Escherichia coli (E. coli)* and avian influenza (AI), which may be transmitted through the air and pose risks of infection. The objective of the dissertation is to provide an insightful understanding the of airborne transmission of *E. coli* and AI which were attached to poultry dust particles.

Chapter I and VI summarized background, gap in knowledge, and discussed the limitations and implications of the study.

Chapter II compared the efficiency of Andersen six-stage impactor, all-glass impinger, and ACD-200 Bobcat, in collecting airborne *E. coli* carried by dust particles. The results showed that the Andersen six-stage impactor and the all-glass impinger outperformed the Bobcat sampler. Airborne *E. coli* were found to mainly aggregate on large particles (>7.0 μm).

The survivability of *E. coli* in poultry litter under different environmental conditions was described in Chapter III. The survivability of airborne *E. coli* was found to have a half-life time of 5.7 ± 1.2 min, while the survivability of settled *E. coli* and *E. coli* in poultry litter were much longer with half-life times of 9.6 ± 1.6 hrs and 15.9 ± 1.3 hrs, respectively.

The effect of ultraviolet (UV) light on the inactivation of airborne *E. coli* carried by poultry dust particles under laboratory conditions was explored in Chapter IV. The inactivation rates varied from over 99.87% and 99.95% at 5.62 s of contact time to 72.90% and 86.60% at 0.23 s of contact time, with 1,707 μW $cm^{-2}$ and 3,422 μW $cm^{-2}$ of UV irradiance, respectively.

Utilizing computational modeling to assess the risk of airborne and deposited AI carried by poultry-litter dust particles was investigated in Chapter V. Results showed that concentrations of airborne AI transmitted to other farms in a day were lower than the minimal infective dose for poultry. The study suggests that factors such as infected location and type of poultry house may influence the risk of airborne transmission of HPAI.

In conclusion, this dissertation explores sampling methods, survivability, mitigation technologies for airborne transmission of pathogens in poultry, and factors affecting infection probabilities in the poultry industry.

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# CHAPTER I

# General Introduction

## 1.1 Background

The U.S. poultry industry is among the world's largest poultry producers. It includes meat products from turkeys and broilers, and eggs from laying hens. The combined values of these products exceeded 35 billion U.S. dollars in 2020 (USDA-NASS, 2020). Poultry products are affordable and important sources of daily protein. In addition, the U.S. poultry industry provides $555.9 billion in economic activity and about 2 million jobs for national populations. However, this important industry is extremely vulnerable to infectious diseases caused by pathogenic microorganisms such as highly pathogenic avian influenza (HPAI). An example is the 2014-15 HPAI outbreak in the Mid-Western U.S., resulting in a significant loss of over 50 million birds and 3.3 billion U.S. dollars (M. Torremorell et al., 2016).

## 1.2 Challenges of Airborne Transmission of Pathogens in Poultry

### 1.2.1 Airborne transmission

The poultry industry in the United States is one of the largest in the world and produces meat products from turkeys and broilers, as well as eggs from laying hens. In 2020, the total value of these products exceeded 35 billion U.S. dollars. Poultry products are an important and affordable source of daily protein for many people in the U.S. and around the world. Additionally, the industry is a major contributor to the economy, generating 555.9 billion U.S. dollars in economic activity and providing around 2 million jobs for domestic population. However, the industry is susceptible to infectious diseases caused by pathogenic microorganisms such as highly pathogenic avian influenza (HPAI). An example of this vulnerability occurred during the 2014-2015 HPAI outbreak in the Mid-Western area of the U.S., which led to the loss of over 50 million birds and 3.3 billion U.S. dollars.

The concentration and size distribution of airborne bacteria are influenced by the type of poultry housing system (Just et al., 2012). In a study conducted over eight months, it was found that the total bacterial concentration was much higher in floor-type systems than in cage-type systems for laying hens (Zhao et al., 2015). The size distribution of airborne bacteria also varies among housing systems, with a skew towards larger sizes in floor-type systems (Zheng et al., 2013). Moreover, bacterial concentration and size distribution also

differ among different poultry types, such as broilers and laying hens, which can be attributed to differences in bacterial shedding, management practices, indoor environmental conditions, and other factors affecting the physical and biological properties of airborne agents (Seedorf et al., 1998).

In the poultry house, the major components of the air include gases, odors, and numerous pathogens may be carried by dust particles or droplet nuclei such as AI viruses (Zhao et al., 2019). AI viruses are first secreted via birds' nasal secretions, feces, and saliva. The bird secretions can either be dried and suspended in the air for a long period of time or deposited on the poultry litter surface. The deposited secretions which carry AI are then mixed with poultry litter particles and re-aerosolized into the air by dust bathing behavior of birds. Both droplet nuclei and dust particles that carry AI may then be distributed into the poultry house environment and transmitted from barn to barn via ventilation system and transport of air.

At susceptible farms, the AI can be sucked in through the ventilation system and be distributed inside the farms. The airborne HPAI viruses are then deposited onto the surface of poultry litter on which they can survive up to 5 days at 24°C (Kurmi et al., 2013). In previous studies, authors have reported that most airborne AI viruses are found in dust particles as small as 1 µm to 5 µm in size (Bertran et al., 2017b; Zhou et al., 2016) at 0.5 m away from poultry housing. It is important to note that fine dust particles (or dust particles with diameters that are generally 2.5 µm and smaller) can travel hundreds of miles (Kwon et al., 2016). With the long dispersion range, the AI viruses carried by fine dust particles can be a possible transmission pathway of HPAI.

### 1.2.2 *Escherichia coli*

The poultry industry is vulnerable to infectious diseases such as *Escherichia coli (E. coli)*, which can be abundant in poultry houses, feeds, and litter. Antibiotics have been used to reduce *E. coli* contamination, but their widespread use can cause the emergence of antibiotic-resistant strains. Alternative methods such as probiotics and UV lights have been developed to reduce microbial contamination, but their effectiveness in reducing airborne bacteria attached to dust particles is not yet clear. The litter is a major reservoir of microorganisms in the poultry environment, and airborne *E. coli* can harm the environment outside the poultry houses and persist on surfaces for a long time. Further studies are needed to investigate the survivability and size distribution of airborne *E. coli* attached to dust particles.

Airborne bacteria are abundant in poultry housing systems, and *E. coli* is the most common Gram-negative species, accounting for 2 to 6% of total bacteria (Zucker et al., 2000). These bacteria may travel through the air, potentially infecting birds and causing diseases to spread over large distances (Lay Jr et al., 2011). Although *E. coli* has been observed to spread through the air in other livestock, such as pigs, it is unknown if this is also true for poultry. To study this hypothesis, we must first collect baseline data on bacterial

concentration and particle size distribution, which impact animal exposure levels and bacterial particle aerodynamics. Unfortunately, this information is currently in short supply.

Based on the above-mentioned research, exposure to airborne *E. coli* might differ among housing systems and poultry types. Therefore, it is important to investigate the concentration and size distribution of airborne *E. coli* in typical US broiler and laying-hen housing systems.

### 1.2.3 Avian influenza

Avian influenza, also known as bird flu, is a disease caused by type A avian influenza viruses that naturally occur in wild aquatic birds worldwide and can infect domestic poultry and other bird and mammal species. Although some wild aquatic birds may carry the virus without getting sick, avian influenza A viruses can infect and cause severe illness and death in certain domesticated bird species, such as chickens, domestic ducks, and turkeys. In the 2022 AI outbreak, approximately 50 million birds, including 265 commercial flocks and 358 backyard flocks, have been affected by the virus. The pathogen can be found in infected birds' saliva, nasal secretions, and feces, and transmission can occur through direct contact or indirect contact with virus-contaminated objects.

Avian influenza type A viruses are divided into two groups, low pathogenic avian influenza (LPAI) and high pathogenic avian influenza (HPAI), based on the severity of the disease they cause (USDA-APHIS, 2022b). LPAI viruses typically cause mild to moderate symptoms in laying hens and broilers, such as ruffled feathers and decreased egg production, while most wild birds infected with LPAI viruses show little signs of illness. However, some LPAI viruses in chickens can evolve into highly pathogenic strains. HPAI viruses, on the other hand, can cause severe sickness and high mortality rates in infected chickens. Only a few avian influenza A(H5) and A(H7) viruses are HPAI, while the majority of circulating avian influenza A(H5) and A(H7) viruses are LPAI. Infections with HPAI A(H5) or A(H7) viruses in hens can cause sickness that affects multiple internal organs, with mortality rates ranging from 90% to 100% within 48 hours. HPAI viruses can be transmitted to wild birds from infected poultry, potentially spreading the virus during migration. HPAI viruses are mainly transmitted through direct contact, but airborne transmission is also possible, as evidenced by bird mortalities near air inlets of poultry houses.

When an outbreak of avian influenza occurs, it can have significant impacts on trade restrictions in the poultry industry. When a region is affected by avian influenza, it is common for trade restrictions to be imposed on poultry products from that area to prevent the spread of the disease. This can have a serious impact on the export market for poultry products from that region, as trade restrictions can lead to a reduction in demand for these products. Trade restrictions can take various forms, including bans on imports or exports of poultry products, increased testing requirements, or restrictions on the movement of live

birds or poultry products. Such restrictions can be imposed by importing countries to protect their own poultry populations and consumers from the risk of infection from avian influenza. For instance, the outbreak of HPAI had a significant impact on the export market of U.S. poultry products. More than 50 countries-imposed trade restrictions on poultry products from the affected region, leading to a decline in the supply of egg and turkey products (Ramos et al., 2017). This shortage caused a rise in prices, further reducing export volume. Factors beyond the poultry sector, such as the strength of the U.S. dollar, also contributed to the decline in the export market. The trade restrictions and decreased supply of products led to a sharp reduction in egg and turkey export volumes, with shipments decreasing by 45% and 41% respectively in July-December 2015, compared to the same months in the previous year. However, the decline in broiler exports was relatively less severe, as production did not decrease. Broiler exports in July-December 2015 were 18% lower than the same period in 2014.

## 1.3 Related Research

### 1.3.1 Sources of airborne microorganisms and dust

The identification of the sources of microorganisms and dust in livestock production systems is crucial to understand the generation of airborne transmission and implement prevention strategies. While sources of dust have been identified and assessed (Aarnink et al., 1999), identifying airborne microorganisms' sources is more complicated due to the complexity and dynamic viability of microbial species in the generation process (Milne et al., 1989).

(1) Microorganisms in poultry industry

The main source of airborne microorganisms from poultry is their feces, which may contain high concentrations of various microorganisms such as bacteria and viruses. *Salmonella* and *E. coli* have been found in feces at concentrations of 2-7 log CFU $g^{-1}$ feces and 2-6 log CFU $g^{-1}$ feces respectively (Gray & Fedorka-Cray, 2001; Omisakin et al., 2003). The microorganisms in feces can become airborne when dried fecal particles are disturbed by air flow or animal activity. Another source of airborne microorganisms in animals is the respiratory tract (Edwards et al., 2004), but this source is less significant than feces. The microorganisms can become airborne through inhalation and exhalation during breathing, coughing, and sneezing. Feed and litter may serve as carriers for a variety of microorganisms, which can be disseminated with feed particles during feeding.

(2) Dust particle source in poultry industry

Airborne dust in animal housing systems can come from a variety of sources, including feed, animal skin and feather debris, feces, litter, microorganisms, pollen, and insect parts. In floor layer systems, the contribution of dust from bedding materials in litter varies from 55 to 68% (Müller & Wieser, 1987), and from 80 to 90% in layer systems with battery system originated from feedstuff (Müller & Wieser, 1987). The contribution of feed to

airborne dust depends on its composition and processing. Feces' contribution to dust is likely related to the housing system.

(3) Concentration and size distribution of airborne *E. coli*

Poultry housing systems have high levels of airborne bacteria, with *E. coli* accounting for 2 to 6% of total bacteria and posing a risk for airborne transmission (Zucker & Müller, 2000). However, information on the concentration and particle size distribution of airborne *E. coli* in poultry is limited. The type of housing system influences the concentration and size distribution of airborne bacteria, with floor-type systems having higher bacterial concentrations and larger size distributions compared to cage-type systems (Zhao et al., 2015). Airborne bacterial concentration and size distribution also differ among poultry types, suggesting that the exposure to airborne *E. coli* may differ among housing systems and poultry types (Seedorf et al., 1998). Investigation of airborne *E. coli* concentration and size distribution is needed for typical US broiler and laying-hen housing systems.

### 1.3.2 Collecting Dust and Airborne *E. coli* in Livestock Facilities

(1) Sampling methods

There are various factors that need to be considered when conducting air sampling for particulate matter and microorganisms in different settings (Zhao et al., 2014). Isokinetic sampling is considered the ideal method (Zhang, 2004) but is practically impossible due to variations in air flow patterns and limitations of some samplers. To reduce sampling bias, legislation stipulates the range of conditions under which non isokinetic samplings may be performed. The sampling location is chosen based on the research purpose, and for human health concerns, sampling should be carried out near the breathing zone. For poultry, stationary samplers in birds' breathing zones are recommended. Sampling near the air outlet is best for emissions of microorganisms and dust, but care should be taken not to place samplers where the airspeed is too high.

To ensure accurate sampling, it is important to consider the recommended sampling duration for various pollutants. For example, ambient air requires a sampling duration of 24 hours, while less than an hour is recommended for airborne microorganisms. However, some samplers are not suitable for extended sampling durations and may only allow for minutes or even seconds of sampling, as is the case with impactors (Thorne et al., 1992). Filtration methods can eliminate issues of evaporation and overloading, but they may not be effective in collecting microorganisms over extended periods due to dehydration (Nguyen et al., 2022). Nevertheless, certain high-volume and high airflow samplers have been developed and can be used for longer periods (Griffin et al., 2011).

(2) Common samplers for sampling *E. coli*

To determine the concentration and size distribution of airborne *E. coli* attached to dry dust particles, a proper sampler that can efficiently collect airborne microorganisms attached to dry dust particles is required. Samplers such as Andersen six-stage impactor (Andersen

impactor), all-glass impinger (AGI-30) and ACD-200 Bobcat (Bobcat) are widely used because of their advantages in airborne sampling. Andersen impactor uses the direct impact technique and is designed as an aerodynamic classifying system for airborne particles. AGI-30 uses the impingement method and is known as a versatile, inexpensive, and easy-to-use method. The high-volume sampler, ACD-200 Bobcat, uses the filtration method and is designed for collecting airborne bacteria at low concentrations. Each sampler operates under different conditions and for different purposes. Thus, when collecting airborne microorganisms attached to dry dust particles in poultry environments, their performance may differ. To evaluate the sampling efficiency of the samplers, the wet aerosolization method was applied previously (Agranovski et al., 2002). Depending on its resistance to the stressors during sampling, the applicability and performance of the bioaerosol samplers were assessed. However, the airborne *E. coli* in the poultry house are aerosolized from dried poultry dust-laden particles generated by bird activities, such as dust bathing (Zhao et al., 2014). The aerosols in the past study (Agranovski et al., 2002) were droplets, while the carrier aerosol in the poultry houses may primarily consist of dried dust particles. So, the results of the study based on wet aerosolization may not be directly applicable nor mimic the actual situation occurring in a poultry house. This requires further investigation.

(3) Common samplers for dust particles

Dust is collected using the filtering principle in Europe (Gravimetric-CEN, 2005), although numerous samplers, including tapered element oscillating microbalance (TEOM), filter, and optical samplers, are utilized in the United States. To gather dust in certain size fractions, a pre-separator is required, and those that use the impaction principle are legally mandated as reference techniques in both Europe and the United States (Kenny et al., 2000). Nevertheless, impaction-based pre-separators may overestimate fine dust concentrations in dusty livestock production systems, whereas cyclone-based pre-separators are less susceptible to overloading (Zhao et al., 2009). Although optical dust samplers that can measure real-time dust concentrations without further processing are now available, they have difficulties in humid conditions and in translating count to mass concentrations.

### 1.3.3 Mitigation techniques for airborne *E. coli*

Airborne *E. coli* can be abundant in poultry production systems, and there are risks for birds downwind receiving the *E. coli* through inhalation and/or contact with the surfaces where airborne *E. coli* settles. Physical deposition and biological inactivation can reduce the airborne *E. coli* concentration, and the electrostatic particulate ionization (EPI) technology has been effective in preventing *Salmonella* transmission among poultry and is adopted by egg producers to prevent airborne avian influenza transmission. Ultraviolet (UV) disinfection can also inactivate microorganisms, but the reduction effect of UV radiation on airborne *E. coli* in poultry environments has not been studied to date. The variation of ventilation, or air speed, leads to a variable contact time (or exposure time of *E. coli* to UV) and inactivation efficacy, which would be different from that in food

industries and human environments. Investigating potential solutions that may reduce airborne *E. coli* in poultry houses has vital implications for control of other emerging pathogens in the poultry industry.

### 1.3.4 Airborne transmission of highly pathogenic avian influenza carried by dust particles

The 2015 highly pathogenic avian influenza outbreak in the U.S. caused significant economic losses to the poultry industry and disrupted egg supply. The disease was spread among farms through unknown means, with traditional biosecurity protocols proving ineffective. Anecdotal evidence suggested that airborne transmission may have played a role. Recent studies confirmed that the virus is airborne transmissible in a ferret model. This study aims to investigate the risk of airborne transmission and offer guidance for effective prevention and containment strategies. In a past study (Zhao et al., 2019), authors mentioned the limitations of using air sampling to investigate long-distance transmission of AI virus and suggested the use of meteorological models, particularly the HYSPLIT model, to study the transmission. The HYSPLIT model is useful because it can account for inhomogeneous and time-varying meteorological conditions, and it can project backward trajectories and visualize the origins of viral particles. The air trajectory and concentration modeling are the core modules of the HYSPLIT model.

## 1.4 Knowledge Gaps

### 1.4.1 Dry aerosolization

While many studies have been conducted to investigate airborne transmission of *E. coli* under wet aerosolization, dry aerosols such as dust particles or drop nuclei have not been well studied. There is still a lack of understanding regarding the transmission of *E. coli* through the air, particularly when it comes to dry aerosols originating from poultry houses. The concentration and size distribution of *E. coli* attached to dry aerosols, such as poultry dust particles, have not been adequately studied. These parameters are important in determining the risk of infection from airborne *E. coli*, as the concentration of *E. coli* reflects the overall extent of exposure, and the size distribution of airborne *E. coli* affects how it travels and where it is deposited in the respiratory tract of birds. Therefore, it is urgent to assess the concentration and size distribution of airborne *E. coli* attached to dry dust particles in the poultry environment.

### 1.4.2 Survivability of airborne *E. coli*

Various factors, such as the type of farm, the size of the flock, and the distance of transmission, can impact the survivability of airborne *E. coli*. Studies which utilize computational modeling are also affected by the pathogen's ability to survive, which is a crucial factor. Poultry houses are kept under stable indoor conditions, which may allow *E. coli* to persist for extended periods in the poultry production environment. As a result, *E. coli* can spread to larger areas via vectors after surviving on surfaces for a long time. All of this raises the question of how long *E. coli* carried by poultry litter particles can survive

in the air and on physical surfaces once it has settled. Although there are many studies investigating the survival of *E. coli* under different conditions such as settled *E. coli* on stainless steel or *E. coli* in poultry litter, there are limited studies conducted under air condition in poultry houses.

### 1.4.3 Mitigation techniques

There are many mitigation techniques that have been well developed to reduce the effects of pathogens in the poultry industry. The most widely used method is the UV-C (UVC) irradiation method. UVC radiation, which has a wavelength range of 100-300 nm, has been extensively researched in the food industry and is known for its ability to inhibit DNA replication, thereby inactivating microorganisms (Ochoa-Velasco et al., 2020). The previous study found that UVC light was highly effective in disinfecting *E. coli* in water, droplets, and surfaces in the food industry, particularly when using a wavelength of 254 nm. However, in poultry houses, airborne *E. coli* can be carried by dust particles, which may prevent them from being exposed to UV light and thereby protect them from irradiation. Additionally, environmental factors such as ventilation systems, air flow, temperature, and relative humidity in poultry houses can affect contact time and resistance to UV light, resulting in varying levels of inactivation efficiency compared to the food industry. Therefore, further investigation is required to determine the effectiveness of UV light in deactivating airborne *E. coli* that is attached to poultry litter particles.

### 1.4.4 Long-distance transmission

The air in poultry houses contains various components such as gases, odors, and multiple pathogens, including Avian Influenza (AI) viruses, which can be carried by dust particles or droplet nuclei. AI viruses are initially secreted through the nasal secretions, feces, and saliva of birds (Zhao et al., 2019). These secretions can either be dried and remain suspended in the air for extended periods or deposited on the surface of poultry litter. The deposited secretions, which contain AI, can mix with poultry litter particles and be re-aerosolized into the air due to the dust bathing behavior of birds (Zhao et al., 2019). Both droplet nuclei and dust particles carrying AI can then be distributed throughout the poultry house environment and can be transmitted from one barn to another through the ventilation system and air transport. However, the concentration of AI after long-distance airborne transmission is relatively low compared to the original source, which may make it challenging to detect with conventional sampling methods. Therefore, a different approach is required to better understand long-distance airborne transmission.

## 1.5 Objectives

In this dissertation, four studies were developed for airborne transmission of *E. coli* and AI in poultry industry. The dissertation is constructed by presenting four studies that have been done during the Ph.D. program. Each study was listed as one chapter. The structure of the dissertation and objectives of each study were shown below.

Chapter I: Reviewing the background of global and U.S. poultry production. Reviews studies of airborne microorganisms in poultry industry, sampling methods for collecting dust and airborne *E. coli*, mitigation techniques, and Airborne transmission of highly pathogenic avian influenza carried by dust particles.

Chapter II: Evaluating bioaerosol samplers for airborne *Escherichia coli* carried by poultry litter particles.

Chapter III: Determining the survival of *Escherichia coli* in airborne and settled poultry litter particles.

Chapter IV: Assessing the effects of Ultraviolet radiation on reducing airborne *Escherichia coli* carried by poultry litter particles.

Chapter V: Modeling long-distance airborne transmission of highly pathogenic avian influenza carried by poultry litter particles.

Chapter VI: Discussions and conclusions.

# CHAPTER II

# Evaluation of Bioaerosol Samplers for Airborne *Escherichia Coli* Carried by Poultry Litter Particles

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## 2.1 Introduction

Broiler chickens are raised on floor housing system in the U.S. While the floor system has been used for years, the living conditions of broilers are increasingly questioned along with current public concerns on farm animal welfare. To address these concerns, animal welfare groups have been progressively promoting environmental enrichments in broiler houses. This movement has resulted in an increasing number of chain restaurants and retailers sourcing poultry meat produced in housing with higher welfare standards. As such, broiler producers are striving to improve the production environment to meet public demands; however, they are facing challenges in multiple dimensions.

The U.S. is the world's largest producer of poultry meat and a major egg producer. It is an industry worth 35 billion dollars in 2020 (USDA-NASS, 2020) and provides about 1 million jobs for Americans. However, that industry is very sensitive to infectious diseases that are caused by pathogenic microorganisms, for example, Avian pathogenic *Escherichia coli* (APEC). The diseases caused by APEC have been distributed widely in many types of poultry houses as well as all ages of birds (Saif et al., 2008). APEC was considered one of the main causes of the economic losses to the global poultry industry (Yu et al., 2016). These bacteria commonly inhabit the lower gastrointestinal tract of poultry and other warm-blooded animals and the macroclimate where the animals reside. APEC causes intestinal manifestations (colibacillosis) such as gastroenteritis, urinary tract infections, neonatal meningitis, hemorrhagic colitis, and Crohn's disease. The economic losses of poultry caused by APEC are due to the cost of poultry losses, mortalities, medication costs, and reduced feed efficiency (Mellata, 2013). A past study mentioned that about 40% of

broiler carcasses condemned were infected with APEC (Hasan et al., 2011), and approximately 30% of broiler flocks in the U.S. are infected by subclinical colibacillosis (Fancher et al., 2020).

Air in the microclimate of the poultry houses contains not only gases and odors but also lots of bacteria, including APEC (Sanz et al., 2021). The *Escherichia coli* (*E. coli*) associated with poultry manure are initially deposited onto poultry litter, and then due to normal poultry movement and their dust-bathing behavior, may become airborne and be carried by poultry dust particles (Zhao et al., 2014). Airborne *E. coli* have been reported to account for 2–6% of total airborne bacteria in poultry houses (Theofel et al., 2020; Zucker et al., 2000). As particles settle out of the air, they contaminate the surfaces on which they settle. Ventilation systems can carry these dust-bound *E. coli* particles throughout a single barn, as well as from barn to barn, creating the potential for widespread impact. Barn-to-barn airborne transmission has been investigated for avian influenza (Zhao et al., 2019). This study showed that airborne transmissibility could be affected by several factors including the type of poultry farm, size of the flock, concentration and size distribution of airborne pathogen, and type of carrier aerosols (dust particles or droplets, for example), and survival of the pathogen. Therefore, under varying conditions, the airborne transmissibility of an agent and the characteristics of its associated airborne particle may differ, thereby impacting the efficiency of various sampling methods. In addition, the airborne transmission of *E. coli* originating from poultry houses is still far from being well understood. This is especially true of dry aerosols for which the concentration (number of microbes counted in-unit air volume) and size distribution (the concentration spectrum over a broad size range of bacteria-laden particles) of airborne *E. coli* attached to dry aerosols (such as poultry dust particles) have not been adequately studied. These parameters are important because they can influence the infection risks of airborne *E. coli* (Ssematimba et al., 2012). More specifically, the airborne *E. coli* concentration reflects the overall extent of *E. coli* exposure, while the size distribution of airborne *E. coli* governs the air transportation and site of deposition along the bird respiratory tract. Therefore, it is an urgent need to evaluate the concentration and size distribution of airborne *E. coli* attached to dry dust particles in poultry environment.

To determine the concentration and size distribution of airborne *E. coli* attached to dry dust particles, a proper sampler that can efficiently collect airborne *E. coli* attached to dry dust particles is required. Samplers such as Andersen six-stage impactor (Andersen impactor), all-glass impinger AGI-30 (AGI-30) and ACD-200 Bobcat (Bobcat) are widely used because of their advantages in airborne sampling. Andersen impactor uses the direct impact technique and is designed as an aerodynamic classifying system for airborne particles. AGI-30 uses the impingement method and is known as a versatile, inexpensive, and easy-to-use method. The high-volume sampler, ACD-200 Bobcat, uses the filtration method and is designed for collecting airborne bacteria at low concentrations. Each sampler operates under different conditions and for different purposes. Thus, when collecting airborne *E.*

*coli* attached to dry dust particles in poultry environments, their performance may differ. To evaluate the sampling efficiency of the samplers, the wet aerosolization method was applied previously (Agranovski et al., 2002). Depending on its resistance to the stressors during sampling, the applicability and performance of the bioaerosol samplers were assessed. However, the airborne *E. coli* in the poultry house are aerosolized from dried poultry dust-laden particles generated by bird activities, such as dust bathing (Zhao et al., 2014). The aerosols in the past study (Agranovski et al., 2002) were droplets, while the carrier aerosol in the poultry houses may primarily consist of dried dust particles. So, the results of the study based on wet aerosolization may not be directly applicable nor mimic the actual situation occurring in a poultry house. In addition, the efficiency of the samplers in terms of collecting samples under dry aerosolization conditions in poultry houses has never been investigated. Therefore, a study to evaluate the performance of the samplers based on the dry aerosolization method is needed. This study aimed to investigate the sampling performance of three bioaerosol samplers, namely an Andersen impactor, an AGI-30, and a Bobcat via dry aerosolization under room thermal conditions. The temperature was about 20.8°C with relative humidity (RH) of 40%. In addition, the size distribution of airborne *E. coli* was also determined by the Andersen impactor.

## 2.2 Materials and Methods

To evaluate and compare sampling performances, the bioaerosol samplers were used to collect *E. coli*-laden dust particles in a testing chamber in a Biosafety Level 2 (BSL-2) laboratory. The laboratory is located at the Animal Science Department, University of Tennessee, Knoxville, TN 37996, U.S.

### 2.2.1 Preparation of *E. coli* solution

The *E. coli* strain used in this study was *Escherichia coli* (ATCC® 25922) which was purchased from American Type Culture Collection (ATCC, Manassas, VA, U.S.). This research has been approved by the Institutional Biosafety Committee at The University of Tennessee under the protocol IBC-21-572-2. *E. coli* strain was cultured at 37°C, 150 rpm for 24 hrs in ATCC® Medium 18 (Tryptic Soy Broth 'TSB' and Tryptic Soy Agar 'TSA', ATCC, Manassas, VA, U.S.). The bacterial concentrations of *E. coli* in the solution after 24 hrs were determined by the traditional serial dilution process. The concentration was approximately 8.80 $\log_{10}$ colony-forming unit ($\log_{10}$ CFU) $mL^{-1}$.

### 2.2.2 Litter preparation

The litter from the commercial broiler farm was first collected and stored in a container placed inside the test chamber. It was then brought back to the Animal Science Department, University of Tennessee, Knoxville. Then, it was autoclaved at 121°C for 20 min and divided into identical-size aluminum boxes with the amount of 6 kg per box. The boxes were sealed by aluminum foil and covered by plastic caps to avoid contamination. They were stored in a 4°C fridge until being used.

It was important to prepare litter so that the bacteria were evenly distributed. To do that, 240 g of litter was needed for this experiment and evenly distributed into 40 ceramic cups. The ability to generate airborne dust was tested in a previous experiment (Nguyen et al., 2021a), and the results showed that 240 g of litter added to the mixer, would produce dust concentrations ranging from 0.9 to 1.1 mg $m^{-3}$ which is within the typical range of dust concentration in a commercial poultry farm (Davis & Morishita, 2005). To prepare litter inoculated with *E. coli*, a set of 43 ceramic cups (40 cups for experiment plus 3 controls) with identical shapes was used to hold the litter. In each cup, 6 g of litter was prepared and mixed with 6 mL of *E. coli* cultured solution. The 6 mL bacteria solution was sprayed evenly onto the litter in each cup. In the meantime, a sterile aluminum spoon was used to gently mix the litter and *E. coli* solution. The mixtures then went through a process of drying at 20.8°C and 30–50% RH for 48 hrs until the dry matter content (DMC) of the mixture reached about 80% that was ready for aerosolization. The *E. coli* concentration in the control cup was measured by adding TSB into the mixture so that the total volume of each cup was up to 15 mL. Then, 1 mL of the solution in each cup was collected by an automatic pipette. The 1 mL solution passed through a traditional serial dilution process to determine the culturable *E. coli* in the 1 mL solution. The concentration of *E. coli* in the cup was approximately 7.80 $\log_{10}$ CFU $g^{-1}$ litter after the drying process. The litter containing *E. coli* was then transferred from 40 ceramic cups to a metal bowl of the mixer for aerosolization. In the bowl, the litter was gently mixed up again before aerosolization.

### 2.2.3 Test chamber

Aerosolization proceeded in an acrylic chamber. This chamber (2100 series, Cleatech, Orange, CA, U.S.) was a non-vacuum unit with two internal access doors with stainless steel frame, and a removable fully gasketed back wall. The dimension of the test chamber was 1.5 m L × 0.6 m W × 0.6 m H. The chamber was well sealed to prevent dust from spilling out. It was also equipped with a temperature and RH sensor for continuously monitoring the inside thermal environment.

### 2.2.4 Aerosolization system

A stand mixer (model DCSM350GBRD02, New York, NY, U.S.) was used for dry aerosolization of airborne *E. coli* in this study. The dimensions of the mixer were 0.3 m L × 0.2 m W × 0.3 m H with a 3.3 L stainless steel bowl. It was operated at the highest speed to ensure the bacteria concentration in the air was high enough so that the samplers were able to detect it. A stir fan was also used to distribute the airborne *E. coli* in the chamber evenly.

### 2.2.5 Dust concentration monitor

To monitor the dust concentration throughout the experiment, a dust concentration monitor (DustTrak DRX aerosol monitor 8533, TSI Inc., Shoreview, MN, U.S.) was used to measure the mass concentration of dust particles of different sizes (Nguyen et al., 2022).

DustTrak was capable of measuring dust particles of $PM_1$, $PM_{2.5}$, $PM_{4.7}$, and $PM_{10}$. The record gap of DustTrak was 1 s. In 20 min of the experiment, a total of 1200 data points were collected to measure accurately dust concentrations. In this study, the dust concentration and particle size were recorded, and the results indicated that the particle concentration was relatively stable between experimental events (Nguyen et al., 2022).

### 2.2.6 Air samplers

To evaluate and compare sampling performances, two sets of three bioaerosol samplers (Figure 2.1) were used to collect *E. coli*-laden dust particles. An Andersen impactor, an AGI-30, and a Bobcat air sampler were tested in this study. The Andersen impactor operates at 28.3 L $min^{-1}$. It can separately collect airborne microorganisms of the different size of > 7.0 µm, 4.7–7.0 µm, 3.3–4.7 µm, 2.1–3.3 µm, 1.1–2.1 µm, 0.65–1.1 µm, respectively, from stage 1 to 6. The AGI-30 operates at 12.5 L $min^{-1}$. The airborne compounds were drawn utilizing a vacuum pump through a fine nozzle in which the particles were accelerated and then impact directly into the 20 mL TSB. The Bobcat operates at 200 L $min^{-1}$. The Bobcat allows the airflow to go through a filter that was attached to the top of the sampler. Dust particles then were collected on a filter. The collected microorganisms can be recovered via a wet foam elution (the single-use filter elution kit).

### 2.2.7 Dry matter content measurement

The DMC of litter, a crucial factor affecting the survival of bacteria (Crane et al., 1983), was measured over time in this study. The DMC of poultry litter is the ratio of the litter mass before and after the litter is completely dried. To determine the DMC of poultry litter, the process was divided into two stages. First, the litter mass (m1) was weighted before going through a 48-h drying process at 105°C until the litter mass was dried. After being dried at 105°C, the litter mass (m2) was weighted again. The litter mass m1 and m2 were cooled down and measured at room temperature (at about 20.8°C, with RH varying from 30–50%). The DMC was then calculated by the litter mass m2 divided by the litter mass m1.

### 2.2.8 System setup and sampling collection

Two hundred and forty grams (240 g) of litter which contained ~ 7.80 $\log_{10}$ CFU [g litter] $^{-1}$ of *E. coli* were prepared and placed in the mixer. The mixer was placed in the center of the chamber to help evenly distribute the dust particles carrying *E. coli*. The mixer was fixed to the chamber surface using suction cups, preventing it from moving during the running process. The stir fan was placed at the corner of the chamber to aid in distributing airborne particles. Each test lasted a total of 40 min. The first 20 min of the test was the aerosolization process of airborne *E. coli* using the mixer and the stirring fan. After the 20-min aerosolization process, the mixer and the stirring fan were stopped, and airborne *E. coli* attached to dust particles were collected by samplers for 20 min. There were two sets

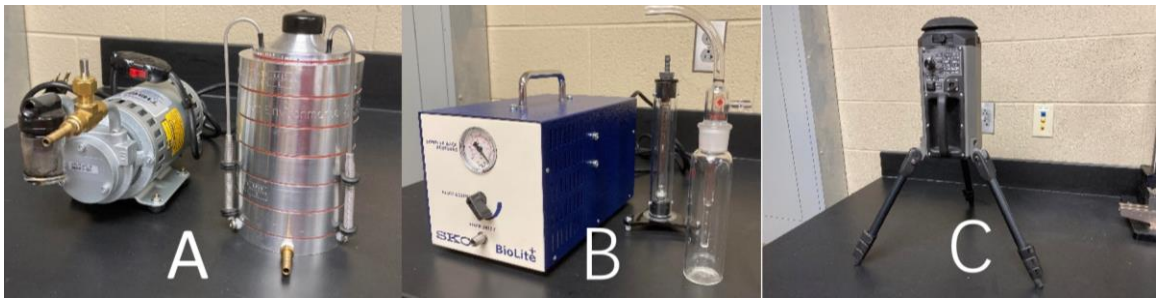

Figure 2.1 The three samplers (from left to right): Andersen impactor (A), AGI-30 (B), and Bobcat (C).

of the three samplers placed side by side next to the mixer (Figure 2.2). The sampling ports of the samplers were adjusted to the fixed height of 27 cm. In addition, the sampler positions were rearranged randomly in each aerosolization event to minimize the location confounding effect. DustTrak was also used to measure the dust concentration. The results showed that the average dust concentrations during the 20-min sampling were between 0.9 and 1.1 mg m-3. The environmental conditions in the chamber were kept at about 20.8°C, with RH varying from 30–50%.

### 2.2.9 Determining airborne *E. coli* collected by Andersen impactor

The Andersen impactor was used to monitor the airborne *E. coli* size distribution and concentration. The Andersen impactor included 6 stages with one Petri dish in each stage. Each Petri dish was prepared with TSA. In the sampling process of the Andersen impactor, the dust particles carrying *E. coli*, after being aerosolized, were drawn into the inlet on top of the Andersen impactor. Then, the particles continuously went through 6 stages. For each stage, dust particles with sizes corresponding to each stage were collected on TSA plates.

When counting the number of culturable *E. coli*, the TSA plates were often overloaded due to the excessive number of airborne *E. coli*. Therefore, directly counting culturable *E. coli* on agar plates was not possible. To resolve the problem, the washing agar plate method was applied. The culturable *E. coli*, after being collected on agar plates, was immediately taken to the BSL-1 laboratory for analysis. The plates were numbered corresponding to the number of stages. Each plate was rinsed with 2 mL of TSB solution with the aid of a glass spreader. Then, 1 mL of the solution was collected by an automatic pipette. The 1 mL solution passed through a traditional serial dilution process to determine the culturable *E. coli* in the 1 mL solution. The agar plates, after washing, were also placed in an incubator letting the remaining *E. coli* on the plate grow. During the air sampling process, the TSA agar plates in the Andersen impactor were dried by airflow in the sampler. Thus, the remaining 1 mL of solution in the washing process was mostly reabsorbed into the agar plates. However, to make sure that there is no residual solution that could affect the test results, the agar plates that have been washed instead of being turned upside down (due to the traditional culture process) will be left-right side up. The total culturable *E. coli* in each stage was the combination of total *E. coli* collected from washing agar plates and total *E. coli* remaining on agar plates corresponding to the washing plates.

### 2.2.10 Determining airborne *E. coli* concentration and size distribution

Each air sample (in liquid form) was used to quantify culturable *E. coli* via traditional culture techniques. After vortexing for 5 s, 0.1 mL serially diluted (1:10) samples were plated onto TSA agar plates. The plates were aerobically incubated at 37°C for 24 hrs. The visible *E. coli* colonies formed on plates (30 to 300 colonies) were determined. Based on the culture results and the sampled air volume, airborne *E. coli* concentrations were

Figure 2.2 The system setup with two sets of samplers (Andersen impactor, AGI-30, and Bobcat).

calculated in logarithm colony-forming units per cubic meter ($\log_{10}$ CFU $m^{-3}$) using equation (2.1).

$$C = log_{10}(\frac{N \times 10^n}{V_p} \times V_s \times \frac{1}{V_a}) \quad (2.1)$$

Where:

C: the airborne bacteria concentration, log10 CFU $m^{-3}$;
N: the number of colonies on a countable plate (30 to 300 colonies);
N: serial dilution factor (n = 0 for undiluted sample, n = 1 for 10-fold diluted sample, etc.);
Vp: the sample volume plated, mL (VP = 0.1 mL in this study);
Vs: the total volume of the original liquid sample, mL;
Va: the total air volume sampled using the bioaerosol samplers, $m^3$.
The airborne *E. coli* size distribution was determined by the Andersen impactor. The size distribution of airborne *E. coli* was presented as the percentage of culturable *E. coli* in each stage divided by the total number of culturable E. coli in all 6 stages.

### 2.2.11 Determining airborne *E. coli* collected by AGI-30

AGI-30 was used to monitor the airborne *E. coli* concentration. Airborne *E. coli* was collected by AGI-30 in liquid form (in TSB medium). The airborne *E. coli* attached to dust particles was drawn into an inlet on top of the AGI-30 and went through a fine nozzle into the TSB solution with the aid of a vacuum pump. Twenty milliliters of TSB medium were prepared in the collection vessel. The solution containing airborne *E. coli* was analyzed to quantify the total culturable airborne *E. coli* collected by the sampler via the traditional culture technique. The total culturable *E. coli* concentrations were calculated by equation (2.1).

### 2.2.12 Determining airborne *E. coli* collected by Bobcat

Bobcat was used to monitor the airborne *E. coli* concentration. The sampler used a dry 52 mm electret filter as the collection media and a collector which sucked air through the filter. The airborne *E. coli* attached to dust particles was collected onto the filter. After 20-min sampling, the filter was removed from the collector. Then, the filter was snapped onto the sample cup and fitted with an elutor cap. To extract *E. coli* attached to dust particles from the filter, a rapid filter elution fluid was used. The fluid contained wet foam elution (8.5 mL of 0.075% Tween 20/25 mM Tris). The elution was released from a canister and passed through the filter evenly to extract any captured *E. coli* attached to dust particles. Then, the solution containing airborne *E. coli* was analyzed to quantify the total culturable airborne *E. coli* collected by the sampler via the traditional culture technique. The total culturable *E. coli* concentrations were calculated by equation (2.1).

### 2.2.13 Statistical analysis

The criterion of the comparison was to identify the samplers that can recover the closest concentration of airborne *E. coli* to the *E. coli* concentration which calculated by the *E. coli* concentration in poultry litter multiplied by the total dust concentration in the chamber. Considering imperfect physical and biological sampling efficiency, none of these samplers (and other aerosol samplers) can recover the airborne microorganisms at 100%. However, the criterion in our study will help us to select the best sampler to estimate the airborne *E. coli* concentration on a commercial farm. The effect of sampler type on the total number of airborne *E. coli* ($\log_{10}$ CFU) and concentration of airborne *E. coli* ($\log_{10}$ CFU $m^{-3}$) were examined using the GLIMMix ANOVA model running on Statistical Analysis System (SAS software, SAS Institute, Cary, North Carolina, U.S.). The total airborne *E. coli* ($\log_{10}$ CFU) was the total CFU detected in the samples of each sampler. The concentration of the airborne *E. coli* was the total airborne *E. coli* normalized by the flow rates of the samplers. The statistical analysis was presented with following model:

$$Y_i = \mu + T_i + \varepsilon \quad (2.2)$$

Where:

$Y_i$: *E. coli* concentration (CFU $m^{-3}$);
$\mu$: actual mean of *E. coli* concentration;
$T$: sampler effect (i = Andersen impactor, AGI-30, and Bobcat);
$\varepsilon$: residual error.
Based on a previous study (Zhao et al., 2011a, 2011b) and our experience, twenty-one replicates were sufficient to perform a powerful statistical analysis for comparing the performance of bioaerosol samplers. The significant level used in the model was 0.05 ($p < 0.05$). Other covariates such as concentration of *E. coli* prepared in ceramic cups, temperature, RH, day, and dust concentration (measured by DustTrak during sampling) were also examined to see if the covariates had any effect on the total airborne *E. coli* and concentration of airborne *E. coli*. Results showed that all covariates were not significant.

## 2.3 Results and discussion

### 2.3.1 Conditions for evaluation of bioaerosol sampler test

Table 2.1 shows the litter dry matter content, initial litter *E. coli* concentration, and environmental conditions during the experiment. The conditions remained stable through experiments.

### 2.3.2 Size distribution of airborne *E. coli* and dust particles

The size distribution of airborne *E. coli* attached to dust was measured by using the Andersen impactor. The size distribution of airborne *E. coli* attached to dust particles during the 20-min sampling process is shown in Figure 2.3. Most airborne *E. coli* were

Table 2.1 Conditions (Mean±SD[a]) for the *E. coli* in bioaerosol sampler test.

| Conditions | Bioaerosol sampler test |
|---|---|
| Dry matter content of litter (%) | 80±2[b] |
| Dust particles concentration (mg $m^{-3}$) | 0.969±0.056 |
| Temperature (°C) | 20.8±0.6 |
| *E. coli* concentration in litter ($log_{10}$ CFU $g^{-1}$) | 7.80±0.29 |
| Relative humidity (%) | 40±4 |

[a] SD means the standard deviation. [b] Dry matter content of litter in ceramic cups after 48 hrs under room conditions.

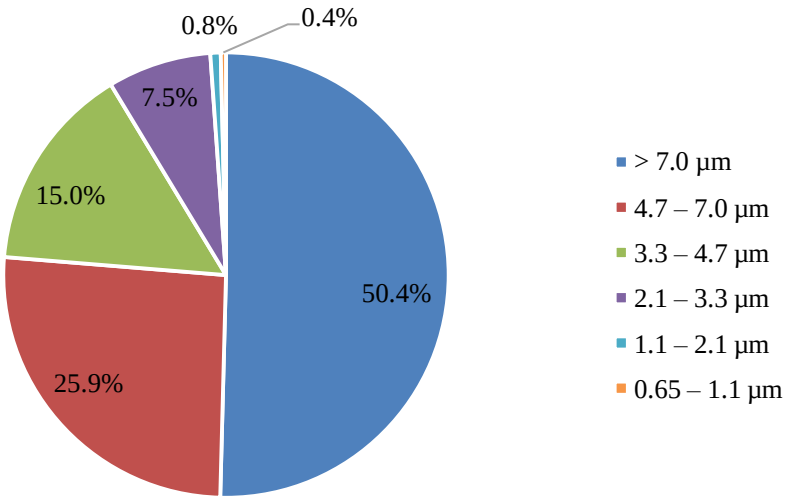


Figure 2.3 Size distribution of the airborne *E. coli* attached to dust particles was measured by an Andersen.

25.9%. The airborne *E. coli* attached to dust particles in the ranges of 3.3–4.7 µm and 2.1–3.3 µm accounted for 15.0% and 7.5% of the total culturable *E. coli*, respectively. The least *E. coli* were found in particles in the ranges of 1.1–2.1 µm and 0.65–1.1 µm which accounted for 0.8% and 0.4% of the total culturable *E. coli*.

The size distribution of airborne dust particles during the 20-min sampling process was monitored by the DustTrak and shown in Table 2.2. Most dust particles have a size smaller than 1.0 µm with a concentration of 0.575±0.118 mg $m^{-3}$. The rest of the dust particles have a size range of 1.0–2.5 µm, 2.5–4.7 µm, 4.7–10.0 µm, and larger than 10.0 µm, with a concentration of 0.014±0.002 mg $m^{-3}$, 0.016±0.007 mg $m^{-3}$, 0.229±0.063 mg $m^{-3}$, and 0.208±0.033 mg $m^{-3}$, respectively. The total dust concentration was 0.969±0.056 mg $m^{-3}$. As shown in Table 2.2, although most dust particles were smaller than 1 µm, the size distribution of bacteria attached to dust particles was mainly larger than 2.1 µm, accounting for 98.8%. This implies that most airborne *E. coli* were attached to dust particles with a size larger than 2.1 µm. In a previous study (Nguyen et al., 2022), authors measured the size distribution of airborne *E. coli* attached to dust particles and the size distribution of dust particles. The results of the study were consistent with the results reported in this study.

In past studies (Hu et al., 2020; Zhao et al., 2011; Zheng et al., 2013), authors examined the size distribution of airborne bacteria attached to aerosols (wet and dry) by using an Andersen impactor. Results of the study also concluded that most airborne bacteria were collected on stage 1 (> 7.0 µm), and the percentage of bacteria gradually decreased from stage 1 to stage 6. This implies that bacteria might be preferentially aggregated on large particles. The distribution could be explained by the survivability of airborne bacteria. Greater abundance on larger particles may reflect more favorable conditions allowing better survival (Stern et al., 2021). Besides, a past study (Zuo et al., 2013) reported a shielding effect on the survivability of airborne viruses. Authors explained that compared with viruses existing as a singlet or attaching to small particles, the virus attached to larger particles could be better protected from changes in the ambient environment (Woo et al., 2012). Therefore, the airborne *E. coli* attached to larger particles (> 7.0 µm) might have better protection compared to smaller ones in our study. Moreover, the small dust particles (< 1.0 µm) may be too small to carry the *E. coli*. A past study (Riley, 1999) reported that the typical size range of *E. coli* was about 1.0–2.0 µm which was bigger than the size of carrier particles. Therefore, the carrier particles with a size range of < 1.0 µm might not be able to carry airborne *E. coli*.

In terms of dispersion, 1.2% of total airborne *E. coli* attached to dust particles were smaller than 2.1 µm. This indicates that dust particles smaller than 2.1 µm can still carry airborne *E. coli*. These dust particles were small and dispersed far from the source. According to a past study (Hayter & Besch, 1974), these airborne *E. coli* carried by particles smaller than 2.1 µm were harmful to poultry. They can deposit in the anterior

Table 2.2 Dust size distribution (Mean±SD[a]) in the bioaerosol sampler test.

| < 1.0 μm ($mg\ m^{-3}$) | 1.0–2.5 μm ($mg\ m^{-3}$) | 2.5–4.7 μm ($mg\ m^{-3}$) | 4.7–10.0 μm ($mg\ m^{-3}$) | > 10.0 μm ($mg\ m^{-3}$) | Total ($mg\ m^{-3}$) |
|---|---|---|---|---|---|
| 0.575±0.118 (59.4%)[b] | 0.014±0.002 (1.5%)[b] | 0.016±0.007 (1.7%)[b] | 0.229±0.063 (23.6%)[b] | 0.208±0.033 (21.4%)[b] | 0.969±0.056 (100.0%)[b] |

[a] SD means the standard deviation. [b] Percentage of the total for each size range.

trachea, lung, posterior air sacs, or even worse in anterior sacs of the poultry respiratory system. With this deep penetration of bacteria into the respiratory system, they could cause a variety of respiratory diseases in chickens (Hayter & Besch, 1974). Moreover, in a study conducted in 2017 (Fathi et al., 2017), the authors mentioned the ability to disperse the air of airborne bacteria. The study reported that the airborne bacteria can travel over 500 m from the source with bacterial concentration reduced only about 5 times compared to the source. Although the ability of airborne *E. coli* to disperse in the air depends on many factors such as the survival ability of ambient influences, its ability to disperse far in the air is a threat to U.S. agriculture.

### 2.3.3 Total airborne *E. coli* and concentration of airborne *E. coli* collected by three samplers

Total airborne *E. coli* collected by the three samplers in 20-min sampling were shown in Table 2.3. The total *E. coli* counts were 4.81±2.08 $\log_{10}$ CFU for Andersen impactor, 5.09±1.92 $\log_{10}$ CFU for AGI-30, and 3.54±0.81 $\log_{10}$ CFU for Bobcat. Based on statistical analysis, Andersen impactor and AGI-30 were not different from one other ($p < 0.05$). Bobcat detected airborne *E. coli* significantly less than Andersen impactor or AGI-30 ($p < 0.05$).

The Bobcat was designed based on the filtration method for measuring airborne microorganisms at low concentrations. The air flow rate of Bobcat (200 L $min^{-1}$) was much higher compared to those of the most common samplers like Andersen impactor (28.3 L $min^{-1}$) and AGI-30 (12.5 L $min^{-1}$). Surprisingly, high flow rate samplers generally collected lower quantities of airborne *E. coli* than low flow samplers. The major problem is that the Bobcat uses a filter-based sampling method, which could have poor recovery of bacteria from the filters.

First, a study in the past mentioned that the recovery efficiency was lowered as the result of a long time remaining on the filter (Lundholm, 1982). In the study, the author compared the most popular samplers including an Andersen impactor, a slit sampler, an impinger, and filter samplers with gelatine filters or membrane filters. The culturable airborne bacteria in the past study (Lundholm, 1982) significantly declined with filtration time, and the 60-min filtration period gave only 55% of the yield of a 1-min period. In addition, the authors also mentioned that even though the filtration period was only 1-min, the efficiency of the filtration method was still not very efficient compared to the impingement and impaction method. Moreover, with a long period of exposure to the filter of Bobcat in our study, the *E. coli* might not adapt well to the filter surface due to the lacking of nutrient availability, which led to the loss of survival (Lundholm, 1982; Petersen & Hubbart, 2020). In contrast, the TSA plates in Andersen impactor and TSB in AGI-30 provided nutritious environments for bacteria immediately after being collected, which preserves *E. coli* survivability better during the 20-min sampling. Further, the loss of bacteria viability could be explained due to the dehydration effect on the filter (Zhao et al., 2011). Zhao et al. (2011) compared the biological efficiency (as a part of sampling efficiency) of three

Table 2.3 Total *E. coli* (Mean±SD[a]) was collected by three samplers.

| Sampler | *E. coli* counts ($log_{10}$ CFU, Mean±SD) |
|---|---|
| Andersen six-stage impactor | 4.81±2.08a |
| All-glass impinger (AGI-30) | 5.09±1.92a |
| ACD-200 Bobcat | 3.54±0.81b |

a, b means in the same column with different superscripts are different ($p < 0.05$). [a] SD means the standard deviation.

samplers, Andersen impactor, AGI-30, and a filter-based sampling method (Airport MD8) for *E. coli* under-sampling stress e.g., dehydration. The study showed that Andersen impactor and AGI-30 both showed high biological efficiency for *E. coli*, while Airport MD8 partially inhibited the bacterial culturability.

Second, Lundholm (1982) mentioned the trapping effect of the filter causing the low recovery efficiency of culturable bacteria. The bacteria might be trapped on the filter, and the washing process might not recover them into a collecting solution. Thus, the culturable bacteria collected in the solution might not coincide with the culturable bacteria on the filter. In our study, the wet foam elution was used to wash out the airborne *E. coli* attached to dust particles from filters. However, the efficiency of the elution was not well studied under dry aerosolization conditions where airborne *E. coli* was attached to dust particles. So, it might have a gap between the number of culturable *E. coli* on the filter and the number of culturable *E. coli* collected by the elution.

Further, the difference in the aerosolization process (wet aerosolization vs dry aerosolization) would yield different performances of samplers. The reason that dry and wet aerosolization would yield performance differences between samplers is that these samplers differ in the sampling efficiencies on aerosols with different sizes. During the sampling process, different flow rates of different samplers might yield different dehydration effects on aerosols. In wet aerosolization, these effects among samplers can cause wet aerosols to evaporate differently leading to particle size changes. Therefore, such particle size changes may affect the efficiencies of the samplers to certain extents depending on the sampler type. In dry aerosolization, the dust particles have relatively stable sizes, and are less affected by the dehydration effect. In a past study (Raynor et al., 2021), authors compared the performance of samplers including Andersen impactor, AGI-30, and Bobcat to collect airborne viruses. Results of the study concluded that high flow rate samplers generally collected greater quantities of the virus than low flow samplers. In our study, the least effective sampler in terms of quantities of *E. coli* collected was Bobcat (the one with the highest flow rate). This could be explained by the water film protection. In the past study, the viral solution was aerosolized by a 6-jet Collison-type nebulizer (BGI Inc., Waltham, MA). The nebulizer generated droplets (wet aerosols) that carried the airborne virus. In contrast, *E. coli* in the present study were mixed with poultry litter and were aerosolized by a mixer which generated dry dust particles (dry aerosols) carrying airborne *E. coli*. The wet aerosolization could provide a protective water film for airborne virus aerosolization (Hoeksma et al., 2015). And thus, during the sampling process of Bobcat, the water film helped the viruses counteract the dehydration effect caused by the high flow rate sampler. Meanwhile, the airborne *E. coli* under dry aerosolization had no protection from water film. Therefore, the airborne *E. coli* cells would be more sensitive to dehydration caused by the high flow rate of Bobcat and lead to significant losses of culturable airborne *E. coli* collected by Bobcat. The low flow rate in Andersen impactor and AGI-30 might not create as much dehydration effect on the bacteria as the high flow

rate in Bobcat. Moreover, the difference in the preparation process between the two studies could be another reason for the discrepancy in sampling performance. In the past study (Raynor et al., 2021), the viruses were cultured in a solution with proper mixtures of nutrients. In the current study, the *E. coli* were prepared in poultry litter which went through an autoclave process. The autoclaving process of poultry litter could affect the quality of poultry litter and produce Maillard reaction product. The temperature used for autoclave sterilization would induce Maillard reactions which could occur at 120°C (Lan et al., 2010). Maillard reaction products have been shown to inhibit the growth of bacteria (Bhattacharjee et al., 2009). However, the effect of the preparation procedure was not well-studied in the present study. Therefore, the effect still needs further investigation. In addition, the biological difference between viruses and bacteria could lead to the different results of Bobcat between the past study (Raynor et al., 2021) and our study. This difference needs further research before getting a conclusion.

Concentrations of airborne *E. coli* measured by the three samplers were respectively 5.05±2.08 $\log_{10}$ CFU $m^{-3}$ for Andersen impactor, 5.69±1.92 $\log_{10}$ CFU $m^{-3}$ for AGI-30, and 2.69±0.81 $\log_{10}$ CFU $m^{-3}$ for Bobcat (Table 2.4). There was no significant difference between Andersen impactor and AGI-30 ($p < 0.05$). Meanwhile, there was a sizeable decrease in the *E. coli* concentration obtained in the Bobcat sampler ($p < 0.05$). The concentrations of airborne *E. coli* collected by the three samplers were then compared to the concentration of *E. coli* in chamber which calculated by the *E. coli* concentration in prepared poultry litter multiplied by the total dust concentration (measured by DustTrak) in the chamber. The theoretical *E. coli* concentration was 5.84 ±1.33 $\log_{10}$ CFU $m^{-3}$. The *E. coli* concentration collected by Andersen impactor and AGI-30 were closer to the theoretical result than the *E. coli* concentration collected by ACD-20. Therefore, Andersen impactor and AGI-30 were more accurate than ACD-200.

In previous research conducted (Thorne et al., 1992), the author compared the performance of the Andersen impactor, the AGI-30, and the Nuclepore filtration-elution (NFE) sampler on collecting airborne bacteria. The performances of these three samplers were compared in 24 swine confinement buildings, with the measurements taken in two seasons, in fall and winter. The authors concluded that AGI-30 was the best sampling method for culturable bacteria in swine houses, and NFE was a satisfactory sampler. The results also showed the Andersen impactor performed worst in terms of sampling bacteria. The reason for the subpar performance of the Andersen impactor was its overloading issue in environments with high concentrations of airborne bacteria. The agar plates were overloaded in a short sampling time (15 s) when the plates were directly cultured. To solve the overloading problem, *E. coli* collected on each TSA plate was rinsed with 2 mL of TSB solution in our study. The *E. coli* concentration in the rinse-off solution was then determined using serial dilution and the standard culture method. By doing that, the *E. coli*

Table 2.4 Concentrations of airborne *E. coli* (Mean±SD[a]) were measured by three samplers.

| Sampler | *E. coli* counts ($log_{10}$ CFU $m^{-3}$, Mean±SD) |
|---|---|
| Andersen six-stage impactor | 5.05±2.08a |
| All-glass impinger (AGI-30) | 5.69±1.92a |
| ACD-200 Bobcat | 2.69±0.81b |

a, b means in the same column with different superscripts are different ($p < 0.05$). [a] SD means the standard deviation.

concentration results collected by Andersen impactor and AGI-30 showed no significant difference in terms of *E. coli* concentration.

In this study, the sampler comparison test was performed in the laboratory environment, i.e., the temperature was about 20.8°C and RH was from 30–50%. However, the environments in commercial poultry houses are much more complicated. The temperature and RH could have much wider ranges in poultry houses and the airspeed in broiler houses could be as high as 0.2–5.0 m $s^{-1}$. It has been reported that airspeed is a factor affecting the efficiency of bioaerosol samplers (Upton et al., 1994). Therefore, validation of the sampling efficiency is recommended to perform at commercial poultry farms.

## 2.4 Conclusions

This study demonstrated a methodology to evaluate bioaerosol sampler efficiency using dry-base carriers of biological agents which are predominant, as compared to wet-based ones, in poultry production systems. The performance of three bioaerosol samplers was compared for sampling total *E. coli* count and *E. coli* concentration. Based on the results, we conclude that 1) airborne *E. coli* might be preferentially aggregated on large particles (> 7.0 μm); 2) there was no significant difference in sampling efficiency between Andersen impactor and AGI-30 in terms of sampling airborne *E. coli*; 3) there was a significant decrease when using Bobcat for sampling. The results of this study will help to select a suitable sampler for the field research where *E. coli* concentration and size distribution are concerned.

# CHAPTER III

# Survival of *Escherichia coli* in Airborne and Settled Poultry Litter Particles

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## 3.1 Introduction

The United States of America (U.S.) is one of the leading countries in poultry production. Poultry products originating in the U.S. primarily consist of meat from broilers and turkeys and eggs from layers. According to the USDA report (USDA-NASS, 2020), the combined value of production from these products in 2020 exceeded USD 35 billion. These products provide important and affordable sources of dietary protein to the domestic population. In addition, approximately 18% of the U.S. poultry products are exported and poultry production in the U.S. was estimated to provide over 1 million jobs. However, the outbreak of infectious diseases is one of the biggest challenges for the poultry industry. For example, the Highly Pathogenic Avian Influenza (HPAI) outbreaks in the U.S., in 2015 resulted in losses of over 50 million birds and 3.3 billion dollars (Torremorell et al., 2016).

*Escherichia coli* (*E. coli*) is a member of the *Enterobacteriaceae* family and is commonly associated with the intestinal tract of warm-blooded animals and the environment in which these animals reside. In poultry, *E. coli* primarily inhabits the lower gastrointestinal tract as an indicator for the poultry environmental quality and exists there as an important commensal species. Typically, *E. coli* is harmless, but some *E. coli* strains may be pathogenic in nature and their virulence may lead to losses in the poultry industry. Pathogenic *E. coli* strains in poultry are commonly referred to as avian pathogenic *E. coli* (APEC) (Saif et al., 2008). The APEC causes the systemic disease colibacillosis in broilers. The severity of APEC disease depends on the health status of the host, virulence characteristics of the *E. coli* strain, and other predisposing factors such as stress. Approximately 30% of broiler flocks in the U.S. are infected by subclinical colibacillosis (Fancher et al., 2020).

*E. coli* can be abundant in poultry house with concentrations up to 4 $\log_{10}$ CFU $m^{-3}$ in the air (Zucker et al., 2000), 3 $\log_{10}$ CFU $g^{-1}$ in feeds (Munoz et al., 2021), and 7 $\log_{10}$ CFU $g^{-1}$ in poultry litter (Martin et al., 1998). To reduce the economic losses caused by *E. coli*, antibiotics, such as tetracyclines and trimethoprim sulfamethoxazole, have been widely utilized in poultry feed (Pitout, 2012). However, the widespread use of antibiotics can cause the emergence and re-emergence of antibiotic resistant bacterial strains. Thus, the use of antibiotics has been limited and many bacteria, including *E. coli*, have reemerged as significant threats to poultry production. Some alternatives were developed to reduce *E. coli* contamination of the farm microclimate such as probiotics (Stęczny & Kokoszyński, 2021) and UV lights (McLeod et al., 2018). These methods do not rely on the use of antibiotics and are relatively effective in reducing microbial contamination in poultry houses. However, these studies have not mentioned the effectiveness of reducing airborne bacteria which attach to dust particles. Therefore, further studies on airborne *E. coli* attached to dust particles such as their survivability or size distribution which directly affects the effectiveness of the methods are needed to investigate.

The litter is a major reservoir of microorganisms in the poultry environment (Carpenter, 1986). The dry matter contents can be about 70–80% of litter mass and it can contain abundant biological organisms and compounds that can affect the quality of the poultry environment (Schulz et al., 2016). Dust particles are aerosolized because of bird activity, so the poultry environment is highly dusty.

Air in the poultry houses may contain abundant microorganisms such as *E. coli* (Sanz et al., 2021). *E. coli* from manure first deposit into poultry litter and are then aerosolized through bird activities (Zhao et al., 2014). Ventilation systems can drive their migration across a poultry house or even from barn to barn. Airborne *E. coli* were shown to account for 2–6% of the total airborne bacteria in poultry houses (Zucker et al., 2000). With the high concentration of *E. coli* and the possibility of barn-to-barn transmission, the airborne *E. coli* can harm the entire wide range of environment outside the poultry houses, and they can deposit on surfaces near the poultry houses. The barn-to-barn airborne transmission of avian influenza was investigated in a study conducted in 2019 (Zhao et al., 2019). The probability of airborne infection is affected by several factors including farm type, flock size, and distance of transmission where the survivability of the pathogen is among the key factors for the modeling accuracy. Moreover, the survivability of *E. coli* on stainless steel under refrigeration conditions and room temperature was reported to exceed 28 days (Wilks et al., 2005). Therefore, it is also possible that *E. coli* can persist for a long time on various surfaces in the poultry production environment. With such a long survival period on the surface, they can spread to larger areas through vectors. These all raise the question of how long the airborne *E. coli*, carried by poultry litter particles, can survive in the air and on the physical surfaces when settled.

To determine the survivability of airborne and settled *E. coli* in laboratory, a proper aerosolization method that may mimic the fate of *E. coli* in the commercial poultry

production environment is required. The wet aerosolization method such as nebulization was widely used to study the survivability of airborne *E. coli* (Chan et al., 2019). However, the airborne *E. coli* in poultry houses are aerosolized from dried litter by bird activities, such as dust bathing (Zhao et al., 2014). So, the results of the study based on wet aerosolization cannot apply to the actual situation in the poultry house. In addition, the survivability of settled *E. coli* after going through the dry aerosolization process has never been investigated. Therefore, a study to determine the survivability of airborne *E. coli* and settled *E. coli* after being aerosolized based on dry aerosolization method needs to be done.

Size distribution of airborne *E. coli* attached to dust particles could affect the survivability of airborne *E. coli*. In a study conducted by Zuo (Zuo et al., 2013), the authors mentioned that carrier particle size had a significant effect on the survivability of airborne viruses. Lighthart (Lighthart & Shaffer, 1997) also reported that test bacterial survivability increased directly with droplet size. However, most of the studies used droplets as aerosol particles to carry bacteria and viruses. The dry dust particles may yield different results compared to droplets. So, the size distribution of airborne *E. coli* attached to dry dust particles also needed to be investigated.

This study aimed to investigate the survivability of airborne and settled *E. coli* via dry aerosolization under room thermal conditions. In addition, the survivability of *E. coli* in poultry litter was also investigated as a reference parameter.

## 3.2 Materials and methods

To investigate the survivability of the airborne *E. coli* and the settled *E. coli*, experiments were run in a test chamber in a Biosafety Level 2 (BSL-2) laboratory. The survivability test of *E. coli* in poultry litter was conducted in Biosafety Level 1 (BSL-1) laboratory. Both laboratories are located at the Animal Science Department, University of Tennessee, Knoxville, TN 37996, U.S.

### 3.2.1 Microorganism and system descriptions

(1) Preparation of *E. coli* solution

The *E. coli* strain used in this study was *Escherichia coli* (ATCC® 25922) which was purchased from American Type Culture Collection (ATCC, Manassas, VA, U.S.). *E. coli* strain was cultured at 37°C, 150 rpm for 24 hrs in ATCC® Medium 18 (Tryptic Soy Broth 'TSB' and Tryptic Soy Agar 'TSA', ATCC, Manassas, VA, U.S.). The bacterial concentrations of *E. coli* in the solution after 24 hrs were from 8 to 9 $\log_{10}$ colony-forming units ($\log_{10}$ CFU) $mL^{-1}$.

(2) Litter preparation

Litter from the commercial broiler farm was first collected and stored in a container. It was then brought back to the Animal Science Department, University of Tennessee, Knoxville. After that, the litter was autoclaved at 121°C in 20 min and divided into identical-size

aluminum boxes with the amount of 6 kg per box. The autoclaved poultry litter was used as a source of organic matter to simulate the biological conditions in poultry environment (Soliman et al., 2018). The sterilization was confirmed to demonstrate a state of freedom from microbial contamination. The boxes were sealed by aluminum foil and covered by plastic caps to avoid contamination. They were stored in a 4°C fridge until being used.

It was important to prepare litter so that the bacteria were evenly distributed. To do that, 240 g of litter needed for the survivability test of airborne *E. coli* and settled *E. coli* experiment were equally distributed into 40 ceramic cups (6 g litter per cup). The amount of airborne dust that can be generated using a mixer was determined in a previous experiment (Nguyen et al., 2021b), and the results showed that 240 g of litter would produce dust concentrations ranging from 0.9 to 1.1 mg $m^{-3}$ which was within a typical range of dust concentration in commercial poultry farm (Davis & Morishita, 2005). To prepare litter inoculated with *E. coli*, litter in each of the 40 cups was mixed with 6 mL of *E. coli* solution. The 6 mL bacteria solution was sprayed evenly onto the litter in each cup. In the meantime, an aluminum spoon was used to gently mix the litter and *E. coli* solution. The mixtures then went through a process of drying at 22°C and 52–67% relative humidity (RH) for 48 hrs until the dry matter content (DMC) of the mixture reached about 70%. The *E. coli* concentration in each cup was approximately 4 $\log_{10}$ CFU $mg^{-1}$ litter after the drying process. The litter containing *E. coli* was then transferred from 40 ceramic cups to a metal bowl of the mixer for aerosolization. In the bowl, the litter was gently mixed up again before aerosolization.

(3) Test chamber

Aerosolization was performed in an acrylic chamber. This chamber (2100 series, Cleatech, Orange, CA, U.S.) was a non-vacuum unit with two internal access doors with stainless steel frame, and a removable fully gasketed back wall. The dimension of the test chamber was 1.5 m L × 0.6 m W × 0.6 m H. The chamber was well sealed to prevent dust-laden particles from spilling out. It was also equipped with a temperature and RH sensor for continuously monitoring the inside thermal environment.

In the settled *E. coli* experiment, the chamber was modified to create a highly dusty environment in order to collect adequate settle dust for analysis. Initial results showed that the aerosolization space of the entire chamber was too large which led to the low concentration of airborne *E. coli* and dust particles. Thus, the chamber was modified by halving the aerosolization space using a partition acrylic film. The aerosolization space after modification was 0.75 m L × 0.6 m W × 0.6 m H.

(4) Aerosolization system

A stand mixer (model DCSM350GBRD02, New York, NY, U.S.) was used for dry aerosolization of airborne *E. coli* in this study. The dimensions of the mixer was 0.3 m L × 0.2 m W × 0.3 m H with a 3.3 L stainless steel bowl. They operated at the highest speed to

ensure the bacteria concentration in the air was high enough. A stir fan was also used to distribute the airborne *E. coli* in the chamber evenly.

(5) Dust concentration monitor

To monitor the dust concentration throughout the experiment, a dust concentration monitor (DustTrak DRX aerosol monitor 8533, TSI Inc., Shoreview, MN, U.S.) was used to provide data on the mass concentration of dust particles with different sizes. DustTrak was capable of measuring dust particles of $PM_1$, $PM_{2.5}$, $PM_{4.7}$, and $PM_{10}$. In this study, the dust concentration and particle size were recorded, and the results indicated that the particle concentration was relatively stable between experimental events.

(6) Air samplers

To evaluate the survivability of the airborne *E. coli*, the AGI-30 impinger (AGI-30) was used to collect *E. coli*-laden dust particles in a test chamber in a BSL-2 laboratory. The AGI-30 operates at 12.5 L $min^{-1}$. The airborne compounds were sucked through a fine nozzle in which the particles were accelerated and then impacted directly into the 20 mL TSB. The AGI-30 was proven to have the highest performance among three commonly used samplers (Andersen impactor, AGI-30 impinger, and BOBCAT ACD-200) for collecting airborne *E. coli* (Nguyen et al., 2021b).

### 3.2.2 Experimental design and procedures

(1) Bacterial size distribution and viable *E. coli* recovering in the airborne *E. coli* survivability test

An Andersen impactor was used to monitor the bacterial size distribution. The Andersen impactor is designed as an aerodynamic classifying system for airborne particles. It operates at 28.3 L $min^{-1}$. Its six stages are designed to sort dust particles with different sizes of >7 µm, 4.7–7 µm, 3.3–4.7 µm, 2.1–3.3 µm, 1.1–2.1 µm, 0.65–1.1 µm, corresponding to stage 1 to stage 6. The dust particles carrying *E. coli*, after being aerosolized, were sucked in the intake on top of the Andersen impactor; then, the particles continuously went through 6 stages. For each stage, dust particles with sizes corresponding to each stage were collected on TSA agar plates.

In the process of sampling with the Andersen impactor, the stages of the sampler were often overloaded due to the excessive number of bacteria collected in each stage. Therefore, counting bacteria on agar plates directly was not possible. To overcome this problem, the agar plate washing method was applied. Bacteria, after being collected on agar plates, were immediately taken to the laboratory for analysis. Each agar plate was rinsed with 2 mL of TSB solution with the aid of a glass spreader, and then 1 mL of solution was collected by pipette. The 1 mL of this solution went through a traditional serial dilution process to determine the total *E. coli* in the solution. The agar plates, after washing, were also placed in an incubator letting the remaining *E. coli* on the plate grow. During the air sampling

process, the agar plates in the Andersen impactor were dried by air flow in the sampler. Thus, the remaining 1 mL of solution in the washing process was mostly reabsorbed into the agar plates. However, to make sure that there is no residual solution that could affect the test results, the agar plates that have been washed instead of being turned upside down (due to traditional culture process) will be left right side up. The total *E. coli* on each stage was the combination of total *E. coli* collected from washing and total *E. coli* remaining on agar plates.

(2) Dry matter content measurement

The moisture content is one variable affecting the survivability of bacteria (Crane et al., 1983). The dry matter content (DMC), which is the inverse term of moisture content, was measured over time in the experiment. The DMC measurement of poultry litter is the ratio of the litter mass before and after the litter is completely dried. To determine DMC, the process was divided into two stages. First, the litter mass (m1) was weighted before going through a 48-hrs drying process until the litter mass was totally dried. After being dried at 105°C, the litter mass (m2) was weighted again. The DMC was then calculated by the litter mass m2 divided by the litter mass m1.

(3) Sample collection for airborne *E. coli*

Two hundred and forty grams (240 g) of litter which contained ~ 4 $\log_{10}$ CFU $mg^{-1}$ litter of *E. coli* were prepared and placed in the mixer. The mixer was placed in the center of the chamber to help evenly distribute the dust particles carrying *E. coli*. The mixer was fixed to the chamber surface by means of suckers, preventing it from moving during the running process. The stir fan was placed at the corner of the chamber to aid in distributing airborne particles. The AGI-30 was placed near the steel bowl of the mixer.

Each test lasted a total of 50 min. The first 20 min of the test was the aerosolization process of airborne *E. coli* using the mixer and stir fan. After the 20-min aerosolization, airborne *E. coli* was collected using the AGI-30 for 10 min and the dust concentration was determined using DustTrak. The second sampling of airborne *E. coli* and dust followed the same protocol but was performed 10 min after the first sampling. This test procedure was repeated 7 times.

(4) Sample collection for settled *E. coli*

Two mixers were used for aerosolization. Two hundred and forty grams (240 g) of litter which contained about 4 $\log_{10}$ CFU $mg^{-1}$ litter of *E. coli* were mixed gently and divided into two parts with 120 g for each mixer. The stir fan was operated during the aerosolization to improve the distribution of airborne *E. coli* in the chamber. Four Petri dishes were placed on both sides of the mixers to collect particles settled from the air. To avoid the position confounding effect, the Petri dishes were arranged randomly in a total of 4 experiment events. Each event started with 15 min aerosolization. After the aerosolization, the four Petri dishes were covered with caps and sealed by parafilm. Two Petri dishes were

immediately analyzed to quantify viable *E. coli* via traditional culture technique. The remaining two Petri dishes were left at laboratory temperature at 20°C, RH at 60% for 24 hrs. After that, they were quantified for viable *E. coli* by the same culture technique. The weight of each Petri dish was determined before and after aerosolization to determine the settle dust weight. The airborne dust concentration during the mixer running time was also monitored by DustTrak.

(5) Viable *E. coli* counting for *E. coli* survivability test in poultry litter

Fifteen (15) ceramic cups, each with six grams (6 g) of poultry litter, were prepared to determine the survivability of *E. coli* in the litter. The six grams of poultry litter were spread in each ceramic cup so that the thickness of the litter was uniform and without large lumps. Then, 6 mL of *E. coli* solution was added to the litter by using a pipette. The solution was sprayed onto the litter, ensuring that the bacterial fluid was distributed as evenly as possible. After that, the mixture of litter and bacterial solution were mixed gently by using an aluminum spoon. The cup was then placed in the BSL-1 under laboratory conditions. The viable *E. coli* in the litter were determined at 0, 12, 24, 48, and 72 hrs after litter samples were prepared in the ceramic cups. At each time point, three cups of samples were used. In addition, two cups of litter added with the TSB solution instead of the bacteria solution were used as a control for *E. coli* analysis and DMC measurement.

To determine the viable *E. coli* counts, TSB was added in each cup so that the total volume of the mixture reached 15 mL. The mixture was mixed evenly. Then, 0.1 mL of the solution (litter-bacteria mixture mixed with TSB) was taken out and transferred to 0.9 mL of TSB. After that, the solution went through a serial dilution process to determine the counts of viable *E. coli*. By doing back-calculation, the bacterial concentration in poultry litter was calculated.

(6) Determining *E. coli* concentration in poultry litter

To determine the viable *E. coli*, the *E. coli* concentrations were calculated in logarithm colony-forming units per gram ($\log_{10}$ CFU $mg^{-1}$) using Equation (3.1).

$$C = \log_{10}\left(\frac{N \times 10^{n}}{V_p} \times V_s \times \frac{1}{m_a}\right), \quad (3.1)$$

Where:

C: the bacteria concentration, $\log_{10}$ CFU $mg^{-1}$;

N: the number of colonies on a countable plate (30 to 300 colonies);

n: serial dilution factor (n = 0 for undiluted sample, n = 1 for 10-fold diluted sample, etc.);

$V_P$: the sample volume plated, mL ($V_P$ = 0.1 mL in this study);

$V_s$: the total volume of the original liquid sample, mL;

$m_a$: the total poultry litter weight in each ceramic cup at the test time, mg.

(7) Determining airborne *E. coli* concentration

Each air sample collected by AGI-30 in liquid form (in TSB medium) was used to quantify viable *E. coli* via traditional culture techniques. After vortexing for 5 s, a 0.1 mL subsample, after going through the serially diluted (1:10) process, was plated onto TSA agar plates. In each experimental event, the subsample was uniformly repeated 3 times to ensure the accuracy of the experiment. The plates were aerobically incubated at 37°C for 24 hrs. The visible *E. coli* colonies formed on plates (30 to 300 colonies) were determined. Based on the culture results and the sampled air volume, airborne *E. coli* concentrations were calculated in logarithm colony-forming units per cubic meter ($\log_{10}$ CFU $m^{-3}$) using Equation (3.1). The parameter $m_a$ converted to $V_a$ which is the total air volume sampled using the bioaerosol samplers, $m^3$.

(8) Determining settled *E. coli* concentration

Each settled sample on an empty Petri dish was used to quantify viable settled *E. coli*. After adding 10 mL of TSB medium (the culture medium) in each Petri dish, the Petri dish was gently shaken to wash the Petri dish surface and draw settled *E. coli* into TSB solution. After that, 0.1 mL of the solution containing *E. coli* was taken by using a pipette and went through a serial dilution process to count viable *E. coli*. Then, the viable *E. coli* was determined as the Equation (3.1). The parameter $m_a$ was the mass of settled dust collected in each dish in each experiment, mg.

### 3.2.3 Calculation of half-life time

The half-life time is the time interval needed for bacteria to decrease by half (Zhao et al., 2011). The bacterial concentrations throughout the experiments would be homogenized and normalized to the dust concentration (CFU $mg^{-1}$). In the survivability of the airborne *E. coli* test, the airborne *E. coli* concentration was calculated based on airborne *E. coli* concentration collected in the air (CFU $m^{-3}$) divided by total dust concentration (mg $m^{-3}$). In the survivability of the settled *E. coli* test, the settled *E. coli* concentration was calculated based on the settled *E. coli* concentration collected on each Petri dish (CFU $mg^{-1}$). The half-life time, then, was calculated by the following Equation (3.2).

$$t_{1/2} = \frac{\log_{10} 2 \times T}{\log_{10}\left(C_{\text{viable bacteria}} / C'_{\text{viable bacteria}}\right)}, \quad (3.2)$$

Where:

$t_{1/2}$: half-life time (min or hrs);

T = 20 (min) for airborne *E. coli* and 24 (hrs) for settled *E. coli* test;

$C_{\text{viable bacteria}}$: *E. coli* concentration for the first sampling event, CFU $mg^{-1}$;

$C'_{\text{viable bacteria}}$: *E. coli* concentration for the second sampling event, CFU $mg^{-1}$.

Linear simple regression was performed to calculate the half-life time of *E. coli*. The half-life time of *E. coli* in poultry litter was calculated based on the *E. coli* death over time by the linear Equation (3.3) (Mubiru et al., 2000):

$$t_{1/2} = \frac{\text{constant} - \log_{10}(\frac{C_{\text{viable bacteria}}}{2})}{k}, \quad (3.3)$$

Where $C_{\text{viable bacteria}}$: the *E. coli* concentration at 0 hrs, CFU $mg^{-1}$; constant: intercept of the linear regression model, $\log_{10}$ CFU $mg^{-1}$; k: the death rate, [$\log_{10}$ CFU $mg^{-1}$] $hrs^{-1}$; and $t_{1/2}$: half-life time, hrs.

### 3.2.4 Statistical analysis

Means and standard deviations for all experiments were calculated by using Rstudio (Rstudio, open-source license, Rstudio, Boston, MA, U.S.). A total of 7 replicates for airborne *E. coli* experiment and 4 replicates for settled *E. coli* yielded decent statical analysis for calculating the half-life time. The conditions such as dust concentration among experiments were tested with the T-test to make sure there was no significant difference in terms of experimental conditions. The *t*-test significance level was 0.05 ($p < 0.05$). For the survivability of *E. coli* in poultry litter, at every time point, the concentration of *E. coli* in poultry litter was tested repeatedly 3 times for reliable viable *E. coli* data.

The half-life time of airborne *E. coli*, settled *E. coli* and *E. coli* in poultry litter were compared, and the differences between the survivability of *E. coli* under different conditions were tested by using a *t*-test run on Rstudio. The *t*-test was used to determine if the means of three sets of data (*E. coli* in poultry litter, airborne *E. coli*, and settled *E. coli*) are significantly different from each other. The *t*-test significance level was 0.05 ($p < 0.05$).

## 3.3 Results

### 3.3.1 Conditions for *E. coli* survivability test

Table 3.1 shows the litter DMC, initial litter *E. coli* concentration and environmental conditions during the experiments for determining survivability of airborne *E. coli*, settled *E. coli* and the *E. coli* in poultry litter. The DMC of litter, *E. coli* concentration and RH in the litter were kept stable throughout the experiments. In the test for settled *E. coli* survivability, instead of using one mixer, two mixers were used. Therefore, the heat generated in the two mixers caused the temperature in the test for settled *E. coli* survivability to be slightly higher than the two other tests.

### 3.3.2 Size distribution of *E. coli* and dust for the airborne *E. coli* survivability test

The size distribution of airborne *E. coli* attached to dust particles and the size distribution of airborne dust particles were tested. The size distribution of airborne *E. coli* attached to dust particles during the 20-min aerosolization process is shown in Figure 3.1. The most *E. coli* were found in the particles larger than 7 µm with a percentage of 47.58%. The second large portion of *E. coli* was those attached to particles in the range of 4.7 to 7 µm,

Table 3.1 Conditions (Mean ± SD) for the *E. coli* in survivability test.

| ***E. coli* concentration and environmental conditions** | **Airborne *E. coli* Survivability** | **Settled *E. coli* Survivability** | ***E. coli* in Poultry Litter Survivability** |
|---|---|---|---|
| DMC [1] of litter (%) | 71 ± 5 | 72 ± 1 | - [2] |
| *E. coli* concentration in litter ($log_{10}$ CFU $mg^{-1}$) | 4.4 ± 0.6 | 4.0 ± 0.5 | - [2] |
| Relative humidity (%) | 54 ± 5 | 63 ± 7 | 36 ± 4 |
| Temperature (°C) | 22.1 ± 1.4 | 27.7 ± 5.1 | 20.5 ± 0.3 |

[1] Dry matter content, [2] DMC and bacteria concentration varied over 72 hrs.

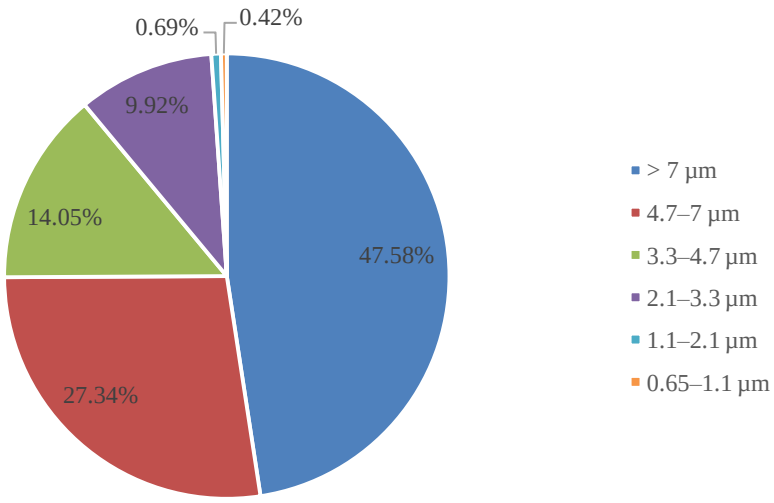


Figure 3.1 Size distribution of the airborne *E. coli* attached to dust particles in the airborne *E. coli* survivability test measured by an Andersen impactor.

accounting for 27.34%. *E. coli* attached to dust particles in the ranges of 3.3–4.7 µm and 2.1–3.3 µm accounted for 14.05% and 9.92% of the total culturable *E. coli*, respectively. The least *E. coli* were found in particles smaller than 2.1 µm which accounted for 1.11% of the total culturable *E. coli*.

The size distribution of airborne dust particles during the 20-min aerosolization process was monitored by the DustTrak and shown in Table 3.2. Most dust particles have the size smaller than 1 µm with a concentration of 0.678 ± 0.108 mg $m^{-3}$. The rest of the dust particles have size range of 1.0–2.5 µm, 2.5–4.7 µm, 4.7–10.0 µm and larger than 10.0 µm, with a concentration of 0.014 ± 0.001 mg $m^{-3}$, 0.016 ± 0.005 mg $m^{-3}$, 0.235 ± 0.042 mg $m^{-3}$ and 0.232 ± 0.032 mg $m^{-3}$, respectively. The total dust concentration was about 1.176 ± 0.120 mg $m^{-3}$. As shown in Table 3.2 and Figure 3.1, although most dust particles were smaller than 1 µm, the size distribution of bacteria attached to dust particles was mainly larger than 2.1 µm, accounting for 98.89%. This indicates that when it comes to airborne *E. coli*, most are attached to dust particles with the size larger than 2.1 µm.

### 3.3.3 *E. coli* survivability in poultry litter

The survivability of *E. coli* in poultry litter was determined in a 72-hrs test under laboratory conditions and delineated in Figure 3.2. The temperature and RH remained stable throughout the test at 20.5 ± 0.3°C and 36 ± 4%. The DMC of litter (containing *E. coli*) changed throughout the test and was presented in Figure 3.2. The *E. coli* concentration decreased from 4.5 $\log_{10}$ CFU $mg^{-1}$ to 2.4 $\log_{10}$ CFU $mg^{-1}$ over 72 hrs. The DMC increased from 38% to 82% due to moisture evaporation. The half-life time of *E. coli* in poultry litter calculated based on the linear regression was 15.9 ± 1.3 hrs.

### 3.3.4 Airborne *E. coli* survivability

The data collected from the first sampling and the second sampling to calculate the half-life time of *E. coli* were listed in Table 3.3. As shown in Figure 3.1, most of the airborne *E. coli* were attached to dust particles larger than 2.1 µm, while only a small amount of total *E. coli* (1.11%) attached to dust particles smaller than 2.1 µm. Therefore, when calculating the concentration of *E. coli* in dust, we only considered the concentration of dust particles larger than 2.1 µm. The DustTrak was able to monitor the dust particles having size range of 1.0–2.5 µm, 2.5–4.7 µm, 4.7–10.0 µm and larger than 10.0 µm. In this study, we assumed that the amount of dust particles larger than 2.1 µm were equivalent to the amount of dust particles larger than 2.5 µm. The half-life time of the airborne *E. coli* based on dust with size >2.5 µm was 5.7 ± 1.2 min.

### 3.3.5 Settled *E. coli* survivability

The survivability of settled *E. coli* was tested over 24 hrs. In 24 hrs, the concentration of settled *E. coli* declined from 3.7 ± 0.1 to 3.0 ± 0.2 $\log_{10}$ CFU $mg^{-1}$, yielding a half-life time of 9.6 ± 1.6 hrs for settled *E. coli*.

Table 3.2 Dust size distribution (Means ± SD) in the airborne E. coli survivability test.

| **<1.0 µm (mg m$^{-3}$)** | **1.0–2.5 µm (mg m$^{-3}$)** | **2.5–4.7 µm (mg m$^{-3}$)** | **4.7–10.0 µm (mg m$^{-3}$)** | **>10.0 µm (mg m$^{-3}$)** | **TOTAL (mg m$^{-3}$)** |
|---|---|---|---|---|---|
| 0.678 ± 0.108 (57.60%) [1] | 0.014 ± 0.001 (1.20%) [1] | 0.016 ± 0.005 (1.40%) [1] | 0.235 ± 0.042 (20.00%) [1] | 0.232 ± 0.032 (19.80%) [1] | 1.176 ± 0.120 (100.00%) [1] |

[1] Percentage of the total for each size range.

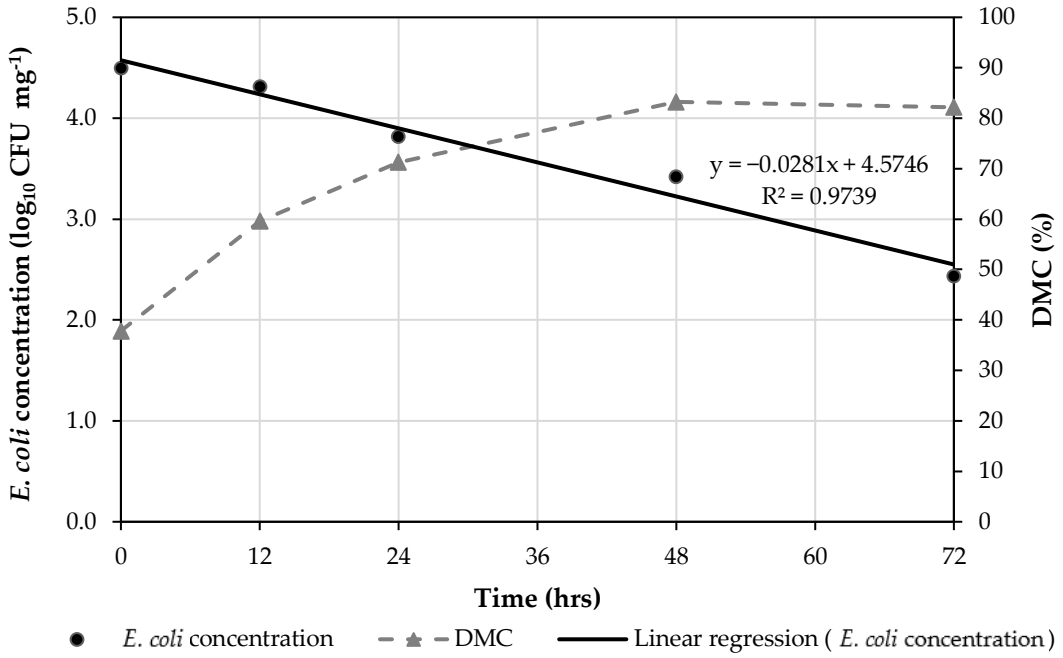


Figure 3.2 *E. coli* concentration and dry matter content (DMC) in poultry litter in a 72-hrs exposure under laboratory environmental condition (20.5°C and 36%).

Table 3.3 Concentrations (Mean ± SD) of dust particles with size larger than 2.5 µm, airborne *E. coli* and airborne *E. coli*-to-dust ratio during air sampling for survivability test of airborne *E. coli*. The 2nd sampling was performed 20 min after the 1st sampling.

| **Concentrations of dust particles and airborne *E. coli*** | **1st Sampling** | **2nd Sampling** |
|---|---|---|
| Dust concentration with size >2.5 µm (mg m$^{-3}$) | 0.032 ± 0.022 | 0.016 ± 0.012 |
| Airborne *E. coli* concentration ($\log_{10}$ CFU m$^{-3}$) | 7.1 ± 0.7 | 5.7 ± 1.0 |
| Airborne *E. coli* concentration carried by dust concentration with size >2.5 µm ($\log_{10}$ CFU mg$^{-1}$) | 8.7 ± 0.7 | 7.5 ± 0.9 |

## 3.4 Discussion

The aim of this study was to determine the survivability of airborne and settled *E. coli* in laboratory under dry aerosolization conditions. Survivability of *E. coli* was determined using half-life time as the indicator. To calculate the half-life time, concentrations of airborne *E. coli* and settled *E. coli* collected at two different time points after the dry aerosolization process were measured and compared. The survivability of *E. coli* in poultry litter that was used for dry aerosolization was also determined in a 72-hrs test under laboratory conditions (20.5 ± 0.3°C and 36 ± 4%). The results show that half-life times of airborne *E. coli*, settled *E. coli*, and *E. coli* in poultry litter were 5.7 ± 1.2 min, 9.6 ± 1.6 hrs, and 15.9 ± 1.3 hrs, respectively.

In the airborne *E. coli* survivability test, the mean half-life time of the bacteria based on dust particles with size larger than 2.5 µm was 5.7 min. Hoeksma (Hoeksma et al., 2015) tested survivability of airborne *E. coli* under wet aerosolization conditions at 20°C and 40–60%. Their results showed that the half-life time of airborne *E. coli* under wet aerosolization conditions was about 2 min, which was much shorter than the half-life time calculated in the present study. The difference between the half-life time of airborne *E. coli* under wet aerosolization conditions and dry aerosolization conditions could be explained by inactivation due to evaporation. After being aerosolized, the wet aerosols lost their water film due to evaporation and become sensitive to ambient influences (Hoeksma et al., 2015). Moreover, the difference in preparation of *E. coli* for aerosolization between the two studies could be another reason for the discrepancy in survivability results. In the current study, the *E. coli* was prepared in poultry litter and exposed at laboratory conditions over 48 hrs before aerosolization. As such, the *E. coli* had already gone through a dehydration process before aerosolization, which might leave only dehydration-resistant *E. coli* for following dry aerosolization. In the study by Hoeksma (Hoeksma et al., 2015), the *E. coli* were aerosolized immediately after preparation via the wet aerosolization. In addition, the autoclaving process of poultry litter could affect the quality of poultry litter and produce Maillard reaction product. The Maillard reaction products were proven to inhibit growth of bacteria (Bhattacharjee et al., 2009). However, the effect of the preparation procedure was not well-studied in the present study. Therefore, the effect still needs further investigation.

Survivability and transmission range of airborne *E. coli* may be affected by the size of particles that *E. coli* attached to. Zuo (Zuo et al., 2013) reported that the carrier particle size had a significant influence in the transmission and survivability of airborne virus. In their study, the authors mentioned that the survivability of virus attached to larger particles was much longer than that attached to smaller particles. The possible explanation presented by Zuo (Zuo et al., 2013) was the shielding effect. In other words, compared with viruses existing as a singlet or attaching to small particles, the virus attached to larger particles could be better protected from changes of ambient environment (Woo et al., 2012). The concentration of *E. coli* should be proportional to the weight of airborne dust in the entire

size spectrum, assuming a uniform mixture of *E. coli* and poultry litter. However, most of dust particles were smaller than 1.0 μm (accounted for 57.60%) and the majority of airborne *E. coli* were found to attach to dust particles larger than 2.1 μm (98.89%). This contradiction could be explained again by the shielding effect. While *E. coli* attached to large particles could be protected from ambient influences, *E. coli* attached to small particles received less protection effect. It led to a rapid death of the *E. coli* attached to small particles during the aerosolization and sampling.

The half-life time of settled *E. coli* in this study was about 9.6 hrs. Wilks (Wilks et al., 2005) tested the survivability of *E. coli* on metal surfaces at laboratory conditions at 20°C. In their study, the total number of viable *E. coli* dropped by 1 log after the first 3 hrs, translating into an approximate 0.9 hrs half-life time. This discrepancy can be explained by differences in *E. coli* preparation methods, surfaces, and substrate (litter vs. liquid solution). As mentioned above, the *E. coli* preparation procedure in our study may affect the *E. coli* quality. Another possible explanation was metal surfaces used by Wilks (Wilks et al., 2005). While the present study used regular plastic Petri dishes to collect settled *E. coli*, Wilks (Wilks et al., 2005) applied *E. coli* directly onto metal surfaces. This different material of surfaces could yield different survivability of *E. coli*. Ketkar (Ketkar et al., 2020) indicated that stainless steel had antimicrobial effects. Further, different substrates (litter vs. liquid solution) used might have yielded different survivability of *E. coli*. While factors like pH and nutrient in poultry litter includes many affecting the survivability of bacteria (Neher et al., 2019; Terzich et al., 2000), liquid solution used by Wilks (Wilks et al., 2005) for culturing *E. coli* was designed as a substrate for bacterial growth.

In the test of *E. coli* survivability in poultry litter, the half-life time was reported to be 15.9 ± 1.3 hrs. Compared with the half-life time of settled *E. coli* (9.6 hrs) and airborne *E. coli* (5.7 min), the half-life time of *E. coli* in poultry litter was significantly longer. A possible explanation was that the *E. coli* in the poultry litter did not go through the aerosolization process which negatively affect the *E. coli* survivability (Zhen et al., 2014). While settled *E. coli* and airborne *E. coli* were aerosolized, *E. coli* in the poultry litter was not aerosolized. In addition, the degree of sample exposure to the environment could affect the survivability of *E. coli* as well. Ruiz (Ruiz-Gil et al., 2020) reported that bacterial survival was highly influenced by ambient influences. The airborne *E. coli* were scattered in the air and the settled *E. coli* were prepared in thin layers where *E. coli* were exposed to ambient environment and were more susceptible to microenvironment changes (Fernandez et al., 2019; Tuson & Weibel, 2013), as compared to *E. coli* in the poultry litter. In contrast, the *E. coli* in poultry litter existed in a chuck form could be more protected from microenvironmental effects (Fernandez et al., 2019; Soupir et al., 2006; Tuson & Weibel, 2013).

## 3.5 Conclusions

The study determined the survivability of airborne, settled, and poultry litter *E. coli* under dry aerosolization conditions in laboratory. Based on the results, we conclude that (1) most *E. coli* could be carried by the dust particles with aerodynamic diameter >2.1 μm, (2) the settled *E. coli* and the *E. coli* in poultry litter can survive much longer than airborne *E. coli*, and the mean half-life time was 5.7 ± 1.2 min for airborne *E. coli*, 9.6 ± 1.6 hrs for settled *E. coli*, and 15.9 ± 1.3 hrs for *E. coli* in poultry litter.

# CHAPTER IV

# Effect of Ultraviolet Radiation on Reducing Airborne *Escherichia coli* Carried by Poultry Litter Particles

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## 4.1 Introduction

The United States is a major producer of eggs and poultry meat worldwide. As a 35 billion dollar sector, the poultry industry provided approximately 1 million jobs for the U.S. in 2020 (USDA-NASS, 2020). However, this sector of the economy is extremely vulnerable to infectious diseases brought on by pathogenic bacteria, such as Avian Pathogenic *Escherichia coli* (APEC). All ages of birds and all types of poultry houses could be affected by the APEC-caused diseases (Saif et al., 2008). One of the key factors contributing to the financial losses of the global poultry sector was thought to be APEC (Dho-Moulin & Fairbrother, 1999). These microorganisms are frequently found in the lower gastrointestinal tracts of chickens and other warm-blooded animals, as well as in the environment where the animals live. Hemorrhagic colitis, gastroenteritis, and urinary tract infections are among the intestinal symptoms (colibacillosis) brought on by APEC. The cost of poultry losses, mortalities, medical expenses, and decreased feed efficiency were the main causes of the economic losses caused by APEC (Mellata, 2013). According to a previous study (Hasan et al., 2011), roughly 40% of broiler carcasses that were condemned included APEC, and 30% of broiler flocks in the United States had subclinical colibacillosis (Fancher et al., 2020).

The air in poultry buildings contains not only smells and pollutants, but also a large number of pathogenic bacteria, such as *Escherichia coli* (*E. coli)*. Young chicks can get infected by vertical transmission from an infected ovary, oviduct, or contaminated eggs passing through the cloacal manures of infected or carrier hens. When birds are infected at a young age, they may have few minimal symptoms of sickness yet still become carriers. In older

birds, infection of *E. coli* has a predisposition for reproductive organs, which frequently leads to infection of ovarian follicles and, as a result, transovarial transmission of the illness. Then, *E. coli* is carried out by poultry manure. The *E. coli* is first excreted onto poultry litter, then plowed up and dispersed into the air by bird activities (Duan et al., 2008). Past studies reported that the concentration of airborne *E. coli* can be up to 4 $\log_{10}$ CFU $m^{-3}$ in poultry houses (Chinivasagam et al., 2009). After being aerosolized into the air, the airborne *E. coli* can migrate into the poultry house following the airflow of ventilation fans. Therefore, there is a high possibility that the birds in the poultry houses can receive the airborne *E. coli* through inhalation and contact with the areas where the airborne *E. coli* settled. The birds become sick by inhaling dust mixed with feces, which can carry up to $10^6$ CFU of *E. coli* per gram (Kabir, 2010). This aerogenic mode of infection is thought to be the primary cause of systemic colibacillosis or colisepticemia (Pourbakhsh et al., 1997). In addition, the airborne *E. coli* can be emitted outside the poultry houses, which poses risks to barn-to-barn airborne transmission (Chinivasagam et al., 2009). The previous study mentioned that there was no significant difference in concentration airborne *E. coli* between the inside and downwind locations within 10 meters. A potential solution that may reduce airborne *E. coli* emitted outside the poultry houses at an affordable cost for farmers is necessary for mitigating the airborne transmission of *E. coli*.

Ultraviolet-C (UVC) which covers the wavelength range from 100–300 nm was well studied in the food industry and is known as a method that can inactivate microorganisms by inhibiting DNA replication (Ochoa-Velasco et al., 2020). The previous study (Ochoa-Velasco et al., 2020) also reported that UVC light was very effective to disinfect *E. coli* in water, droplets, and surfaces in the food industry processing. Specifically, UV with a wavelength of 254 nm showed the highest performance in terms of disinfecting pathogens (Ochoa-Velasco et al., 2020). In poultry houses, the airborne *E. coli* can be carried by dust particles (Nguyen et al., 2022; Y. Zhao et al., 2014) that might prevent UV light exposure, and thus the dust particles can protect the airborne *E. coli* from being irradiated (Guerrero-Beltr· n & Barbosa-C· novas, 2004). In addition, the variation of environmental conditions in poultry houses such as ventilation systems or air flows, temperature, and relative humidity (RH) lead to variable contact time and resistance to UV light. The inactivation efficiency in the poultry houses might be different from the food industry. Therefore, the inactivation efficiency of UV light on poultry litter-based airborne *E. coli* needs further investigation.

The objective of this study was to investigate the inactivation efficiency of UV light (wavelength of 254 nm) on airborne *E. coli* carried by poultry dust particles in laboratory conditions. The laboratory conditions remained stable at about 22.6°C with an RH of 60%. A system that simulated the conditions of the poultry house was designed to evaluate the inactivation rate. The tested wind speeds were from 0.11 to 2.61 m $s^{-1}$, corresponding to the contact time from 5.6 s to 0.23 s. In addition, the UV intensity, dust concentrations, and size distribution of *E. coli* carried by poultry dust particles were also recorded.

## 4.2 Materials and Methods

To evaluate the effect of UV light, the experiment was conducted in a Biosafety Level 2 (BSL-2) laboratory located at the Animal Science Department, University of Tennessee, Knoxville, TN 37996, U.S. The Institutional Biosafety Committee at The University of Tennessee has approved this study under the protocol IBC-21-572-2.

### 4.2.1 Microorganism and system descriptions

(1) Preparation of *E. coli* solution

The *E. coli* strain utilized in this investigation was *Escherichia coli* (ATCC® 25922), which was obtained from the American Type Culture Collection (ATCC, Manassas, VA, U.S.). *E. coli* strain was cultured at 37°C, 150 rpm for 24 hrs in ATCC® Medium 18 (Tryptic Soy Broth 'TSB' and Tryptic Soy Agar 'TSA', ATCC, Manassas, VA, U.S.). The bacterial concentrations of *E. coli* in the solution after 24 hrs were determined by the traditional serial dilution process (Nguyen et al., 2022; Nguyen et al., 2022). The concentration was approximately 9 $\log_{10}$ colony-forming unit ($\log_{10}$ CFU) $mL^{-1}$.

(2) Litter preparation

The litter preparation was performed in the same way as in our previous studies (Nguyen et al., 2022; Nguyen et al., 2022). Litter was taken from a commercial broiler farm. It was subsequently returned to the Biosafety Level 1 (BSL-1) laboratory for an analysis of the dry matter content (DMC). It was then autoclaved at 121°C for 20 minutes before being separated into identical-size aluminum boxes weighing 6 kg each. The boxes were packed with aluminum foil and coated with plastic lids to prevent contamination. They were kept in a 4°C fridge until they were utilized.

It was necessary to prepare the litter so that the bacteria were distributed evenly. This experiment required 240 g of litter, which was evenly distributed among 40 ceramic cups. In a prior experiment, the capacity to generate airborne dust was examined (Nguyen et al., 2022), and the findings revealed that 240 g of litter put to the mixer produced dust concentrations ranging from 0.9 to 1.1 mg $m^{-3}$ which is within the average range of dust concentration in a commercial chicken farm (Davis & Morishita, 2005). To prepare litter inoculated with *E. coli*, a set of 43 ceramic cups (40 cups for experiment plus 3 controls) which were identical in shape was used to hold the litter. In each cup, 6 g of litter was prepared and mixed with 6 mL of *E. coli* cultured solution. The 6 mL bacteria solution was sprayed equally onto the litter in each cup. Meanwhile, a sterile metal spoon was used to gently mix the litter and *E. coli* solution. After that, the mixtures were dried for 48 hours at 20.8°C and 40–65% RH till the DMC reached about 80% and was appropriate for aerosolization. The concentration of *E. coli* in the control cup was determined by adding TSB to the mixture until the total volume of each cup reached 15 mL. An automated pipette was then used to collect 1 mL of the solution in each cup. A conventional serial dilution approach was used to determine the culturable *E. coli* in the 1 mL solution. The

concentration of *E. coli* in the cup was approximately 8 $\log_{10}$ CFU $g^{-1}$ litter after the drying process. The *E. coli*-containing litter was then moved from 40 ceramic cups to the mixer's metal bowl for aerosolization. Before aerosolization, the litter was gently mixed again in the bowl.

(3) Test chambers

Two connected acrylic chambers were used in this study. The upstream chamber (2100 series, Cleatech, Orange, CA, U.S.) was a non-vacuum unit with two internal access doors with stainless steel frame, and a detachable completely gasketed rear wall. The dimension of the test chamber was 1.5 m L × 0.6 m W × 0.6 m H. The dimension of the downstream chamber (2200 series, Cleatech, Orange, CA, U.S.) was 0.7 m L × 0.6 m W × 0.6 m H. The two chambers were connected by an aluminum tube installed with two UV lamps. The scrubber helped the air inside of two chambers to circulate. The dimension of the scrubber was 0.24 m D × 0.6 m H. The chambers were well sealed to prevent dust from spilling out. Temperature and RH sensors were equipped for continuously monitoring the inside thermal environment.

(4) Aerosolization system

In this study, a stand mixer (model DCSM350GBRD02, New York, NY, U.S.) was used to dry aerosolize airborne *E. coli*. The dimensions of the mixer was 0.3 m L × 0.2 m W × 0.3 m H with a 3.3 L stainless steel bowl. It was operated at maximum speed to ensure the bacteria concentration in the air was high enough for the samplers to be able to detect it. A swirl fan was used to spread the airborne *E. coli* in the chamber equally.

(5) Dust monitoring

To monitor the dust concentration throughout the experiment, a dust concentration monitor (DustTrak DRX aerosol monitor 8533, TSI Inc., Shoreview, MN, U.S.) was used to measure the mass concentration of dust particles of different sizes (Nguyen et al., 2022). DustTrak was capable of measuring dust particles of < 1.0 µm, 1.0–2.5 µm, 2.5–4.7 µm, 4.7–10.0 µm, and > 10.0 µm. The record intervals of DustTrak were 1 s. In 10 min of the experiment, a total of 600 data points were collected to monitor dust concentrations. The dust concentration and particle size were measured in this study, and the findings showed that the particle concentration was relatively consistent between experimental events (Nguyen et al., 2022).

(6) Air samplers

An All-Glass Impinger (AGI-30, Ace Glass, Vineland, U.S.) and an Andersen six-stage impactor (Andersen impactor TE-10-800, Thermo Fisher Scientific, Inc., Franklin, Mass.) were used in this study (Figure 4.1). The AGI-30 was proven to be an efficient sampler used for dry-aerosolization conditions (Nguyen et al., 2022). The AGI-30 runs at a rate of 12.5 L $min^{-1}$. The airborne compounds were pulled using a vacuum pump via a fine nozzle, where they were accelerated before impacting directly into the 20 mL TSB. The size


(**a**) (**b**)

Figure 4.1 Two samplers: (**a**) Andersen six-stage impactor; (**b**) AGI-30 impinger.

distribution of airborne *E. coli* carried by poultry dust particles were monitored using the Andersen impactor. The sampler operates at 28.3 L $min^{-1}$. It can separately collect airborne microorganisms of varied sizes of > 7.0 μm, 4.7–7.0 μm, 3.3–4.7 μm, 2.1–3.3 μm, 1.1–2.1 μm, 0.65–1.1 μm, respectively, from stages 1 to 6. The impactor separates dust particles (carrying *E. coli*) into seven different size ranges and collects them onto seven agar plates/stages via impaction mechanism. This impactor creates different air speeds. When dust particles in the air stream impact onto an agar plate at a speed, only particles above a certain size can be impacted on the plate. Smaller particles are transported by the air stream to the next stage where a higher air speed is created, allowing collection of smaller particles on another agar plate. Details regarding the cascade impaction mechanism were published in the paper by Andersen (Andersen, 1958).

### 4.2.2 Experimental design and procedures

(1) System design

A system was designed to simulate the conditions of the environment in the poultry houses. A sketch map of the system was shown in Figure 4.2. Two treatments were applied, one with UV lamps (one and two UV lamps) and the other without. Each system was made up of two chambers connected by an aluminum scrubber. The aerosolization system, which was described in 2.1.4., was used to aerosolize airborne *E. coli* attached to dust particles in the upstream chamber. To collect viable airborne *E. coli* generated from the aerosolization system, samplers were put in the upstream chamber. Two UV lamps (UVC lamp, Konideke, Yongchang, China) with a wavelength of 254 nm were installed in the aluminum scrubber. The two UV lamps are installed symmetrically in the scrubber and positioned on wall of scrubber (Figure 4.2). With one UV lamp positioned on wall of the scrubber, only one side of the airborne dust particles is exposed under UV irradiation. Symmetrically positioning two UV light bulbs on the wall of the scrubber will increase the irradiation area on both sides of the dust particles, increasing the possibility of *E. coli* being exposed to UV rays. Another set of samplers was put in the downstream chamber to capture viable *E. coli* attached to dust particles after being irradiated with UV light. Air-in ports with high-efficiency particulate air (HEPA) filters were added to both chambers. The downstream chamber's outflow was connected to a vacuum pump. The air filters served to keep airborne *E. coli* and dust particles out of the laboratory, while the vacuum pump helped to direct and control the airflow. The decrease rate was obtained by comparing the concentrations of airborne *E. coli* in the two chambers. In the system, the decrease of airborne *E. coli* was studied at varying air speeds (from 0.11 to 2.61 m $s^{-1}$, a typical airspeed range in the poultry houses (Yao et al., 2018)) and UV irradiance levels. Airborne *E. coli* may deposit on the surface of the test system, referred to as physical loss, which should be determined and excluded from the calculation of biological inactivation by UV light. The same operation was done in the testing system without the UV lamp, and the findings provided data on the physical deposition of airborne *E. coli* during air movement in the testing system. The

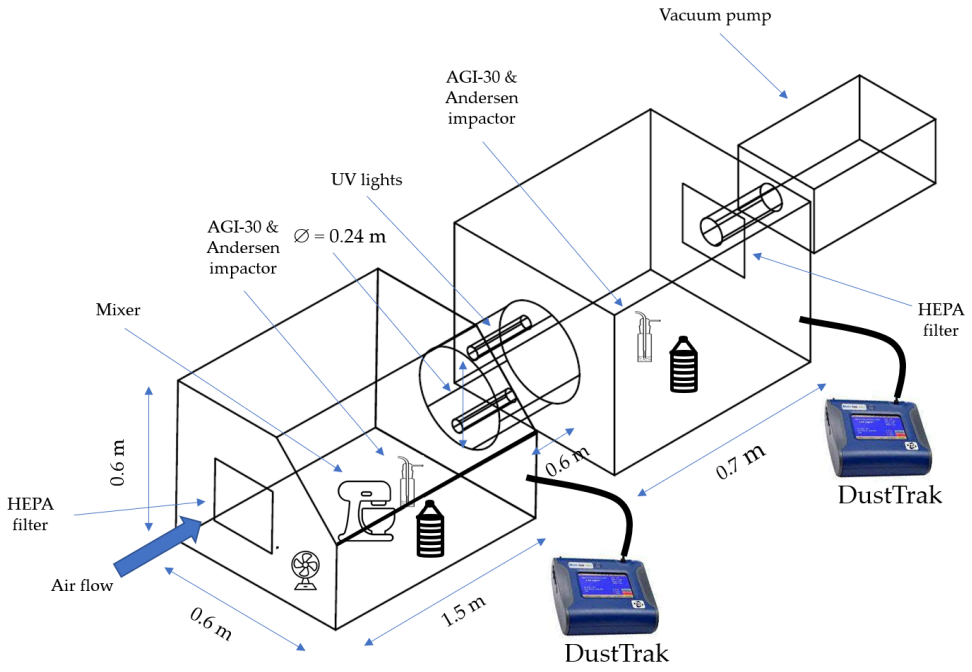



Figure 4.2 Testing system designed for examining the inactivation efficiency of UV light on airborne *E. coli*.

physical loss was calculated by comparing the concentrations in the upstream and downstream chambers.

(2) System setup and sampling collection

A total of 240 grams (240 g) of litter containing 8 $\log_{10}$ CFU (g litter)$^{-1}$ of *E. coli* was produced and added to the mixer. To assist in equally spreading the dust particles carrying *E. coli*, the mixer was put in the center of the chamber. Suction cups were used to secure the mixer to the chamber surface, preventing it from sliding throughout the running process. The stir fan was positioned in the chamber's corner to aid in the distribution of airborne particles. The test would take ten minutes to complete. The samplers, mixer, DustTrak, and fan were all turned on at the same time. The samplers' sampling ports were adjusted to a set height of 27 cm. In addition, in each aerosolization event, the sampler positions were switched at random to reduce the location impact. The dust concentration in the two chambers was also measured using DustTrak. The temperature in the chamber was fixed at around 22.6°C, with RH of ~60%.

(3) UV light intensity distribution

A mathematical model, UVCalc software (UVCalc®, Bolton Photosciences Inc., Edmonton, Alberta, Canada) was used to simulate the UV light intensity distribution in the UV scrubber. The UVCalc software is widely used to support the design of UV reactor in the most accurate way (Li et al., 2013). However, because of the optical complexity of the scrubber, a UV light meter was used to validate the accuracy of the model. A UV light meter (Amtast U.S. Inc., Lakeland, FL, U.S.) was used to measure the UV light intensity. UV light in the range of 248 nm to 262 nm was measured by the UV meter. The measuring range for irradiance is 0.001 mW cm$^{-2}$ to 39.99 mW cm$^{-2}$. After installing the UV lamps in the aluminum scrubber, they were measured at various distances to obtain the most UV intensity distribution in the connection tube.

### 4.2.3 Calculation of *E. coli* concentration and inactivation rates

(1) Determining size distribution of airborne *E. coli* carried by poultry dust particles

The size distribution of airborne *E. coli* carried by poultry dust particles was monitored using an Andersen impactor. The Andersen impactor determines the counts of *E. coli* that are carried by different (seven) size ranges of poultry dust particles. The Andersen impactor has six stages, each with one Petri dish. TSA was used to prepare each Petri dish. After being aerosolized, poultry dust particles carrying *E. coli* were sucked into the inlet of the Andersen impactor during the sampling process. The particles which carry *E. coli* then went through six stages of the sampler. TSA plates were used to capture dust particles carrying *E. coli* with sizes that corresponded to each stage. The *E. coli* on the agar plates were placed in an incubator for 24 hrs, at 37°C and allowed *E. coli* to grow.

(2) Determining airborne *E. coli* concentration

AGI-30 was used to collect *E. coli* from the air (in TSB medium). With the use of a vacuum pump that was directly connected to AGI-30, airborne *E. coli* carried by dust particles were pulled into the intake of the AGI-30 and passed via a fine nozzle into the TSB solution. In the collection vessel, 20 mL of TSB medium was prepared. The total culturable airborne *E. coli* collected by the sampler was quantified using the traditional culture procedure. In the traditional culture procedure, each air sample (in liquid form) was utilized to quantify culturable *E. coli*. A total of 0.1 mL serially diluted with the ratio of 1:10 samples were plated onto TSA agar plates after vortexing for 5 seconds. The plates were aerobically incubated for 24 hrs at 37°C. On plates, the visible *E. coli* colonies (30 to 300 colonies) were counted. Airborne *E. coli* concentrations, in logarithm colony-forming units per cubic meter ($\log_{10}$ CFU $m^{-3}$), were determined based on Equation (4.1).

$$C = \log_{10}\left(\frac{N \times 10^{n}}{V_p} \times V_s \times \frac{1}{V_a}\right), \quad (4.1)$$

Where:

C: the airborne bacteria concentration, $\log_{10}$ CFU $m^{-3}$;

N: the number of colonies on a countable plate (30 to 300 colonies);

n: serial dilution factor (n = 0 for undiluted sample, n = 1 for 10-fold diluted sample, etc.);

$V_p$: the sample volume plated, mL ($V_p$ = 0.1 mL in this study);

$V_S$: the total volume of the original liquid sample, mL;

$V_a$: the total air volume sampled using bioaerosol samplers, $m^3$.

(3) Inactivation rates

The inactivation rate refers to the inactivation or the loss of airborne *E. coli* after passing through the UV light scrubber. The rate of the inactivation was calculated by Equation (4.2):

$$\text{Inactivation rate} = \left(1 - \frac{C2}{C1} \times \frac{1}{1-a}\right) \times 100\%, \quad (4.2)$$

Where:

Inactivation rate: biological loss caused by the UV lamps, %;

C2: the airborne bacteria concentration in the downstream chamber, CFU $m^{-3}$;

C1: the airborne bacteria concentration in the upstream chamber, CFU $m^{-3}$;

a: physical loss caused by the system, %.

The k-value was an additional metric used to represent how UV light affected microbiological survival (Hijnen et al., 2006). The k-value is the inactivation rate of

bacteria normalized by UV irradiance and contact time. The k-value was determined by using Equation (4.3):

$$k = -\frac{\log_{10}\left(\frac{C2}{C1} \times \frac{1}{1-a}\right)}{F}, \quad (4.3)$$

Where:

k: k-value, $cm^2\ mJ^{-1}$;

C2: the airborne bacteria concentration in the downstream chamber, CFU $m^{-3}$;

C1: the airborne bacteria concentration in the upstream chamber, CFU $m^{-3}$;

a: physical loss caused by the system, %;

F: is the product of UV irradiance, mW $cm^{-2}$, and the contact time (from 5.6 s to 0.23 s in this study).

(3) Reynolds number

At high wind speeds, the flow of air in the system was affected by turbulent flow which can lead to deviations of the k-value. To verify whether the flow of air in the system was affected by turbulent flow, the Reynolds number which is an indicator for turbulent flow was calculated (Guo & Ghalambor, 2005). The Reynolds number in a pipe was calculated by the following Equation (4.4):

$$N_{Re} = \frac{\rho v d}{\mu}, \quad (4.4)$$

Where:

$N_{Re}$: Reynolds number;

ρ: the density of the fluid, kg $m^{-3}$;

v: the flow speed, m $s^{-1}$;

d: the hydraulic diameter of the pipe, m;

μ: the kinematic viscosity, kg $m^{-1}$ $s^{-1}$. In this system, since the airflow was circulated through the UV scrubber, the hydraulic diameter is equal to the inside pipe diameter.

### 4.2.4 Statistical analysis

The system was tested with three doses of UV light which were zero, one, and two UV lamps. With each dose of UV light, there were 4 wind speed levels being tested at 0.11, 0.51, 1.74, and 2.61 m $s^{-1}$ corresponding to the contact times of 5.62, 1.17, 0.34, and 0.23 s. Temperature and RH were kept stable during experiments. With each wind speed level, the test was repeated three times which makes the total observations of 36 data points. The GLIMMix ANOVA model running on Statistical Analysis System (SAS 9.4, SAS Institute

Inc., Cary, NC, U.S.) was used in statistical analysis to assess the inactivation rate of airborne *E. coli* and the k-values as influenced by the as influenced by the airborne *E. coli* and initial bacterial concentrations. A significant level was applied as the *p*-value of 5%.

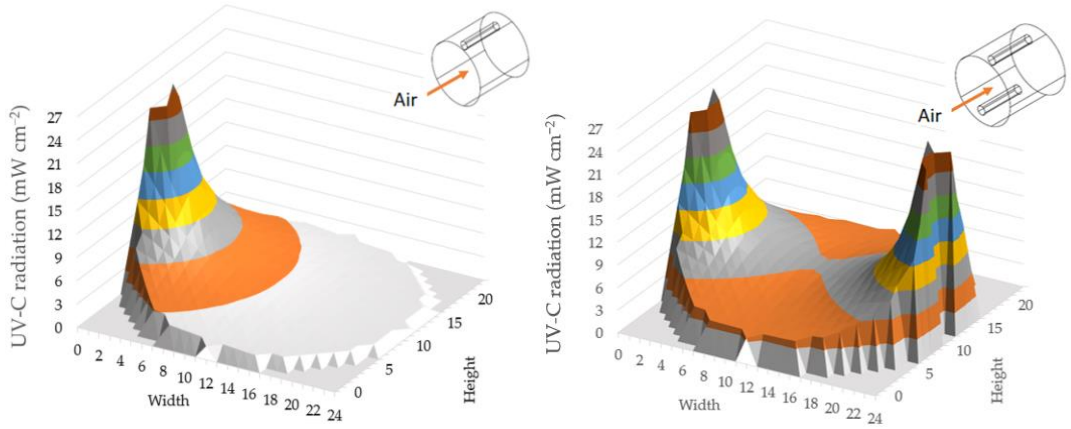
## 4.3 Results

### 4.3.1 UV light intensity distribution

Two UV lamps were positioned oppositely inside the tube. In a dusty environment, airborne *E. coli* can be carried by dust particles which prevent UV light from irradiating *E. coli*. Placing two symmetrical UV lamps can increase the UV irradiance exposure to *E. coli*. The UV light intensity distribution of one and two UV lamps simulated by UVCalc software is shown in Figure 4.3. The distributions of UV intensity were not uniform. With one UV lamp installation, the UV fluence rate decreased as the distance away from the UV lamps increased. The overall means of UV irradiations in the central plane were 3,687 µW $cm^{-2}$ and 7,434 µW $cm^{-2}$ for one UV lamp and two UV lamps, and in the total scrubber were 1,707 µW $cm^{-2}$ and 3,422 µW $cm^{-2}$ for one UV lamp and two UV lamps, respectively. The UV fluence rate measurement was validated by the UV light meter at different distances away from the UV lamps. A total of 12 points at different distances were measured for each UV lamp setup. The average fluence rate of the 12 points measurement were 4,884 µW $cm^{-2}$ and 9,860 µW $cm^{-2}$ for one and two UV lamps. The average fluence rate of 12 points with the corresponding distances calculated by the model is 5,048 µW $cm^{-2}$ and 10,869 µW $cm^{-2}$. The relative accuracy of the measured data and the data taken from the model has a difference of about 3% and 9% for one and two UV lamps, respectively. This shows the reliability of the model as the UV light meter has an accuracy of ±5%. In two UV lamp setup, there is a difference of more than 5% compared to the model data. This can be explained by limitations in the measurement process. UV meter sensor can only cover a certain irradiance angle. Therefore, when measuring the UV intensity of two lamps symmetrically positioned on wall, it will not be able to cover the entire incident light, leading to a slight decrease in fluence rate. In one UV lamp setup, the UV meter sensor covers the irradiance angle better than in two UV lamp setups, making a better accuracy rate.

### 4.3.2 Size distribution of *E. coli* attached to dust particles and dust particles

The size distribution of airborne *E. coli* carried by poultry dust particles is shown in Table 4.1. In the upstream chamber, most *E. coli* were found in particles larger than 7 µm. The second sizable portion of *E. coli* was those attached to particles in the range of 4.7 to 7 µm. The least *E. coli* was found in particles smaller than 2.1 µm which accounted for the total culturable *E. coli*.




(**a**) (**b**)

Figure 4.3 UV light intensity distribution of (**a**) one UV lamp; (**b**) two UV lamps.

Table 4.1 Size distribution of airborne *E. coli* carried by poultry dust particles in upstream and downstream chambers.

| **Contact Time (s, mean ± SD)** | **Chamber** | **>7.0 µm (%)** | **4.7–7.0 µm (%)** | **3.3–4.7 µm (%)** | **2.1–3.3 µm (%)** | **1.1–2.1 µm (%)** | **0.65–1.1 µm (%)** |
|---|---|---|---|---|---|---|---|
| 5.62 ± 0.91 | Upstream | 47.52 | 30.60 | 10.05 | 10.64 | 0.23 | 0.96 |
| | Downstream | 25.85 | 27.56 | 24.85 | 19.41 | 0.74 | 1.59 |
| 1.2 ± 0.06 | Upstream | 34.32 | 24.70 | 6.76 | 15.57 | 10.03 | 8.62 |
| | Downstream | 31.30 | 29.09 | 7.85 | 24.32 | 5.38 | 2.06 |
| 0.34 ± 0.01 | Upstream | 34.76 | 20.86 | 9.63 | 9.09 | 19.25 | 6.41 |
| | Downstream | 23.74 | 37.40 | 10.78 | 7.98 | 15.88 | 4.22 |
| 0.23 ± 0.01 | Upstream | 42.75 | 23.64 | 5.72 | 8.86 | 15.25 | 3.78 |
| | Downstream | 62.30 | 13.09 | 10.30 | 5.52 | 4.80 | 3.96 |

The mass size distribution of dust particles was measured by DustTrak, and the results are shown in Figure 4.4. With a proportion of 62.3%, the majority of dust particles were less than 1 µm in size. The rest of the dust particles had size ranges of 1.0–2.5 µm, 2.5–4.7 µm, 4.7–10.0 µm, and larger than 10.0 µm, with proportions of 2.1%, 3.2%, 16.8%, and 15.7%, respectively. As shown in Table 4.1 and Figure 4.4, although most dust particles were smaller than 1 µm, the size distribution of bacteria attached to dust particles was mainly larger than 2.1 µm. This demonstrated that most airborne *E. coli* are associated with dust particles greater than 2.1 µm in size. It can be explained by shielding effect (Zuo et al., 2013). In a previous study (Zuo et al., 2013), authors mentioned that the virus associated with bigger particles may be more protected from changes in the ambient environment than viruses that live as a singlet or bind to smaller particles. Thus, *E. coli* in this study may be shielded from environmental ambient when carried by large particles. Small particles had less of a protective impact on bacteria adhering to them. This could be one reason of the quick death of airborne *E. coli*.

### 4.3.3 Contact time effect on airborne *E. coli*

The contact time affected the concentration of viable *E. coli*. As the wind speed increased, the contact time decreased. Additionally, when contact time decreased, the concentration of *E. coli* in the system also decreased as shown in Figure 4.5. This was consistent with the real situation in the poultry house. When wind speed increases, the concentration of dust particles and pathogens decreases (Wang et al., 2014).

### 4.3.4 Physical loss of testing system

The physical loss was calculated by comparing the concentrations in the upstream and downstream chambers. In this system, the physical loss of *E. coli* was approximately 83% or 0.8 $\log_{10}$ reduction. The physical loss of the testing system is shown in Figure 4.6.

### 4.3.5 Inactivation efficiency of UV light

Temperatures and RH are shown in Table 4.2. The temperatures and RH remained stable over the experiments. However, there was a slight decrease in RH at the short contact time (0.23 s). An explanation could be that dehydration affected RH. At a short contact time or high wind speed, the dehydration effect in the air inside of chambers would increase leading to the decrease of RH (Nguyen et al., 2022).

The inactivation efficiencies of UV light are shown in Table 4.3. The concentrations of airborne *E. coli* were reduced significantly for all treatments with UV lamps. The inactivation rates (biological loss), after removing the physical loss caused by the system, varied from 99.87% and 99.95% at 5.62 s of contact time with irradiance levels of 1,707 µW $cm^{-2}$ and 3,422 µW $cm^{-2}$ to 72.90% and 86.60% at 0.23 s of contact time with irradiance levels of 1,707 µW $cm^{-2}$ and 3,422 µW $cm^{-2}$. Results also showed that the inactivation rates decreased according to the contact times. As wind speed increased from 0.11 to 2.61 m $s^{-1}$, the time that airborne *E. coli* was exposed to light decreased from 5.6 s

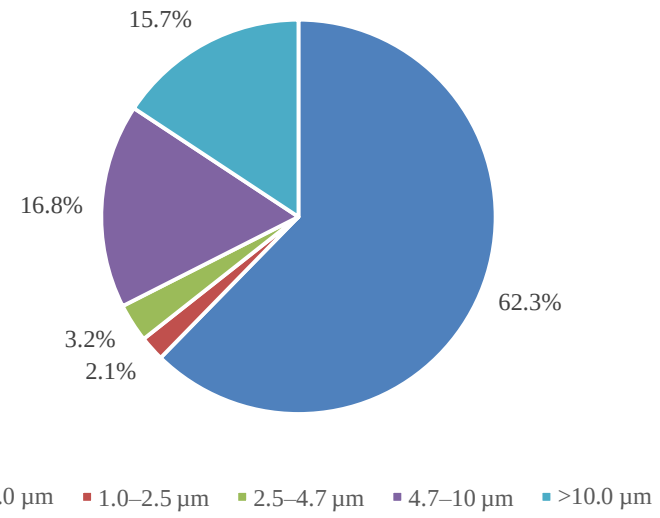



Figure 4.4 Size distribution of poultry dust particles.

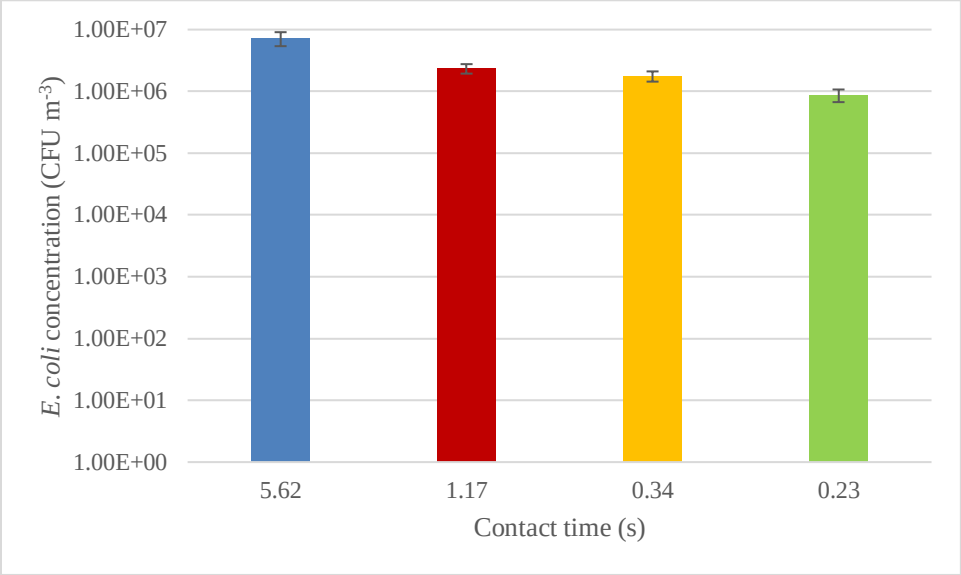



Figure 4.5 Contact time effect on *E. coli* concentration.

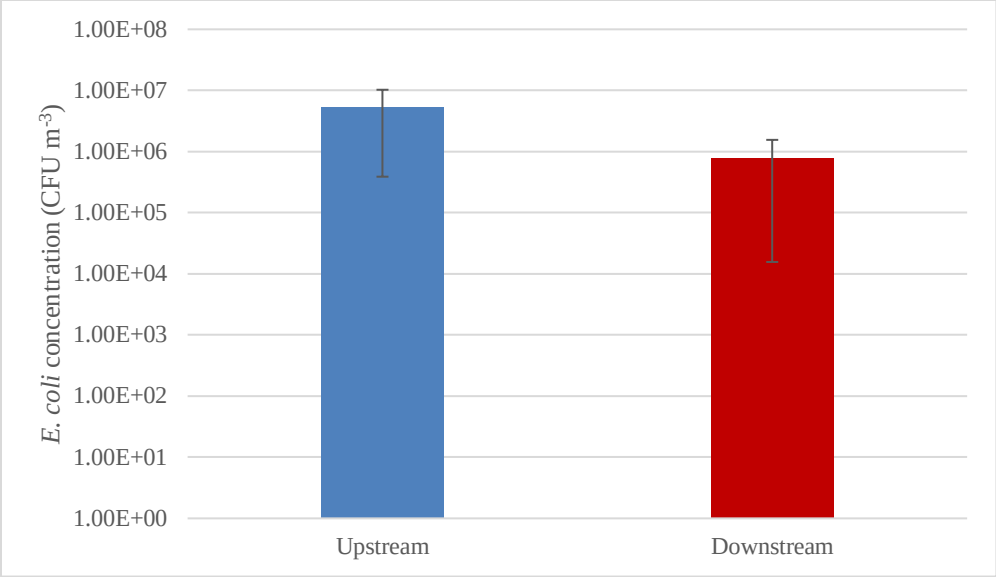



Figure 4.6 Physical loss of airborne *E. coli*.

Table 4.2 Temperature and relative humidity correspond to contact times and the number of UV lamps.

| Contact Times (s, Mean ± SD) | Number of UV Lamps | Temperature (°C, Mean ± SD) | Relative Humidity (%, Mean ± SD) |
|---|---|---|---|
| 5.62 ± 0.91 | 1 | 23.0 ± 0.7 [a] | 61 ± 7 [a] |
| | 2 | 23.0 ± 0.7 [a] | 61 ± 7 [a] |
| 1.2 ± 0.06 | 1 | 22.0 ± 1.4 [a] | 60 ± 2 [a] |
| | 2 | 22.0 ± 1.4 [a] | 60 ± 2 [a] |
| 0.34 ± 0.01 | 1 | 22.5 ± 0.5 [a] | 61 ± 3 [a] |
| | 2 | 22.5 ± 0.5 [a] | 61 ± 3 [a] |
| 0.23 ± 0.01 | 1 | 22.7 ± 1.3 [a] | 56 ± 5 [b] |
| | 2 | 22.7 ± 1.3 [a] | 56 ± 5 [b] |

Note: [a], [b] mean in the same column with different letters are different ($p < 0.05$). SD means standard deviation.

Table 4.3 Inactivation rates of UV light correspond to contact times and the number of UV lamps.

| Contact Times (s, mean ± SD) | Number of UV Lamps | Inactivation Rates (%, mean ± SD) | Log Reduction ($log_{10}$) |
|---|---|---|---|
| 5.62 ± 0.91 | 1 | 99.87 ± 0.07 [a] | 2.9 ± 0.3 |
| | 2 | 99.95 ± 0.04 [a] | 3.5 ± 0.5 |
| 1.2 ± 0.06 | 1 | 93.97 ± 0.36 [b] | 1.2 ± 0.0 |
| | 2 | 96.85 ± 1.23 [c] | 1.6 ± 0.2 |
| 0.34 ± 0.01 | 1 | 92.60 ± 0.63 [d] | 1.1 ± 0.0 |
| | 2 | 95.40 ± 0.59 [e] | 1.3 ± 0.1 |
| 0.23 ± 0.01 | 1 | 72.90 ± 2.57 [f] | 0.6 ± 0.0 |
| | 2 | 86.60 ± 1.35 [g] | 0.9 ± 0.1 |

Note: Means with the same letter are not significant different ($p < 0.05$). SD means standard deviation.

to 0.23 s. Therefore, the UV irradiance doses exposed to airborne *E. coli* also decreased, leading to a decrease in the inactivation rates.

The k-values are shown in Table 4.4. The k-values were not similar among different treatments. When the contact times and the number of UV lamps changed, the k-values changed accordingly. Typically, in the same bacteria strain, the k-value would be unchanged when they were exposed to the same disinfectant. However, in this study, the k-value varied when the contact time changed. An explanation was that the turbulent flow of high wind speed affected the k-value. To verify that, we calculated the Reynolds number which is an indicator for turbulent flow. The results showed that at the 5.62 s contact time, the Reynolds number was 1,738 which was smaller than 2,000 which means the laminar flow (Guo & Ghalambor, 2005). However, for 1.17, 0.34, and 0.23 s contact times, the Reynolds numbers were 8,863, 29,370, and 44,576, respectively, which were significantly greater than 4,000, indicating turbulent flow (Guo & Ghalambor, 2005).

## 4.4 Discussion

The designed system was assessed for the effect of UV light on the inactivation of airborne *E. coli* carried by poultry dust particles. The inactivation rate was examined at different air speeds with the aid of a vacuum pump from 0.11 m $s^{-1}$ to 2.61 m $s^{-1}$ corresponding to the contact time from 5.62 s to 0.23 s, and different UV radiation intensity (1,707 µW $cm^{-2}$ and 3,422 µW $cm^{-2}$). Before conducting the experiment, to ensure the expected UV irradiation intensity, UVCalc software was applied to simulate the UV light intensity distribution in the UV scrubber. The maximum UV light irradiance was observed to be close to the UV lamps, indicating that the UV irradiance in the UV scrubber was not spread uniformly. UV light irradiance reached up to 24,759 µW $cm^{-2}$ for one UV lamp or 25,864 µW $cm^{-2}$ for two UV lamps when close to the lightbulb, and it gradually drops to 946 µW $cm^{-2}$ for one UV lamp or 3,450 µW $cm^{-2}$ for two UV lamps when 24 cm away from the UV bulb for one UV lamp or 17 cm away from the UV bulbs for two UV lamps. The UV light irradiance was measured in laboratory conditions with a clear environment. In poultry house conditions, UV light irradiance may not reach such a level. Especially with the dusty conditions of the poultry environment, UV light bulbs can be covered with dust and reduce their ability to sterilize. Prior research found that the dust concentration might reach 81.33 mg $m^{-3}$ in poultry houses (Ellen et al., 2000). When summer comes, the ventilation system must work harder resulting in increased airflow through the UV system. This would increase the amount of dust particles flowing through the UV tube, leading to a reduction of disinfectant ability.

In a past study (Ochoa-Velasco et al., 2020), UV light has been extensively explored and was well recognized as a technique that can inactivate germs by preventing DNA replication. According to earlier research (Ochoa-Velasco et al., 2020), UV radiation was particularly effective in killing *E. coli* in water, droplets, and surfaces used in the processing of food. The same was true for airborne *E. coli* carried by poultry litter particles. In general, a positive relationship between UV irradiance level and the

Table 4.4 K-values correspond to contact times and the number of UV lamps.

| **Contact Times (s, mean ± SD)** | **Number of UV Lamps** | **k-values ($cm^2$ $mJ^{-1}$, mean ± SD)** |
|---|---|---|
| 5.62 ± 0.91 | 1 | 0.300 ± 0.106 [a] |
| | 2 | 0.171 ± 0.059 [ab] |
| 1.2 ± 0.06 | 1 | 0.608 ± 0.145 [c] |
| | 2 | 0.378 ± 0.065 [a] |
| 0.34 ± 0.01 | 1 | 1.928 ± 0.35 [d] |
| | 2 | 1.136 ± 0.16 [e] |
| 0.23 ± 0.01 | 1 | 0.144 ± 0.072 [b] |
| | 2 | 0.114 ± 0.080 [b] |

Note: Means with the same letter are not significant different ($p < 0.05$). SD means standard deviation.

inactivation rate was observed in this study. The positive relationship between UV irradiance and the inactivation rate was also reported in previous studies (Hijnen et al., 2006; Xu et al., 2005). At the high wind speed (2.61 m $s^{-1}$ or 0.23 s of contact time), the inactivation of the airborne *E. coli* drastically increased when more UV irradiances were applied (3422 µW $cm^{-2}$ versus 1707 µW $cm^{-2}$). While a single lamp (average of 1707 µW $cm^{-2}$) killed 72.90 ± 2.57% (or 0.6 ± 0.0 $log_{10}$ reduction) of the bacteria, two lamp (average of 3422 µW $cm^{-2}$) inactivation rate up to 86.60 ± 1.35% (or 0.9 ± 0.1 $log_{10}$ reduction) of the bacteria. When the number of UV lamps was raised from one to two at lower wind speeds (≤1.74 m $s^{-1}$), a positive relationship was still seen, but the difference was not as obvious as it was at higher wind levels. At low wind speed, the exposure time of airborne *E. coli* to UV light increased significantly from 0.23 s (at 2.61 m $s^{-1}$) to 5.62 s (at 0.11 m $s^{-1}$), resulting in about 3 $log_{10}$ of the bacteria being inactivated. As a result, it is difficult to observe the difference as clearly as at high wind levels where the exposure period was short. In addition, the results also pointed out that the inactivation of airborne *E. coli* was not linearly related to the UV irradiance used. A previous study (Xu et al., 2005) also reported a similar result. The study (Xu et al., 2005) reported that an increase in UV irradiation (within a similar contact time) above 5 µW $cm^{-2}$ did not yield a proportional increase in inactivation rate. A possible explanation is that the dust particles which carry *E. coli* can block a certain amount of UV irradiation to *E. coli*. This can lead to a decrease in UV irradiation efficiency and makes the increase in irradiance not proportional to inactivation rates.

In a previous study (Nguyen et al., 2022), we examined the survivability of airborne *E. coli* carried by poultry dust particles in laboratory conditions. The airborne *E. coli* had a half-life time of over 5.7 minutes. The half-life time is the amount of time required for bacteria to decline by half or 50%. In this study, the survival time of the bacteria when exposed to UV light was much shorter. Specifically, the inactivation rate was 72.90 ± 2.57% with irradiance level of 1,707 µW $cm^{-2}$ or 86.60 ±1.35% with irradiance level of 3,422 µW $cm^{-2}$ at the 0.23 s contact time, and up to approximately 100% with irradiance levels of 1,707 µW $cm^{-2}$ and 3,422 µW $cm^{-2}$ at the 5.6 s contact time. In 1,707 µW $cm^{-2}$ and 3,422 µW $cm^{-2}$ UV irradiance levels, 99.87 ± 0.07% and 99.95% ± 0.04 (or 2.9 ± 0.3 $log_{10}$ reduction and 3.5 ± 0.5 $log_{10}$ reduction) of *E. coli* were eliminated in 5.6 s of contact time compared to only 50% in 5.7 min in the normal condition. Therefore, it can be affirmed that the use of UV light to reduce airborne *E. coli* carried by poultry dust particles was extremely effective under the experimental conditions.

Based on the obtained results, the k-value was calculated accordingly. In principle (Hijnen et al., 2006; Zhao et al., 2014), the k-value should be the same for the same bacteria strain exposed to the same disinfectant. However, by the effect of turbulent flow generated at the high wind, the k-value was impacted. The results showed that, at the contact time of 5.62 s, the Reynolds number was smaller than 2,000 which means the laminar flow (Guo & Ghalambor, 2005). In contrast, at shorter contact times (1.17, 0.34, and 0.23 s), the

turbulent flow appeared. This turbulent flow is an unstable airflow and might affect the k-values. The bacteria in the laminar flow exposes to just one site to UV radiation. On the contrary, when the airflow is unsteady, dust particles might spin around. As a result, it increases the chance that *E. coli* are exposed to UV radiation. The inactivation rate is affected by wind speed by two means. On one hand, higher wind speed reduces the contact time, which compromises the inactivation rates; on the other hand, higher wind speed increases turbulence that alters UV exposure by *E. coli* and thus inactivation rates. The latter is an interesting assumption that has never been reported by other studies and requires further research. In addition, when installed UV lamps varied, k-values also varied. One UV bulb may effectively eliminate microorganisms. However, doubling the number of UV lamps did not raise inactivation rates proportionally. The k-value is defined as the inactivation rates adjusted by irradiance and contact periods. So, since the inactivation rates did not rise according to the number of UV lamps, we may calculate the various k-values.

To apply the UV system on an industrial scale, contact time, wind speed of the ventilation system, UV irradiance level, and dust concentrations need to be considered. In commercial poultry houses, the ventilation rates will vary depending on the season and variety of environmental conditions. Thus, when applying to the industrial scale, the varies in conditions may affect inactivation effectiveness. In addition, the poultry houses are typically dusty which can affect the UV system in the long-term run. The poultry dust can cover the UV lamp surfaces which reduces the UV irradiance, and thus, reduces the effect of the UV system. A periodic cleaning schedule is suggested when applying the system in poultry houses. Next, even though UVA (wavelength of 315–400 nm) was well studied and proven that it had positive effects on poultry (Rana & Campbell, 2021), UVC (wavelength of 200–300 nm) was not well studied yet. Therefore, the application of UVC, in this case, 254 nm, into the poultry environment also needs to consider its impact on the poultry. Finally, study results suggested that one UV lamp was able to create the irradiance level of 1707 μW $cm^{-2}$ which effectively (92.6% of inactivation rates) killed airborne *E. coli* generated in the system at the contact time of 0.34 s or longer. The upstream chamber of the system has a volume of 0.54 $m^3$ with a concentration of 6.7 $\log_{10}$ CFU $m^{-3}$ of airborne *E. coli* which is consistent with a previous study (Nguyen et al., 2022). Given the typical size of a poultry house, it is necessary to install multiple UV light systems corresponding to the volume of the house to ensure complete coverage of the poultry house. In addition, an increasing number of UV lamps would not proportionally increase the inactivation rates. The poultry house, however, is a dusty environment where airborne dust particles can prevent some UV exposure to *E. coli*. Thus, it is necessary to increase the irradiation source, or in other words increase the number of UV lamps. The system would be installed before the outlet of ventilation system to reduce airborne *E. coli* emitted outside the poultry houses.

## 4.5 Conclusions

This study investigated the effect of UV light on the inactivation of airborne *E. coli* carried by poultry dust particles in laboratory conditions. The laboratory conditions remained stable at about 22.6°C with an RH of 60%. In this study, a system that simulated the actual conditions of the poultry houses was designed to evaluate the inactivation efficiency. Based on the results, we conclude that (1) the inactivation rates reduced from approximately 99.87% and 99.95% at 5.62 s of contact time with 1,707 μW $cm^{-2}$ and 3,422 μW $cm^{-2}$ of irradiance levels to 72.90% and 86.60% at 0.23 s of contact time with 1,707 μW $cm^{-2}$ and 3,422 μW $cm^{-2}$ of irradiance levels; (2) the average of UV irradiation were 1,707 μW $cm^{-2}$ and 3,422 μW $cm^{-2}$ for one UV lamp and two UV lamps, respectively; (3) turbulent flow might affect the inactivation efficiency of the UV system. The results of this study will help to bring up an idea of an affordable mitigating system for airborne pathogens.

# CHAPTER V

# Modeling Long-Distance Airborne Transmission of Highly Pathogenic Avian Influenza Carried by Dust Particles

This chapter is submitted to ***Scientific Reports***.

**Nguyen, X. D.,** Zhao, Y., Evans, J. D., Lin, J., Voy, B., Purswell, J. L., Hawkins, S., & Evans, J. D. (2023). Modeling Long-Distance Airborne Transmission of Highly Pathogenic Avian Influenza Carried by Dust Particles.

## 5.1 Introduction

The U.S. poultry industry is among the world's largest poultry producers. It includes meat products from turkeys, broilers, and eggs from laying hens. The combined values of these products exceeded 35 billion U.S. dollars in 2020 (USDA-NASS, 2020). Poultry products are affordable and important sources of daily protein. In addition, about 18% of U.S. poultry products are exported annually, and the industry provides over 1 million jobs for national populations. However, this important industry is extremely vulnerable to infectious diseases caused by pathogenic microorganisms such as highly pathogenic avian influenza (HPAI). The HPAI virus is one of the biggest challenges facing the poultry industry. In 2015, an outbreak of HPAI in the Mid-Western U.S. resulted in a significant loss of over 50 million birds and 3.3 billion U.S. dollars (Torremorell et al., 2016). This showed the vulnerability of the U.S. poultry industry to viral infectious diseases such as HPAI.

Avian influenza (AI) or bird flu refers to the infectious disease caused by the infection of type A avian influenza. These viruses are found in wild aquatic birds worldwide and can infect domestic poultry and other bird and mammal species. Although avian influenza A viruses can infect wild aquatic birds' intestines and respiratory tracts, other species, such as wild ducks, may not become ill. Avian influenza A viruses, on the other hand, are highly infectious among commercial poultry, and some of these viruses can sicken and even kill certain domesticated bird species such as chickens, domestic ducks, and turkeys. As of November 2022, approximately 50 million birds including 265 commercial flocks and 358 backyard flocks have been affected by the 2022 AI outbreak (USDA-APHIS, 2022a). AI

type A viruses can be found in infected birds' saliva, nasal secretions, and feces. Transmission of the high concentration of pathogens to naive (susceptible) birds can result from direct contact between birds or by indirect contact with virus-contaminated fomites (Swayne & Suarez, 2000).

Due to the severity of the disease, AI type A viruses are divided into two groups including low pathogenic avian influenza (LPAI) and high pathogenic avian influenza (HPAI). Low pathogenic avian influenza viruses produce little or moderate illness in laying hens and broilers (such as ruffled feathers and a drop in egg production). The majority of avian influenza A viruses are low pathogenic, causing little symptoms of illness in infected wild birds. Some low-pathogenic viruses in chickens can evolve into highly pathogenic avian influenza viruses. On the other hand, infected chickens suffer from severe sickness and a high death rate due to HPAI viruses. Only a few avian influenza A(H5) and A(H7) viruses are HPAI type A viruses, while the vast majority of avian influenza A(H5) and A(H7) viruses circulating in birds are LPAI viruses. In hens, HPAI type A(H5) or A(H7) virus infections can produce sickness that affects several internal organs, with mortality rates ranging from 90% to 100%, frequently within 48 hours (World Health, 2005). Infections of HPAI A(H5) and A(H7) viruses in poultry can transmit to wild birds, resulting in the additional geographic spread of the virus when the birds migrate. HPAI viruses are transmitted mainly through direct contact infection. However, with the initial bird mortalities reported near air inlets of poultry houses, there is a high chance that HPAI viruses were transmitted into poultry houses via ventilation system by airborne transmission.

In the poultry house, the major components of the air include gases, odors, and numerous pathogens may be carried by dust particles or droplet nuclei such as AI viruses. AI viruses are first secreted via birds' nasal secretions, feces, and saliva. The bird secretions can either be dried and suspended in the air for a long period of time or deposited on the poultry litter surface. The deposited secretions which carry AI are then mixed with poultry litter particles and re-aerosolized into the air by dust bathing behavior of birds. Both droplet nuclei and dust particles that carry AI may then be distributed into the poultry house environment and transmitted from barn to barn via ventilation system and transport of air.

At susceptible farms, the AI can be sucked in through the ventilation system and be distributed inside the farms. The airborne HPAI viruses are then deposited onto the surface of poultry litter on which they can survive up to 5 days at 24°C (Kurmi et al., 2013). In previous studies, authors have reported that most airborne AI viruses are found in dust particles as small as 1 µm–5 µm in size (Bertran et al., 2017a; Zhou et al., 2016) at 0.5 m away from poultry housing. It is important to note that fine dust particles (or dust particles with diameters that are generally 2.5 µm and smaller) can travel hundreds of miles(Kwon et al., 2016). With the long dispersion range, the AI viruses carried by fine dust particles can be a possible transmission pathway of HPAI.

To determine the possibility of long-distance airborne transmission of HPAI carried by poultry dust particles, this study aims at simulating the airborne transmission of HPAI by using the Hybrid Single-Particle Lagrangian Integrated Trajectory model (HYSPLIT) to assess the risk of airborne and deposited AI carried by poultry litter dust particles. Compared to other models such as Computational fluid dynamics (CFD) which is also able to simulate the flow of the air, the advantage of HYSPLIT is to integrate the meteorological data into the model which improves the accuracy of the simulation. In the study, 72 infected commercial poultry farms (72 % of the national total of the infected commercial farms) in the Mid-Western U.S. were focused on, and only data from the period of February 08$^{th}$, 2022 to May 25$^{th}$, 2022, when cases appeared in the area were included in the model. Infected backyard birds accounted for a small number of infected birds with a low number of infected farms, and therefore, were not included in this study.

## 5.2 Materials and Methods

### 5.2.1 Infected farm data

Data from 168 infected cases in the U.S. were obtained from the Animal and Plant Health Inspection Service (APHIS) and Watt Poultry (WattPoultry.com). Each confirmed case datum included physical address, county and state, infection confirmation date, and the number of birds infected. In this study, 168 infected commercial poultry cases (72% of the national total of commercial farm infections) in the Mid-Western U.S. were focused on, and only data from the period of February 08$^{th}$, 2022 to May 22$^{nd}$, 2022, were included in the model. The dispersion simulation modeling was performed to examine if the confirmed cases in the Mid-West received air from other farms before being infected and estimate the concentration of airborne AI received. The reason for selecting data from the commercial farms in the Mid-Western area was that this area accounted for a total of 68.5% of the national total infected commercial farms and the region that accounted for the largest number of birds affected during the previous AI outbreak since 2015. Although the number of backyard flocks infected was higher than commercial flocks, the number of infected birds per backyard flock was significantly lower than the number of birds infected per commercial farm.

### 5.2.2 HYSPLIT modelling

The HYSPLIT model (Hybrid Single-Particle Lagrangian Integrated Trajectory model, National Oceanic, and Atmospheric Administration, Washington, D.C., U.S.) is a computational model used to compute air parcel trajectories, which determines how far and in which direction a parcel of air, and hence air contaminants, will move. HYSPLIT modeling is also able to estimate air pollutant dispersion, chemical transformation, and deposition. In this study, the HYSPLIT model was used to simulate the air movement of $PM_{2.5}$ which hypothetically carries AI to examine if the $PM_{2.5}$ particles travel passing through other farms before the farms became infected. The modeling also computed the

concentration of airborne and deposited AI carried by $PM_{2.5}$ dust particles (or fine dust particles) in the farms. The AI survival time of 24 hrs at 28°C and a period of 21 days prior to the infection confirmation dates (Zhao et al., 2019) was applied in the modeling. Three different periods, namely 8 a.m., 4 p.m., and 12 a.m. (Local Standard Time) were calculated separately to reduce the temporal wind speed and direction-varied effects. Airborne and deposited AI were examined at the height of 6 m above ground level (m agl), considering the typical height of the poultry houses. The height of typical air inlets which is 1.5 m was applied in the model.

The concentration of airborne AI carried by $PM_{2.5}$ was assessed by using both default and ceiling input data in the HYSPLIT model. The AI concentration has been reported to be detected predominantly from fine dust particles (Zhou et al., 2016), and with the long range of transmission, the $PM_{2.5}$ size can be a big concern for public health. The default data stands for the representative data from scientific references, and the ceiling data stands for parameters that might happen in the worst scenarios. The required parameters were provided in Table 5.1.

### 5.2.3 Model processing

Forward concentration modeling was used to assess the possibility of infections from infected poultry farms to other farms. Data from 168 infected cases from February 08$^{th}$, 2022 to May 22$^{nd}$, 2022, in the Mid-Western area were utilized in the study. First, the AI data collected daily from APHIS and Watt Poultry websites were imported into HYSPLIT modeling. The AI data were divided into four categories including default $PM_{2.5}$, ceiling $PM_{2.5}$, deposited default $PM_{2.5}$, and deposited ceiling $PM_{2.5}$. Default $PM_{2.5}$ and ceiling $PM_{2.5}$ stands for the airborne AI concentration carried by $PM_{2.5}$ in the default scenario and worst scenario, respectively. Deposited default $PM_{2.5}$ and deposited ceiling $PM_{2.5}$ are the AI concentration carried by $PM_{2.5}$, after being aerosolized, being deposited on the surface of poultry facilities in the default scenario and worst scenario respectively. Hypothetically, the deposited AI, after being transmitted into the poultry house and deposited on surfaces, would be picked up by birds in the poultry house. Meteorological data was downloaded from the National Oceanic and Atmospheric Administration (NOAA, Washington, D.C., U.S.) website for each day. To reduce the variability of wind direction as well as meteorological conditions, the model is run every 8-hrs interval which results in three trajectories covering the air arrival time at 8 a.m., 4 p.m., and 12 a.m. Local Standard Time (LST). After processing the data, airborne AI concentration data collected from the modeling were then exported as kmz files which were then loaded in Google Earth Pro (Google LLC, Mountain View, CA, U.S.). With concentration modeling, the clear viral pattern movement of AI can be observed on Google Earth. The AI concentration data corresponding to the 4 scenarios and types of poultry farms would be reported. The modeling concentrations of AI were then compared to the minimal infective doses of

Table 5.1 Input parameters for the HYSPLIT model.

| Parameter | Unit | Value |
|---|---|---|
| Total run time per cycle | hrs | 24 |
| Trajectory modeling direction | - | Forward |
| Top of the model | m | 1,500 |
| Incubation period | day | 7 (USDA-APHIS, 2022c) |
| Virus emission duration | hrs | 24 |
| Height of concern | m | 0-6 (airborne) & 0 (deposit) |
| $PM_{2.5}$ emission rate | mg $bird^{-1}$ $d^{-1}$ | 1 (laying hen) (Cambra-López et al., 2010; Li et al., 2011; Shepherd et al., 2015) |
| | | 38 (turkey) (Cambra-López et al., 2010; Li et al., 2011; Shepherd et al., 2015) |
| PM density | g $cm^{-3}$ | 1.5 (Rosenthal et al., 2007) |
| PM deposition velocity | m $s^{-1}$ | 0.001 ($PM_{2.5}$) (Lin et al., 1994) |
| Half-life | day | 1.0/1.5 (default/ceiling) (Shaman & Kohn, 2009) |
| Percentage of manure in dust | % | 5 (laying hen) (Cambra-López et al., 2010; Zhao et al., 2014)<br>40 (turkey) (Cambra-López et al., 2010; Zhao et al., 2014) |

Table 5.1 Continued.

| | | |
|---|---|---|
| Viral shedding rate | Log $EID_{50}$ [g feces]$^{-1}$ | 4/5 (default/ceiling) (Forrest et al., 2010) |
| Viral survival reduction | % | 60 (after 24 hrs) (Kurmi et al., 2013) |

airborne AI viruses and deposited AI viruses calculated based on Formula (5.1) (5.2) and were utilized to assess the possibility of infection for each case.

### 5.2.4 Minimal infective doses for airborne transmission

Minimal infective dose for airborne transmission (MIDa) is the quantity of airborne AI (measured in $EID_{50}$ $m^{-3}$) that is necessary to cause infection in a healthy bird (Spackman et al., 2016). MIDs of poultry were studied by Spackman and DeJesus. The 50% egg infective dose ($EID_{50}$) or egg/embryo infective dose 50 is a unit for the concentration of a certain virus. Specific pathogen-free (SPF) eggs/embryos are employed as the culture medium. The original viral sample is serially diluted first. Each dilution is injected into a small number of eggs. Then, this dilution is used to determine the $EID_{50}$ when 50% of the eggs in a dilution are infected. The MIDa were calculated based on the general minimal infective dose (MIDt). MIDt were $10^3$ $EID_{50}$ for turkeys (Spackman et al., 2016) and $10^{3.5}$ $EID_{50}$ for laying hens (DeJesus et al., 2016). The same MIDa was used for laying hens, broilers, breeders, and pullets. The MIDa values were calculated by the following Formula (5.1):

$$\text{MIDa} = \text{MIDt} \times \frac{1}{v \times r \times 24}, \quad (5.1)$$

Where:

MIDt: the general infective dose, $EID_{50}$;

v: the tidal volume of the bird, $m^3$;

r: respiratory rate, time $hrs^{-1}$;

MIDa: the Minimal infective dose for airborne transmission, $EID_{50}$ $m^{-3}$ for a day (24 hrs).

Minimal infective dose for deposited (MIDd) AI is the quantity of deposited AI (measured in $EID_{50}$ $m^{-2}$) that is necessary to cause infection in a healthy bird. The same MIDd was used for laying hens, broilers, breeders, and pullets. The MIDd values were calculated by the following Formula (5.2):

$$\text{MIDd} = \text{MIDt} \times \frac{\mathbf{1}}{\boldsymbol{s}}, \quad (5.2)$$

Where:

MIDt: the general infective dose, $EID_{50}$;

s: the area that a bird needs in the house, 0.11 $m^2$ per bird (or stocking density of 9 birds per $m^2$) for laying hen (Krause & Schrader, 2019) and 0.35 $m^2$ per bird (or stocking density of about 3 birds per $m^2$) for turkey (Bartz et al., 2020), $m^2$ $bird^{-1}$;

MIDd: the Minimal infective dose for deposited AI viruses, $EID_{50}$ $m^{-2}$.

### 5.2.5 Infection probability

The probability of farm infection (Formula 5.4) is determined by the probability of individual-bird infection (Formula 5.3) at a given dosage (d) and flock size (nf). We assumed that 95% of birds might get infected at the (d) dosage, θ was 0.00069 for turkeys and 0.00022 for laying hens (Zhao et al., 2019).

$$Pi = 1 - (1 - \theta)^{d}, \tag{5.3}$$

$$Ph = 1 - (1 - Pi)nf, \tag{5.4}$$

Where:

$P_i$: probability of individually bird infection, %;

θ: probability of one $ID_{50}$ infect to bird, %;

d: dosage of viruses exposed to a bird, $EID_{50}$ $bird^{-1}$ $day^{-1}$;

$P_h$: probability of farm infection, %;

nf: size of flock, bird.

## 5.3 Results

### 5.3.1 Minimal infective doses

The infective dosage, lung capacity, breathing rate, and exposure duration were all used to compute the MIDa and MIDd. Lung capacity and respiratory rate have been studied in a previous study, they are in turkeys are $7.7 \times 10^{-5}$ $m^3$ and $2.4 \times 10^3$ times $hrs^{-1}$, respectively, and $1.4 \times 10^5$ $m^3$ and $1.6 \times 10^3$ times $hrs^{-1}$ in laying hens (Zhao et al., 2019). Over a day of exposure, the resulting MIDa values were 210 $EID_{50}$ $m^{-3}$ for turkeys and 5,880 $EID_{50}$ $m^{-3}$ for laying hens. MIDd values were 2,837 $EID_{50}$ $m^{-2}$ for turkeys and 28,460 $EID_{50}$ $m^{-2}$ for laying hens.

### 5.3.2 Viral concentrations in different types of poultry houses

The viral concentrations of AI in different types of poultry houses are reported in Figure 5.1 (airborne AI), Figure 5.2 (deposited AI), and Figure 5.3 (combined concentrations of airborne and deposited AI). The figures show the viral concentrations carried by $PM_{2.5}$ dust particles in 5 different types of poultry houses with 4 different scenarios including airborne ceiling, deposited ceiling, airborne default, and deposited default. Results show that in all categories, the viral concentrations were lower than MIDa and MIDd lines. This implies that under all scenarios, these commercial farms are likely not to have received viral loads above their MIDa and MIDd. This suggests that there is little risk of sludge development under both normal conditions and worst conditions.

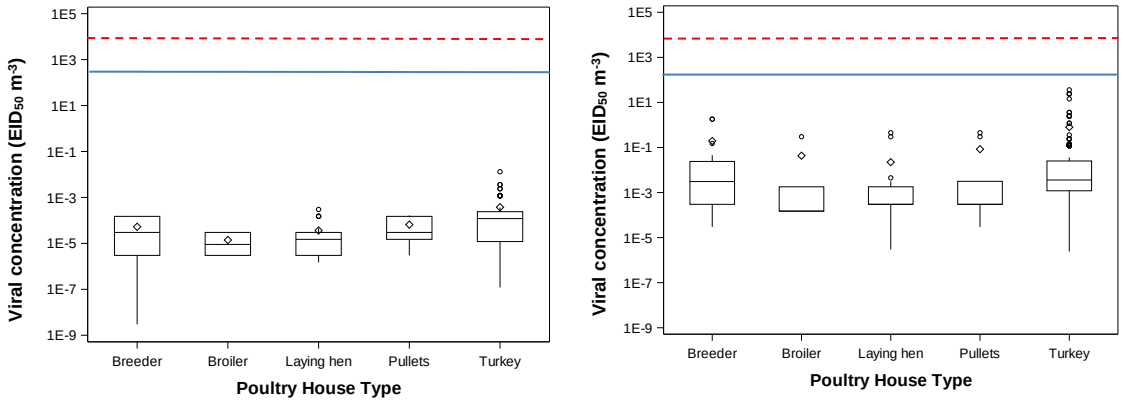
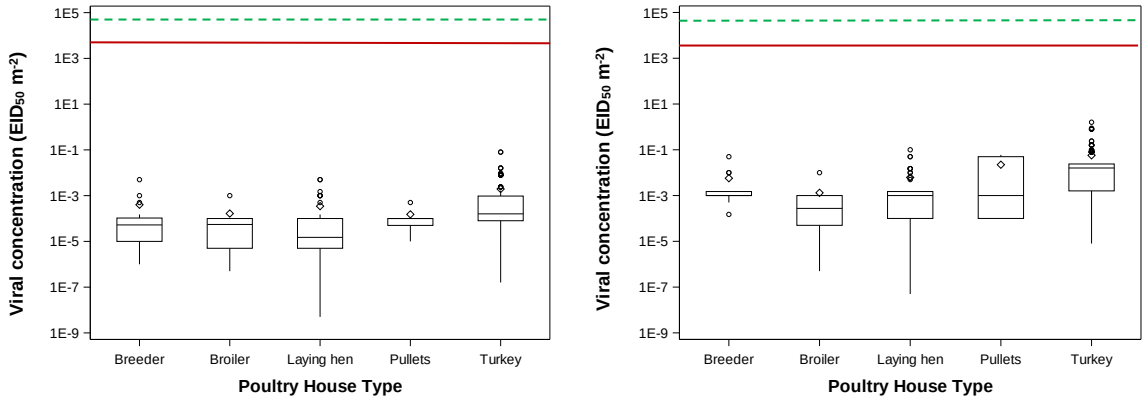
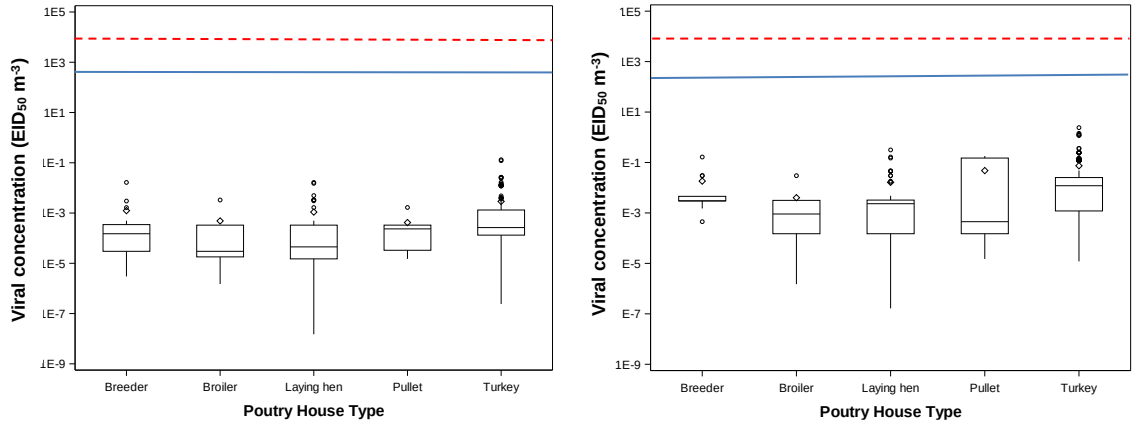



Figure 5.1 Concentrations of airborne highly pathogenic avian influenza (HPAI) viruses at the recipient farms. The concentration of avian influenza (AI) viruses is reported according to two categories (ceiling and default). The concentration of (a) airborne AI viruses in the default scenario, 50% egg infective dose ($EID_{50}$) $m^{-3}$; and (b) concentration of airborne AI viruses in the ceiling scenario, $EID_{50}$ $m^{-3}$. The blue solid line stands for the minimal infective dose of airborne AI (MIDa) values of turkey and the red dashed line stands for the MIDa values of laying hen.




(a) (b)

Figure 5.2 Concentrations of deposited highly pathogenic avian influenza (HPAI) viruses at the recipient farms. The concentration of avian influenza (AI) viruses is reported according to two categories (ceiling and default). The concentration of (a) airborne AI viruses in the ceiling scenario, 50% egg infective dose ($EID_{50}$) $m^{-2}$; and (b) deposited AI viruses in the default scenario, $EID_{50}$ $m^{-2}$. The red solid line stands for the minimal infective dose of deposited AI (MIDd) values of turkey and the green dashed line stands for the MIDd values of laying hen.




(a) (b)

Figure 5.3 Combined concentrations of airborne and deposited highly pathogenic avian influenza (HPAI) viruses at the recipient farms. The concentration of avian influenza (AI) viruses is reported according to two categories (ceiling and default). The concentration of (a) AI viruses in the default scenario, 50% egg infective dose ($EID_{50}$) $m^{-3}$; and (b) concentration of AI viruses in the ceiling scenario, $EID_{50}$ $m^{-3}$. The blue solid line stands for the minimal infective dose of airborne AI (MIDa) values of turkey and the red dashed line stands for the MIDa values of laying hen.

### 5.3.3 Farm infection probabilities in different states

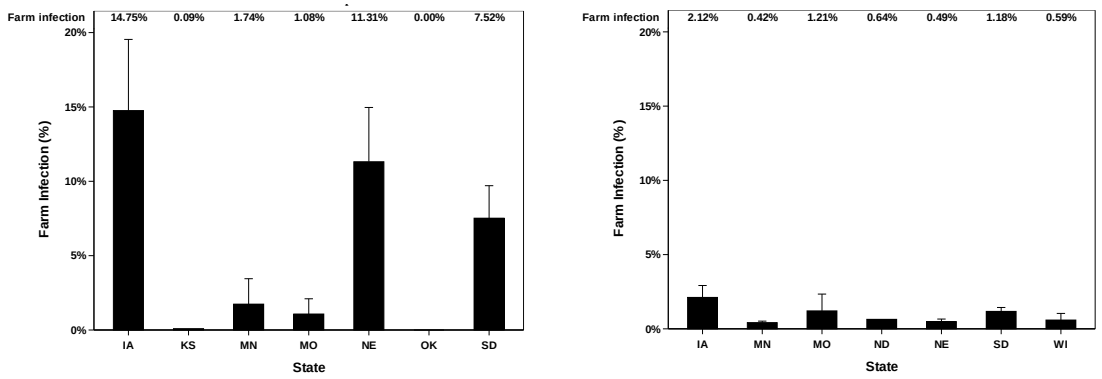
The farm infection probabilities of poultry farms that have received AI viruses from different locations in the Mid-Western area have been reported based on two categories, scenarios (default or ceiling) and transmission states (airborne or deposited). First, in the default scenario (Figure 5.4), deposited AI data show three remarkable results Iowa, Nebraska, and South Dakota farms have 14.8%, 11.3%, and 7.5% chance of being infected by AI viruses from the previously infected farm from the Mid-Western area. Minnesota, Missouri, Oklahoma, and Kansas farms have unremarkable percentages with the highest infection probability of 1.7% of having the chance to be infected from the Mid-Western area. Airborne AI data in the default scenario show low infection probability that Iowa farms, South Dakota farms, Missouri farms, and North Dakota farms have 2.1%, 1.2%, 1.2%, and 0.6% chance to be infected. Kansas, Minnesota, Nebraska, and Wisconsin have lower than 0.5% of chance that can be infected by AI.

The remaining results of ceiling scenarios (Figure 5.5) of airborne and deposited AI data show significantly high infection probabilities with the highest probability of 79.0% in South Dakota. However, the chance of the ceiling scenario happening is relatively low compared to the default scenario. The remarkable infection probabilities of deposited AI viruses imply that in the ceiling scenario, susceptible farms in South Dakota can be infected by AI viruses from previously infected farms with a probability up to approximately 79.0%.

### 5.3.4 Farm infection probabilities at different poultry house types

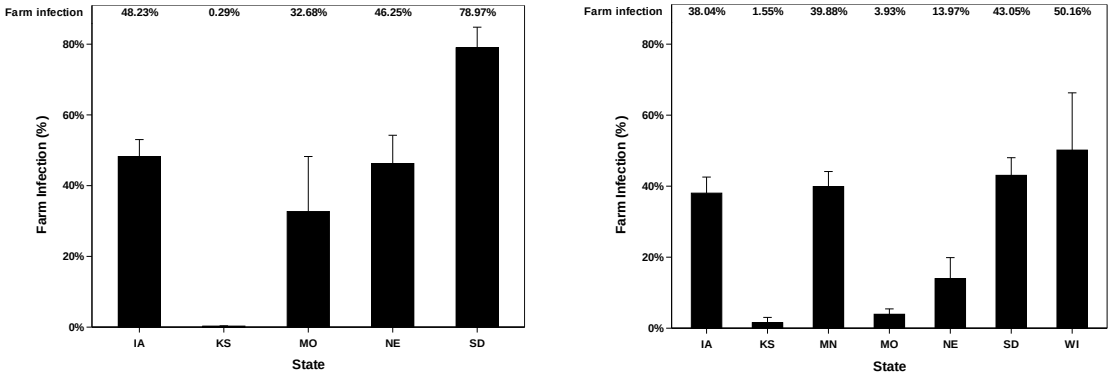
The farm infection probabilities of poultry farms in different poultry house types have been reported based on two categories, scenarios (default or ceiling) and transmission states (airborne or deposited). There were five poultry house types including breeder, broiler, laying hen, pullet, and turkey that were reported in this study. First, in the default scenario (Figure 5.6), deposited AI data show two remarkable results that laying hen farms have 11.9% (the infection probability was measured each day) and turkey farms have 10.3% probability of being infected by AI viruses from the previously infected farm from the Mid-Western area. Broiler, breeder, and pullet farms have unremarkable percentages with the highest infection probability of 1.7% having the chance to be infected by AI from the Mid-Western area. Airborne AI data in the default scenario show low infection probability that turkey farms have 1.4%, laying hen farms have 0.8%, and pullet farms have 0.2% of being infected.

The remaining results of ceiling scenarios (Figure 5.7) of airborne and deposited data show significantly high infection probabilities with the highest probability of 64.8% in turkey farms. However, the chance of the ceiling scenario happening is relatively low compared to the default scenario. In all scenarios, turkey farms are the poultry house type that has the highest chance to get infected by AI with one exception in the deposited default scenario.



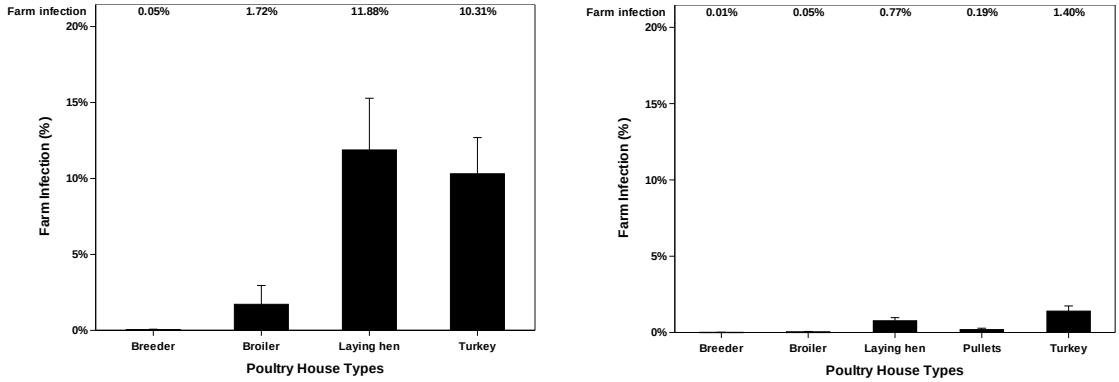

(a) (b)

Figure 5.4 Farm infection probabilities of highly pathogenic avian influenza (HPAI) viruses at the recipient farms in default scenario. The farm infection probabilities of avian influenza (AI) viruses are reported according to two categories (deposited and airborne). Farm infection probabilities of (a) deposited AI viruses in default scenario, %; and farm infection probabilities of (b) airborne AI viruses in default scenario, %. The error bar stands for standard deviations. X- axel abbreviations stand for states as follows IA (Iowa), KS (Kansas), MO (Missouri), NE (Nebraska), SD (South Dakota), and WI (Wisconsin).



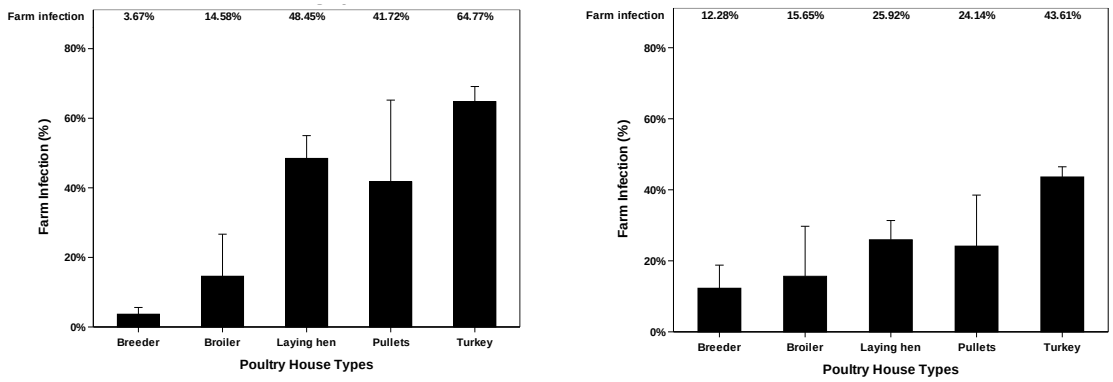

(a) (b)

Figure 5.5 Farm infection probabilities of highly pathogenic avian influenza (HPAI) viruses at the recipient farms in ceiling scenario. The farm infection probabilities of avian influenza (AI) viruses are reported according to two categories (deposited and airborne). Farm infection probabilities of (a) deposited AI viruses in ceiling scenario, %; and farm infection probabilities of (b) airborne AI viruses in ceiling scenario, %. The error bar stands for standard deviations. X- axel abbreviations stand for states as follows IA (Iowa), KS (Kansas), MO (Missouri), NE (Nebraska), SD (South Dakota), and WI (Wisconsin).




(a) (b)

Figure 5.6 Farm infection probabilities of highly pathogenic avian influenza (HPAI) viruses at different poultry house types in default scenario. The farm infection probabilities of avian influenza (AI) viruses are reported according to two categories (deposited and airborne). Farm infection probabilities of (a) deposited AI viruses in default scenario, %; and farm infection probabilities of (b) airborne AI viruses in default scenario, %. The error bar stands for standard deviations.




(a) (b)

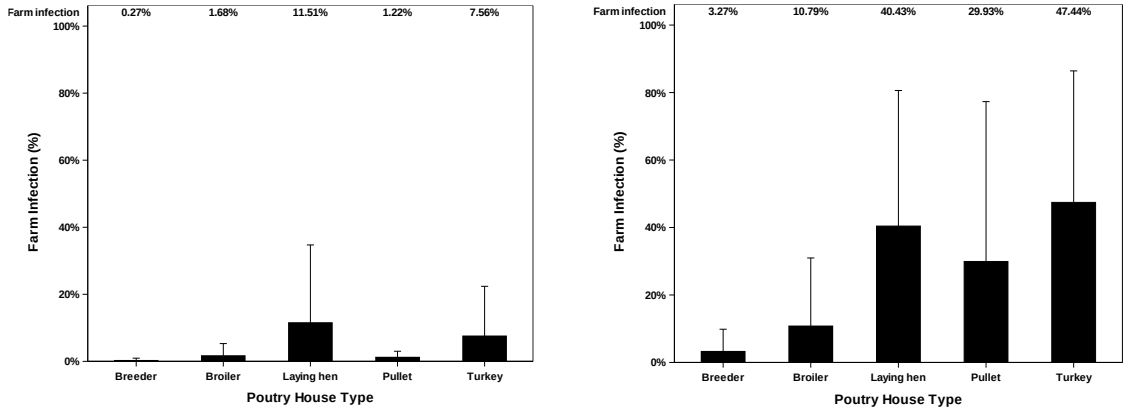
Figure 5.7 Farm infection probabilities of highly pathogenic avian influenza (HPAI) viruses at different poultry house types in ceiling scenario. The farm infection probabilities of avian influenza (AI) viruses are reported according to two categories (deposited and airborne). Farm infection probabilities of (a) deposited AI viruses in ceiling scenario, %; and farm infection probabilities of (b) airborne AI viruses in ceiling scenario, %. The error bar stands for standard deviations.

### 5.3.5 Farm infection probabilities caused by combined AI concentration

The farm infection probabilities caused by combined AI concentrations were reported in this study. The combined AI concentration is the combined concentration of airborne and deposited AI. The farm infection probabilities were reported based on different poultry house types (Figure 5.8) and different states (Figure 5.9). Airborne AI, after traveling for a long distance, gets in poultry facilities and stays both airs suspended and deposited. The combined AI concentration simulated a more accurate situation happening in poultry facilities. With the combined concentration of AI, the farm infection probabilities are still low in the default scenario with the highest farm infection probabilities in laying hen facilities (11.9%) and in IA state (12.9%). In the ceiling scenario, the highest farm infection probabilities are in turkey facilities (47.4%) and in Iowa state (47.8%).

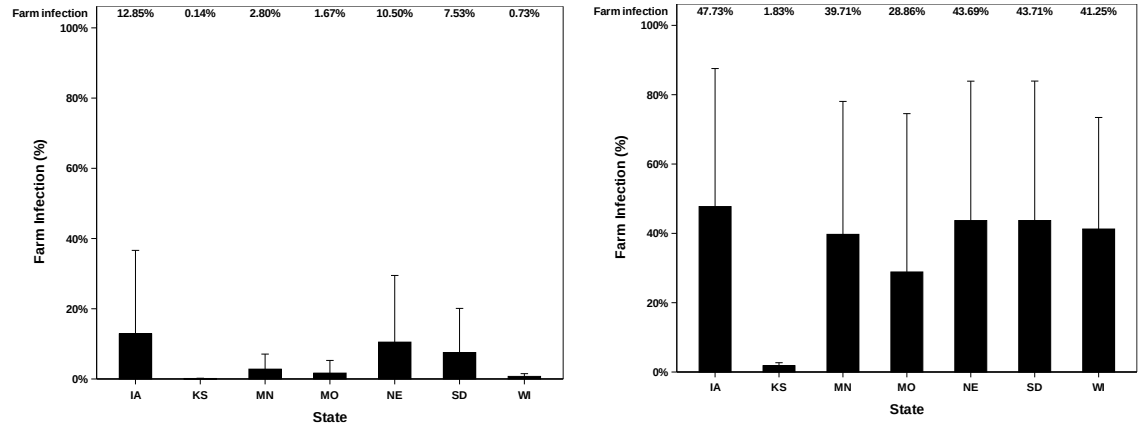
## 5.4 Discussion

In this study, the HYSPLIT model was used to simulate the long-distance airborne transmission of HPAI and to assess the risk of airborne and deposited AI carried by poultry-litter dust particles. The study reported the viral concentrations simulated by HYSPLIT modeling in AI-infected poultry houses. The resulting hypothetical virus carried by $PM_{2.5}$ concentrations generated by the HYSPLIT model was relatively low in all types of poultry farms. The concentration of airborne, deposited, and even combined concentration of airborne and deposited AI in these poultry farms are insignificant compared to the MIDa and MIDd values. Most poultry farms are hard to spread AI to surrounding poultry farms and causing infection. Compared to previous studies (Zhao et al., 2019), a similar conclusion was reported that the AI concentrations received by poultry farms from infected farms were relatively much lower than the minimal infective dose that a bird received. When traveling through the air for a long distance, the AI virus can be greatly affected by outdoor conditions. This causes them to be inactivated extremely quickly. A previous study (Shahid et al., 2009) reported that the AI virus could live for more than 100 days at 4°C but was inactivated after 24 hrs at 28°C and 30 minutes at 56°C. The outbreak of AI in 2022 happened in late spring and early summer when the temperature is typically above 25°C which can be a possible explanation for the short survival of the AI virus. The study also examined the farm infection probabilities at different farm locations. In the default scenario, when the conditions were more similar to the actual conditions, farm infection probabilities were generally low with the highest farm infection probabilities accounting for 14.8% in deposited AI and 2.1% in airborne AI. In the ceiling scenario, a remarkable farm infection probability was reported in South Dakota, which accounted for 79.0%. Although our results revealed that AI concentrations carried by $PM_{2.5}$ were much lower than MID, the farm infection data in the ceiling scenario showed a significantly high probability (79.0%) of farm infection. The ceiling scenario stands for the worst situation when all conditions are optimal for the infection of airborne AI. The chance of a ceiling scenario happening is low, and it does not represent the actual situation in real poultry farms.




a) (b)

Figure 5.8 Farm infection probabilities of combined concentration of highly pathogenic avian influenza (HPAI) viruses at the recipient farms at different poultry house types. Farm infection probabilities of (a) combined AI concentration in default scenario, %; and farm infection probabilities of (b) combined AI concentration in ceiling scenario, %. The error bar stands for standard deviations.




a) (b)

Figure 5.9 Farm infection probabilities of combined concentration of highly pathogenic avian influenza (HPAI) viruses at the recipient farms in different states. The farm infection probabilities of avian influenza (AI) viruses are reported according to two categories (default and ceiling). Farm infection probabilities of (a) combined AI concentration in default scenario, %; and farm infection probabilities of (b) combined AI concentration in ceiling scenario, %. The error bar stands for standard deviations. X- axel abbreviations stand for states as follows IA (Iowa), KS (Kansas), MO (Missouri), NE (Nebraska), SD (South Dakota), and WI (Wisconsin).

Farm infection probabilities at different poultry house types were calculated in this study. In most scenarios, the turkey farm type showed the highest chance of being infected by long-distance airborne transmission of AI, except in default deposited and default combined, where the turkey farm type has the second highest chance of being infected by AI. These results are consistent with the real statistical report of infected farms according to USDA APHIS (USDA-APHIS, 2022a). From February 08$^{th}$ to May 25$^{th}$, a total of 167 AI-infected cases over 125 infected cases are turkey farms compared to 42 cases are other poultry house types. The fact that the majority of turkey farms are infected by airborne AI can be explained by the ventilation rate (VR) of poultry farms. The typical ventilation rate for turkey is 9.2 $m^3$ $hrs^{-1}$ $bird^{-1}$, for broiler breeder is 7.8 $m^3$ $hrs^{-1}$ $bird^{-1}$, for broiler is 3.9 $m^3$ $hrs^{-1}$ $bird^{-1}$, for laying hen is 2.0 $m^3$ $hrs^{-1}$ $bird^{-1}$, and for pullet is 1.0 $m^3$ $hrs^{-1}$ $bird^{-1}$ (Al-Zaidi, 2022; Zhao et al., 2015; Zuidhof et al., 1993). The VR of turkey is higher than the VRs of other poultry house types. With the same number of birds and similar ventilation time, the turkey facilities get more $m^3$ air than other poultry house-type facilities. Thus, it increases the chance turkey farms get airborne AI transmitted from infected farms. In addition, the poultry housing structure of turkey farms makes the birds easier to expose to airborne AI. Turkey house type has an open sidewall structure which let the birds expose more to outside air (Emekli et al., 2012), while the laying hen house structure is closed and secured. With this open structure of the house, turkeys have more chance to receive airborne AI than laying hens.

In general, the deposited AI showed higher infection probabilities in all locations and all poultry house types. Deposited AI, after long-distance travel, can be deposited on any surface in the poultry farm and be picked up directly by birds. Meanwhile, airborne AI can infect birds by being inhaled by birds. Even though airborne AI can be suspended in poultry houses for long periods of time, the survivability may be compromised by the dehydration effect caused by the ventilation system which reduced the survivability of microorganisms in poultry facilities (Nguyen et al., 2022). On the other hand, deposited AI can stay longer in poultry facilities by depositing on poultry litter or bird feces. This environment can provide preferential culture conditions for deposited AI, thus, supporting the survival of deposited AI. These discussions are consistent with previous studies. In a study conducted by Baleshwari (Kurmi et al., 2013), authors reported that at 24°C, the viral concentration in feces would reduce only about 20% after 24 hrs. While other authors (Shaman & Kohn, 2009; Weber & Stilianakis, 2008) reported that human or pig-adapted influenza A virus subtypes showed a significant decrease (10-fold) in their viability in the air in 24 hrs. With better survivability, deposited AI had more chance to cause infection in birds rather than airborne AI. In addition, birds are often kept in confined spaces with limited ventilation and are in close contact with contaminated surfaces, feed, and water. The virus can then spread easily from bird to bird through contact with contaminated surfaces, feed, or water. In contrast, after getting in the poultry house, airborne AI requires the infected birds to be

in close proximity to one another and for the virus to remain airborne for a sufficient period of time.

In the study, the type of poultry house, flock size, and distance from infected farms to recipient farms were the factors, besides the concentration of virus, that play a crucial role in the probability of infection in poultry. Larger flocks were found to have a higher chance of infection, while smaller flocks were at low infection risk. For instance, turkey poultry facilities generally have the highest chance of becoming infected. Yet, in the default deposited scenario, even though fewer laying hen farms were affected by AI, the infection probabilities of these farms still showed a higher chance than those of turkeys. This could be explained by that in this scenario, most of the infected laying hen farms were located in Iowa and Nebraska and had large ranges of flock sizes. The smallest flock had 915,900 birds, while the largest had 5,011,700 birds (USDA-APHIS, 2022b). These flock sizes were significantly larger than those in turkey facilities. The study also found that as the distance from a source farm increases, the concentration of the virus decreases, reducing the probability of airborne infection. For instance, in the deposited ceiling scenario, the South Dakota farm infection probabilities were significantly high (about 79.0%). The infected and recipient farms were close to each other and mainly located in South Dakota, which made the recipient farms highly susceptible to the AI virus from infected farms.

Although utilizing the HYSPLIT model can address the risk of long-distance travel of AI, this is a meteorological model which mainly focuses on the effects of meteorological factors on the virus. Some other factors including the interaction between dust particles or between dust particles and viruses, wild birds, humans, vehicles, and so on, were excluded from the model. In addition, the minimum accuracy distance of the model was 12 km, thus any farms within 12 km were not well simulated in the model. The HYSPLIT model helps in understanding the possibility and risk of airborne transmission in different locations as well as different poultry house types, but confirmed airborne transmission cannot be concluded without genetic analysis. In addition, the study reported the AI levels found in the recipient farms. The model predicted airborne viral concentrations of less than $10^{-3.5}$ $EID_{50}$ $m^{-3}$ in the default scenario, both for airborne and deposited AI. However, in real-life poultry houses, there is no such air sampler that can accurately collect viable airborne and deposited AI. As a result, it is challenging to confirm the accuracy of the meteorology model, including HYSPLIT. As a result, utilizing HYSPLIT may be a more sensitive method to explain and predict the potential outbreak of AI caused by airborne transmission. Finally, the findings of the infection probabilities in various poultry house types and across different states can provide a comprehensive understanding of which poultry house types have a high chance of being exposed to airborne avian influenza, thereby allowing for proper preventive measures to be taken.

## 5.5 Conclusions

This study used the HYSPLIT model to simulate the long-distance airborne transmission of highly pathogenic avian influenza (HPAI) and assessed the risk of airborne and deposited AI carried by poultry-litter dust particles. The study found that viral concentrations simulated by the HYSPLIT model were relatively low in all types of poultry farms and the concentration of airborne, deposited, and even combined AI in these farms were insignificant compared to their minimum infective doses. The study showed that the turkey farm type had the highest chance of being infected by long-distance airborne transmission of AI due to its high ventilation rate and open sidewall structure. Additionally, the deposited AI showed higher infection probabilities in all locations and all poultry house types due to their longer survivability on contaminated surfaces, feed, and water. Overall, the findings suggest that the predicted concentrations were below the minimal infective dose of poultry; however, the probability of airborne infection could still be high at sites with large bird populations. The results also highlight the importance of managing the deposition of AI in poultry facilities to prevent infection.

# CHAPTER VI

# Discussions and Conclusions

## 6.1 Sampling Method for Dry Aerosolization Condition

Poultry industry is a dusty environment where the dust particles can be generated and aerosolized into the air by the bird activities. Poultry manure, which carries many types of microorganisms including *E. coli* and AI, can be deposited onto the poultry litter and then be aerosolized with poultry dust particles by attaching to them. Besides, many wet aerosolization methods were previously studied to assess the samplers' performance, but the carrier aerosol in the poultry house may primarily consist of dried dust particles which were not widely studied. To have better understanding about the transmission mechanism, selecting an optimal sampling method is crucial objective to study about airborne transmission of pathogens in poultry industry under dry aerosolization conditions. Among three commonly used sampler for aerosols including Andersen six-stage impactor (Andersen impactor), all-glass impinger (AGI-30) and ACD-200 Bobcat (Bobcat), the Andersen impactor and AGI-30 outperformed the Bobcat, implying that they are suitable for collecting aerosols under dry aerosolization condition. However, this study simulated the poultry indoor environment with high concentrations of dust and airborne *E. coli*. At low concentrations of dust and airborne *E. coli*, the performance of the three samplers may be different. Andersen impactor and AGI-30 operated at 28.3 and 12.5 L $min^{-1}$ airflow rates which were much lower than Bobcat (200 L $min^{-1}$). With the much higher airflow rate, Bobcat yielded 0.005 CFU $L^{-1}$ $min^{-1}$ minimal detection limit which represented for the sensitivity of sampler. The lower minimal detection limit is, the more sensitive sampler is. Compared to Andersen impactor (0.08 CFU $L^{-1}$ $min^{-1}$ minimal detection limit) and AGI-30 (0.035 CFU $L^{-1}$ $min^{-1}$ minimal detection limit), the Bobcat seems to be suitable for low dust and airborne *E. coli* concentration condition because of its low minimal detection limit. This requires further investigation to confirm the effect of dust and airborne *E. coli* on the performance of the three samplers.

## 6.2 Half-life Time of Airborne Pathogens

The half-life time of pathogens refers to the time it takes for half of the initial population of pathogens to decay or become inactive. This concept is commonly used in microbiology and environmental science to describe the persistence or survival of pathogens in different environments. The half-life time can vary depending on the specific pathogen, the environmental conditions (such as temperature, humidity, and sunlight), and the type of

surface or material that the pathogen is on. Understanding the half-life time of pathogens is important in evaluating the risks of exposure and developing strategies for controlling or mitigating their spread. Especially with the rapid development of technologies, there are many computational models that are applied to simulate the movement of pathogens in the environmental conditions. The half-life time is an important indicator which governs the transmission range of airborne pathogens. Determining half-life time of pathogens can provide more information to support further studies. In this study, the poultry litter went through an autoclave process which may affect the quality of poultry litter and produce Maillard reaction product. The Maillard reaction products were proven to inhibit growth of bacteria. However, the effect of the preparation procedure was not well-studied in the present study. Therefore, this effect still needs further investigation.

## 6.3 Mitigation Technologies

The poultry industry has been facing challenges in controlling the spread of airborne pathogens including *E. coli* and AI, which can lead to significant economic losses and potential public health risks. To address this issue, various mitigation technologies have been developed and implemented.ne approach is to improve ventilation systems in poultry houses. Properly designed and maintained ventilation systems can help to reduce the concentration of airborne pathogens by increasing the air exchange rate and removing contaminated air. This can be achieved through the use of fans, air inlets, and exhaust vents. Additionally, the installation of air filters can further reduce the number of airborne pathogens, although the effectiveness of this approach may vary depending on the type and efficiency of the filters used. Physical deposition and biological inactivation are two methods that can reduce the concentration of airborne pathogens. Electrostatic particulate ionization (EPI) technology has been effective in reducing the transmission of *Salmonella* and AI in poultry houses. Ultraviolet (UV) radiation can directly kill pathogens. In terms of cost, the UV method seems to be widely used with more affordable cost compared to EPI. To apply the UV system into poultry housing system, the durability of the system needs to be considered. With dusty environment, the UV lamps can be covered by dust, and thus, compromise the effectiveness of UV irradiance. This issue can be solved by measuring the time intervals for system maintenance, for instances, cleaning UV lamps or replace UV bulbs. Further investigation needs to be done to determine these time intervals.

## 6.4 Computational Modeling

Computational modeling is a viable tool for simulating disease spread in the poultry business. Computer modeling allows researchers to explore the complicated connections between environmental parameters such as ventilation rate, air temperature, and humidity, and the concentration of airborne pathogens. Computational models can replicate the behavior of airborne viruses and their transport dynamics in poultry houses using precise and extensive input data. These models may be used to determine crucial parameters that drive disease transmission, such as the dispersion of infectious particles, the number of

vulnerable birds, and bird-to-bird contact rates. Moreover, computer modeling can forecast the efficiency of mitigation methods such as air filtration or UV irradiation in lowering airborne pathogen concentrations. the Hybrid Single-Particle Lagrangian Integrated Trajectory model (HYSPLIT) is a computer model used to compute air parcel trajectories, which predict how far and in which direction a parcel of air, and hence air pollutants, will move. HYSPLIT may also estimate air pollutant dispersion, chemical transformation, and deposition. To determine the possibility of long-distance airborne transmission of pathogens carried by poultry dust particles, utilizing HYSPLIT model can simulate the movement of pathogens and so can assess the risk of airborne and deposited pathogens carried by poultry litter dust particles. Compared to other models such as Computational fluid dynamics (CFD) which is also able to simulate the flow of the air, the advantage of HYSPLIT is to integrate the meteorological data into the model which improves the accuracy of the simulation. However, the computational model based on the average parameter inputs which might not represent for the actual situation happening in some farms. For instance, in the study, the concentration of airborne AI emitted from previous infected farms were calculated based on average $PM_{2.5}$ emission rate, density, percentage of manure in dust, and flock size which might not represent for some certain situations. Therefore, further studies need to be done to have a better understanding of AI emission concentrations.

## 6.5 Future Study

To have better understanding of long-distance transmission of AI carried by dust particles in poultry industry, the adhesion ability of HPAI to fine dust particles is an important indicator governing the distance of movement. The adhesion ability can be studied by applying Molecular Dynamics (MD) simulation to simulate the interaction between AI and particles' surfaces. Besides, the effect of farm size and distance between farms on the infection probability also needs to be assessed.

# LIST OF REFERENCES


Aarnink, A. J. A., Roelofs, P., Ellen, H., Gunnink, H. (1999). *Dust sources in animal houses.* Paper presented at the Proceedings of the International Symposium on Dust Control in Animal Production Facilities, 30 May-2 June, Aarhus (D).

Agranovski, I. E., Agranovski, V., Grinshpun, S. A., Reponen, T., Willeke, K. (2002). Collection of airborne microorganisms into liquid by bubbling through porous medium. *Aerosol Science Technology, 36*(4), 502-509. doi:https://doi.org/10.1080/027868202753571322

Al-Zaidi, A. A. M. A. (2022). Ventilation system design for poultry buildings. *Net Journal of Agricultural Science, 10*(1), 37-42. doi: 10.30918/NJAS.101.22.008

Andersen, A. A. (1958). New sampler for the collection, sizing, and enumeration of viable airborne particles. *Journal of Bacteriology, 76*(5), 471-484. doi:10.1128/jb.76.5.471-484.1958

Bartz, B. M., Anderson, K. A., Oviedo-Rondón, E. O., Livingtson, K., Grimes, J. L. (2020). Effects of stocking density on large white, commercial tom turkeys reared to 20 weeks of age: 1. growth and performance. *Poultry Science, 99*(11), 5582-5586. doi:10.1016/j.psj.2020.08.024

Bertran, K., Balzli, C., Kwon, Y.-K., Tumpey, T. M., Clark, A., Swayne, D. E. (2017a). Airborne transmission of highly pathogenic influenza virus during processing of infected poultry. *Emerging Infectious Diseases, 23*(11), 1806. doi:10.3201/eid2311.170672

Bertran, K., Balzli, C., Kwon, Y. K., Tumpey, T. M., Clark, A., Swayne, D. E. (2017b). Airborne Transmission of Highly Pathogenic Influenza Virus during Processing of Infected Poultry. *Emerging Infectious Diseases, 23*(11), 1806-1814. doi:10.3201/eid2311.170672

Bhattacharjee, M. K., Sugawara, K., Ayandeji, O. T. (2009). Microwave sterilization of growth medium alleviates inhibition of Aggregatibacter actinomycetemcomitans by Maillard reaction products. *Journal of Microbiological Methods, 78*(2), 227-230. doi:10.1016/j.mimet.2009.06.004

Cambra-López, M., Aarnink, A. J. A., Zhao, Y., Calvet, S., Torres, A. G. (2010). Airborne particulate matter from livestock production systems: A review of an air pollution problem. *Environmental Pollution, 158*(1), 1-17. doi:https://doi.org/10.1016/j.envpol.2009.07.011

Carpenter, G. A. (1986). Dust in livestock buildings—review of some aspects. *Journal of Agricultural Engineering Research, 33*(4), 227-241. doi:https://doi.org/10.1016/S0021-8634(86)80038-5

Chan, W. L., Chung, W. T., Ng, T. W. (2019). Airborne Survival of *Escherichia coli* under Different Culture Conditions in Synthetic Wastewater. *International Journal of Environmental Research and Public Health, 16*(23), 4745. doi:https://doi.org/10.3390/ijerph16234745

Chinivasagam, H. N., Tran, T., Maddock, L., Gale, A., Blackall, P. J. (2009). Mechanically ventilated broiler sheds: a possible source of aerosolized *Salmonella, Campylobacter*, and *Escherichia coli*. *Applied and Environmental Microbiology, 75*(23), 7417-7425. doi:10.1128/AEM.01380-09

Crane, S. R., Moore, J. A., Grismer, M. E., Miner, J. R. (1983). Bacterial pollution from agricultural sources: a review. *Transactions of the ASAE, 26*(3), 858-0866. doi:doi: 10.13031/2013.34036

Davis, M., Morishita, T. Y. (2005). Relative ammonia concentrations, dust concentrations, and presence of *Salmonella* species and *Escherichia coli* inside and outside commercial layer facilities. *Avian Diseases, 49*(1), 30-35. doi:https://doi.org/10.1637/0005-2086(2005)49[30:RACDCA]2.0.CO;2

DeJesus, E., Costa-Hurtado, M., Smith, D., Lee, D. H., Spackman, E., Kapczynski, D. R., . . . Pantin-Jackwood, M. J. (2016). Changes in adaptation of H5N2 highly pathogenic avian influenza H5 clade 2.3.4.4 viruses in chickens and mallards. *Virology, 499*, 52-64. doi:10.1016/j.virol.2016.08.036

Dho-Moulin, M., Fairbrother, J. M. (1999). Avian pathogenic *Escherichia coli* (APEC). *Veterinary Research, 30*(2-3), 299-316.

Duan, H., Chai, T., Cai, Y., Zhong, Z., Yao, M., Zhang, X. (2008). Transmission identification of *Escherichia coli* aerosol in chicken houses to their environments using ERIC-PCR. *Science in China Series C: Life Sciences, 51*(2), 164-173. doi:10.1007/s11427-008-0021-0

Edwards, D. A., Man, J. C., Brand, P., Katstra, J. P., Sommerer, K., Stone, H. A., . . . Scheuch, G. (2004). Inhaling to mitigate exhaled bioaerosols. *Proceedings of the National Academy of Sciences, 101*(50), 17383-17388.

Ellen, H. H., Bottcher, R. W., Von Wachenfelt, E., Takai, H. (2000). Dust levels and control methods in poultry houses. *Journal of Agricultural Safety Health, 6*(4), 275. doi:10.13031/2013.1910

Emekli, N. Y., Caglayan, N., Ozmerzi, A., Canakci, M. (2012). Structural characteristics and heat requirements of modern greenhouses in southern of Turkey. *Akdeniz Üniversitesi Ziraat Fakültesi Dergisi, 25*(2), 93-101.

Fancher, C. A., Zhang, L., Kiess, A. S., Adhikari, P. A., Dinh, T. T., Sukumaran, A. T. (2020). Avian pathogenic *Escherichia coli* and Clostridium perfringens: Challenges in no antibiotics ever broiler production and potential solutions. *Microorganisms, 8*(10), 1533. doi:https://doi.org/10.3390/microorganisms8101533

Fathi, S., Hajizadeh, Y., Nikaeen, M., Gorbani, M. (2017). Assessment of microbial aerosol emissions in an urban wastewater treatment plant operated with activated sludge process. *Aerobiologia, 33*(4), 507-515. doi:https://doi.org/10.1007/s10453-017-9486-2

Fernandez, M. O., Thomas, R. J., Garton, N. J., Hudson, A., Haddrell, A., Reid, J. P. (2019). Assessing the airborne survival of bacteria in populations of aerosol droplets with a novel technology. *Journal of the Royal Society Interface, 16*(150), 20180779. doi:https://doi.org/10.1098/rsif.2018.0779

Forrest, H. L., Kim, J.-K., Webster, R. G. (2010). Virus shedding and potential for interspecies waterborne transmission of highly pathogenic H5N1 influenza virus in sparrows and chickens. *Journal of Virology, 84*(7), 3718-3720. doi: https://doi.org/10.1128/JVI.02017-09

Gravimetric-CEN. (2005). Measurement Method for the Determination of the PM 2.5 Mass Fraction of Suspended Particulate Matter. *European. BSI Group*.

Gray, J. T., Fedorka-Cray, P. J. (2001). Survival and infectivity of *Salmonella* choleraesuis in swine feces. *Journal of Food Protection, 64*(7), 945-949. doi: https://doi.org/10.4315/0362-028X-64.7.945

Griffin, D. W., Gonzalez, C., Teigell, N., Petrosky, T., Northup, D. E., Lyles, M. (2011). Observations on the use of membrane filtration and liquid impingement to collect airborne microorganisms in various atmospheric environments. *Aerobiologia, 27*, 25-35. doi:https://doi.org/10.1007/s10453-010-9173-z

Guerrero-Beltr· n, J., Barbosa-C· novas, G. (2004). Advantages and limitations on processing foods by UV light. *Food Science Technology International, 10*(3), 137-147. doi:https://doi.org/10.1177/1082013204044359

Guo, B., Ghalambor, A. (2005). Chapter 11 - Transportation. In B. Guo A. Ghalambor (Eds.), *Natural Gas Engineering Handbook (Second Edition)* (pp. 219-262): Gulf Publishing Company.

Hasan, B., Faruque, R., Drobni, M., Waldenström, J., Sadique, A., Ahmed, K. U., . . . Alam, M. (2011). High prevalence of antibiotic resistance in pathogenic *Escherichia coli* from large-and small-scale poultry farms in Bangladesh. *Avian Diseases, 55*(4), 689-692. doi:10.1637/9686-021411-Reg.1

Hayter, R., Besch, E. (1974). Airborne-particle deposition in the respiratory tract of chickens. *Poultry Science, 53*(4), 1507-1511. doi:https://doi.org/10.3382/ps.0531507

Hijnen, W. A. M., Beerendonk, E. F., Medema, G. J. (2006). Inactivation credit of UV radiation for viruses, bacteria and protozoan (oo) cysts in water: a review. *Water Research, 40*(1), 3-22. doi:https://doi.org/10.1016/j.watres.2005.10.030

Hoeksma, P., Aarnink, A. J. A., Ogink, N. W. M. (2015). *Effect of temperature and relative humidity on the survival of airborne bacteria = Effect van temperatuur en relatieve luchtvochtig-heid op de overleving van bacteriën in de lucht* (1570-8616). Retrieved from Wageningen : Wageningen UR Livestock Research (Livestock Research report 864) - 27: https://edepot.wur.nl/348736

Hu, W., Murata, K., Fan, C., Huang, S., Matsusaki, H., Fu, P., Zhang, D. (2020). Abundance and viability of particle-attached and free-floating bacteria in dusty and nondusty air. *Biogeosciences, 17*(17), 4477-4487. doi:https://doi.org/10.5194/bg-17-4477-2020

Just, N. A., Létourneau, V., Kirychuk, S. P., Singh, B., Duchaine, C. (2012). Potentially pathogenic bacteria and antimicrobial resistance in bioaerosols from cage-housed and floor-housed poultry operations. *Annals of Occupational Hygiene, 56*(4), 440-449. doi:https://doi.org/10.1093/annhyg/mer105

Kenny, L. C., Gussman, R., Meyer, M. (2000). Development of a sharp-cut cyclone for ambient aerosol monitoring applications. *Aerosol Science & Technology, 32*(4), 338-358. doi:https://doi.org/10.1080/027868200303669

Ketkar, G. N., Malaiappan, S., Muralidharan, N. (2020). Comparative Evaluation of Inherent Antimicrobial Properties and Bacterial Surface Adherence between Copper and Stainless Steel Suction Tube. *Journal of Pharmaceutical Research International*, 149-156. doi:10.9734/jpri/2020/v32i1930721

Krause, E. T., Schrader, L. (2019). Suggestions to Derive Maximum Stocking Densities for Layer Pullets. *Animals (Basel), 9*(6). doi:10.3390/ani9060348

Kurmi, B., Murugkar, H. V., Nagarajan, S., Tosh, C., Dubey, S. C., Kumar, M. (2013). Survivability of Highly Pathogenic Avian Influenza H5N1 Virus in Poultry Faeces at Different Temperatures. *Indian Journal of Virology, 24*(2), 272-277. doi:10.1007/s13337-013-0135-2

Kwon, K. S., Lee, I. B., Ha, T. (2016). Identification of key factors for dust generation in a nursery pig house and evaluation of dust reduction efficiency using a CFD technique. *Biosystems Engineering, 151*, 28-52. doi:https://doi.org/10.1016/j.biosystemseng.2016.08.020

Lan, X., Liu, P., Xia, S., Jia, C., Mukunzi, D., Zhang, X., . . . Xiao, Z. (2010). Temperature effect on the non-volatile compounds of Maillard reaction products derived from xylose–soybean peptide system: Further insights into thermal degradation and cross-linking. *Food Chemistry, 120*(4), 967-972. doi:doi.org/10.1016/j.foodchem.2009.11.033

Lay Jr, D. C., Fulton, R. M., Hester, P. Y., Karcher, D. M., Kjaer, J. B., Mench, J. A., . . . O'Sullivan, N. P. (2011). Hen welfare in different housing systems. *Poultry Science, 90*(1), 278-294. doi:https://doi.org/10.3382/ps.2010-00962

Li, H., Xin, H., Burns, R. T., Jacobson, L. D., Noll, S., Hoff, S. J., . . . Hetchler, B. P. (2011). Air emissions from tom and hen turkey houses in the US Midwest. *Transactions of the ASABE, 54*(1), 305-314. doi:10.13031/2013.36258

Li, M., Qiang, Z., Bolton, J. R. (2013). In situ detailed fluence rate distributions in a UV reactor with multiple low-pressure lamps: Comparison of experimental and model results. *Chemical Engineering Journal, 214*, 55-62. doi:https://doi.org/10.1016/j.cej.2012.10.024

Lighthart, B., Shaffer, B. T. (1997). Increased airborne bacterial survival as a function of particle content and size. *Aerosol Science Technology, 27*(3), 439-446. doi:https://doi.org/10.1080/02786829708965483

Lin, J. J., Noll, K. E., Holsen, T. M. (1994). Dry deposition velocities as a function of particle size in the ambient atmosphere. *Aerosol Science and Technology, 20*(3), 239-252.

Lundholm, I. M. (1982). Comparison of methods for quantitative determinations of airborne bacteria and evaluation of total viable counts. *Applied and Environmental Microbiology, 44*(1), 179-183. doi:10.1128/AEM.44.1.179-183.1982

Lutful Kabir, S. M. (2010). Avian colibacillosis and salmonellosis: a closer look at epidemiology, pathogenesis, diagnosis, control and public health concerns.

*International Journal of Environmental Research and Public Health, 7*(1), 89-114. doi:10.3390/ijerph7010089

Martin, S. A., McCann, M. A., Waltman II, W. D. (1998). Microbiological survey of Georgia poultry litter. *Journal of Applied Poultry Research, 7*(1), 90-98. doi:https://doi.org/10.1093/japr/7.1.90

McLeod, A., Hovde Liland, K., Haugen, J. E., Sørheim, O., Myhrer, K. S., Holck, A. L. (2018). Chicken fillets subjected to UV-C and pulsed UV light: Reduction of pathogenic and spoilage bacteria, and changes in sensory quality. *Journal of Food Safety, 38*(1), e12421. doi:10.1111/jfs.12421

Mellata, M. (2013). Human and avian extraintestinal pathogenic *Escherichia coli*: infections, zoonotic risks, and antibiotic resistance trends. *Foodborne Pathogens Disease, 10*(11), 916-932. doi:https://doi.org/10.1089/fpd.2013.1533

Milne, A., Theodorou, M. K., Jordan, M. G. C., King-Spooner, C., Trinci, A. P. J. (1989). Survival of anaerobic fungi in feces, in saliva, and in pure culture. *Experimental Mycology, 13*(1), 27-37. doi:https://doi.org/10.1016/0147-5975(89)90005-4

Mubiru, D., Coyne, M. S., Grove, J. H. (2000). *Escherichia coli* pathogen O157: H7 does not survive longer in soil than a nonpathogenic fecal coliform. *Agronomy Notes, 32*, 3.

Müller, W., Wieser, P. (1987). Dust and microbial emissions from animal production. *World Animal Science (Netherlands)*.

Munoz, L. R., Pacheco, W. J., Hauck, R., Macklin, K. S. (2021). Evaluation of commercially manufactured animal feeds to determine presence of *Salmonella*, *Escherichia coli*, and Clostridium perfringens. *Journal of Applied Poultry Research, 30*(2), 100142. doi:https://doi.org/10.1016/j.japr.2021.100142

Neher, D. A., Cutler, A. J., Weicht, T. R., Sharma, M., Millner, P. D. (2019). Composts of poultry litter or dairy manure differentially affect survival of enteric bacteria in fields with spinach. *Journal of Applied Microbiology, 126*(6), 1910-1922. doi: https://doi.org/10.1111/jam.14268

Nguyen, X. D., Zhao, Y., Evans, J. D., Lin, J., Purswell, J. L. (2022). Survival of *Escherichia coli* in Airborne and Settled Poultry Litter Particles. *Animals, 12*(3), 284. doi:https://doi.org/10.3390/ani12030284

Nguyen, X. D., Zhao, Y., Evans, J. D., Lin, J., Schneider, L., Voy, B., . . . Purswell, J. L. (2022). Evaluation of Bioaerosol Samplers for Airborne *Escherichia Coli* Carried by Poultry Litter Particles. *Journal of the ASABE, 65*(4), 825-833. doi:10.13031/ja.15057

Nguyen, X. D., Zhao, Y., Evans, J. D., Lin, J., Schneider, L., Voy, B., . . . Purswell, J. L. (2021a). *Evaluation of bioaerosol samplers for collecting airborne E. coli carried by dust particles from poultry litter*. Paper presented at the 2021 ASABE Annual International Virtual Meeting.

Nguyen, X. D., Zhao, Y., Evans, J. D., Lin, J., Schneider, L., Voy, B., . . . Purswell, J. L. (2021b). *Evaluation of bioaerosol samplers for collecting airborne E. coli carried by dust particles from poultry litter.* Paper presented at the 2021 ASABE Annual International Virtual Meeting.

Ochoa-Velasco, C. E., Ávila-Sosa, R., Hernández-Carranza, P., Ruíz-Espinosa, H., Ruiz-López, I. I., Guerrero-Beltrán, J. Á. (2020). Mathematical modeling used to evaluate the effect of UV-C light treatment on microorganisms in liquid foods. *Food Engineering Reviews, 12*(3), 290-308. doi:https://doi.org/10.1007/s12393-020-09219-y

Omisakin, F., MacRae, M., Ogden, I. D., Strachan, N. J. C. (2003). Concentration and prevalence of *Escherichia coli* O157 in cattle feces at slaughter. *Applied and Environmental Microbiology, 69*(5), 2444-2447. doi:https://doi.org/10.1128/AEM.69.5.2444-2447.2003

Petersen, F., Hubbart, J. A. (2020). Physical Factors Impacting the Survival and Occurrence of *Escherichia coli* in Secondary Habitats. *Water, 12*(6), 1796. doi:https://doi.org/10.3390/w12061796

Pitout, J. D. (2012). Extraintestinal Pathogenic *Escherichia coli*: A Combination of Virulence with Antibiotic Resistance. *Front Microbiol, 3*, 9. doi:10.3389/fmicb.2012.00009

Pourbakhsh, S. A., Boulianne, M., Martineau-Doizé, B., Dozois, C. M., Desautels, C., Fairbrother, J. M. (1997). Dynamics of *Escherichia coli* infection in experimentally inoculated chickens. *Avian Diseases*, 221-233. doi:https://doi.org/10.2307/1592463

Ramos, S., MacLachlan, M., Melton, A. (2017). *Impacts of the 2014-2015 Highly Pathogenic Avian Influenza Outbreak on the U.S. Poultry Sector*. Retrieved from Economic Research Service: https://www.ers.usda.gov/webdocs/outlooks/86282/ldpm-282-02.pdf?v=539#:~:text=HPAI%20resulted%20in%20substantially%20lower,which%20further%20reduced%20export%20volume.

Rana, M. D., Campbell, D. L. M. (2021). Application of ultraviolet light for poultry production: a review of impacts on behavior, physiology, and production. *Frontiers in Animal Science*, 39. doi:https://doi.org/10.3389/fanim.2021.699262

Raynor, P. C., Adesina, A., Aboubakr, H. A., Yang, M., Torremorell, M., Goyal, S. M. (2021). Comparison of samplers collecting airborne influenza viruses: 1. Primarily impingers and cyclones. *PLoS One, 16*(1), e0244977. doi:10.1371/journal.pone.0244977

Riley, M. (1999). *Correlates of smallest sizes for microorganisms.* Paper presented at the Size Limits of Very Small Microorganisms Proceedings of a Workshop.

Rosenthal, E., Schneider, T., Büscher, W., Diekmann, B. (2007). Sedimentation von tierartspezifischen Stäuben im Stallinnenraum. *Landtechnik, 62*(2), 102-103.

Ruiz-Gil, T., Acuna, J. J., Fujiyoshi, S., Tanaka, D., Noda, J., Maruyama, F., Jorquera, M. A. (2020). Airborne bacterial communities of outdoor environments and their associated influencing factors. *Environment International, 145*, 106156. doi:https://doi.org/10.1016/j.envint.2020.106156

Saif, Y. M., Barnes, H., Glisson, J. R., Fadly, A. M., McDougald, L. R., Swayne, D. E. (2008). Diseases of poultry. 12. *Ames, Iowa: Blackwell Pub Professional*, 452-514.

Sanz, S., Olarte, C., Hidalgo-Sanz, R., Ruiz-Ripa, L., Fernández-Fernández, R., García-Vela, S., . . . Torres, C. (2021). Airborne Dissemination of Bacteria (Enterococci, Staphylococci and Enterobacteriaceae) in a Modern Broiler Farm and Its Environment. *Animals, 11*(6), 1783. doi:https:// doi.org/10.3390/ani11061783

Schulz, J., Ruddat, I., Hartung, J., Hamscher, G., Kemper, N., Ewers, C. (2016). Antimicrobial-resistant *Escherichia coli* survived in dust samples for more than 20 years. *Frontiers in Microbiology, 7*, 866. doi:https://doi.org/10.3389/fmicb.2016.00866

Seedorf, J., Hartung, J., Schröder, M., Linkert, K. H., Phillips, V. R., Holden, M. R., . . . Pedersen, S. (1998). Concentrations and emissions of airborne endotoxins and microorganisms in livestock buildings in Northern Europe. *Journal of Agricultural Engineering Research, 70*(1), 97-109.

Shahid, M. A., Abubakar, M., Hameed, S., Hassan, S. (2009). Avian influenza virus (H5N1); effects of physico-chemical factors on its survival. *Virology Journal, 6*(1), 38. doi:10.1186/1743-422X-6-38

Shaman, J., Kohn, M. (2009). Absolute humidity modulates influenza survival, transmission, and seasonality. *Proceedings of the National Academy of Sciences, 106*(9), 3243-3248.

Shepherd, T. A., Zhao, Y., Li, H., Stinn, J. P., Hayes, M. D., Xin, H. (2015). Environmental assessment of three egg production systems—Part II. Ammonia, greenhouse gas, and particulate matter emissions. *Poultry Science, 94*(3), 534-543. doi:https://doi.org/10.3382/ps/peu075

Soliman, E. S., Sallam, N. H., Abouelhassan, E. M. (2018). Effectiveness of poultry litter amendments on bacterial survival and Eimeria oocyst sporulation. *Veterinary World, 11*(8), 1064. doi:10.14202/vetworld.2018.1064-1073

Soupir, M., Mostaghimi, S., Yagow, E., Hagedorn, C., Vaughan, D. (2006). Transport of fecal bacteria from poultry litter and cattle manures applied to pastureland. *Water, Air, Soil Pollution, 169*(1), 125-136. doi:https://doi.org/10.1007/s11270-006-1808-x

Spackman, E., Pantin-Jackwood, M. J., Kapczynski, D. R., Swayne, D. E., Suarez, D. L. (2016). H5N2 Highly Pathogenic Avian Influenza Viruses from the US 2014-2015 outbreak have an unusually long pre-clinical period in turkeys. *BMC Veterinary Research, 12*(1), 260. doi:10.1186/s12917-016-0890-6

Ssematimba, A., Hagenaars, T. J., De Jong, M. C. (2012). Modelling the wind-borne spread of highly pathogenic avian influenza virus between farms. *PLoS One, 7*(2), e31114. doi:https://doi.org/10.1371/journal.pone.0031114

Stęczny, K., Kokoszyński, D. (2021). Effect of probiotic preparations (EM) on productive characteristics, carcass composition, and microbial contamination in a commercial broiler chicken farm. *Animal Biotechnology, 32*(6), 758-765. doi:https://doi.org/10.1080/10495398.2020.1754841

Stern, R. A., Mahmoudi, N., Buckee, C. O., Schartup, A. T., Koutrakis, P., Ferguson, S. T., . . . Sunderland, E. M. (2021). The Microbiome of Size-Fractionated Airborne Particles from the Sahara Region. *Environmental Science & Technology, 55*(3), 1487-1496. doi:https://doi.org/10.1021/acs.est.0c06332

Swayne, D. E., Suarez, D. L. (2000). Highly pathogenic avian influenza. *Revue Scientifique et Technique-office International des Epizooties, 19*(2), 463-475. doi:10.20506/rst.19.2.1230

Terzich, M., Pope, M. J., Cherry, T. E., Hollinger, J. (2000). Survey of pathogens in poultry litter in the United States. *Journal of Applied Poultry Research, 9*(3), 287-291. doi:https://doi.org/10.1093/japr/9.3.287

Theofel, C. G., Williams, T. R., Gutierrez, E., Davidson, G. R., Jay-Russell, M., Harris, L. J., Schaffner, D. W. (2020). Microorganisms Move a Short Distance into an Almond Orchard from an Adjacent Upwind Poultry Operation. *Applied and Environmental Microbiology, 86*(15), e00573-00520. doi:doi:10.1128/AEM.00573-20

Thorne, P. S., Kiekhaefer, M. S., Whitten, P., Donham, K. J. (1992). Comparison of bioaerosol sampling methods in barns housing swine. *Applied and Environmental Microbiology, 58*(8), 2543-2551. doi:10.1128/AEM.58.8.2543-2551.1992

Torremorell, M., Alonso, C., Davies, P. R., Raynor, P. C., Patnayak, D., Torchetti, M., McCluskey, B. (2016). Investigation into the Airborne Dissemination of H5N2 Highly Pathogenic Avian Influenza Virus During the 2015 Spring Outbreaks in the Midwestern United States. *Avian Diseases, 60*(3), 637-643. doi:10.1637/11395-021816-Reg.1

Torremorell, M., Alonso, C., Davies, P. R., Raynor, P. C., Patnayak, D., Torchetti, M., McCluskey, B. (2016). Investigation into the airborne dissemination of H5N2 highly pathogenic avian influenza virus during the 2015 spring outbreaks in the Midwestern United States. *Avian Diseases, 60*(3), 637-643. doi:https://doi.org/10.1637/11395-021816-Reg.1

Tuson, H. H., Weibel, D. B. (2013). Bacteria–surface interactions. *Soft Matter, 9*(17), 4368-4380. doi:10.1039/C3SM27705D

Upton, S. L., Mark, D., Douglass, E. J., Hall, D. J., Griffiths, W. D. (1994). A wind tunnel evaluation of the physical sampling efficiencies of three bioaerosol samplers. *Journal of Aerosol Science, 25*(8), 1493-1501. doi:https://doi.org/10.1016/0021-8502(94)90220-8

USDA-APHIS. (2022a). *2022 Confirmations of Highly Pathogenic Avian Influenza in Commercial and Backyard Flocks*. Retrieved from https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/animal-disease-information/avian/avian-influenza/hpai-2022/2022-hpai-commercial-backyard-flocks

USDA-APHIS. (2022b). 2022 Confirmations of Highly Pathogenic Avian Influenza in Commercial and Backyard Flocks. Retrieved from https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/animal-disease-information/avian/avian-influenza/hpai-2022

USDA-APHIS. (2022c). *Case Definition - Avian Influenza (AI)*. Retrieved from https://www.aphis.usda.gov/animal_health/monitoring_surveillance/avian-influenza-case-definition.pdf: https://www.aphis.usda.gov/animal_health/monitoring_surveillance/avian-influenza-case-definition.pdf

USDA-NASS. (2020). *Poultry - Production and Value 2020 Summary*. Retrieved from https://www.nass.usda.gov/Publications/Todays_Reports/reports/plva0421.pdf

Wang, Z., Gao, T., Jiang, Z., Min, Y., Mo, J., Gao, Y. (2014). Effect of ventilation on distributions, concentrations, and emissions of air pollutants in a manure-belt layer house. *Journal of Applied Poultry Research, 23*(4), 763-772. doi:https://doi.org/10.3382/japr.2014-01000

Weber, T. P., Stilianakis, N. I. (2008). Inactivation of influenza A viruses in the environment and modes of transmission: a critical review. *Journal of Infection, 57*(5), 361-373.

Wilks, S. A., Michels, H., Keevil, C. W. (2005). The survival of *Escherichia coli* O157 on a range of metal surfaces. *International Journal of Food Microbiology, 105*(3), 445-454. doi:https://doi.org/10.1016/j.ijfoodmicro.2005.04.021

Woo, M.-H., Grippin, A., Anwar, D., Smith, T., Wu, C.-Y., Wander, J. D. (2012). Effects of relative humidity and spraying medium on UV decontamination of filters loaded with viral aerosols. *Applied Environmental Microbiology, 78*(16), 5781-5787. doi:https://doi.org/10.1128/AEM.00465-12

World Health, O. (2005). *Avian influenza: assessing the pandemic threat*. Retrieved from https://apps.who.int/iris/bitstream/handle/10665/68985/WHO_CDS_2005.29.pdf

Xu, P., Kujundzic, E., Peccia, J., Schafer, M. P., Moss, G., Hernandez, M., Miller, S. L. (2005). Impact of environmental factors on efficacy of upper-room air ultraviolet germicidal irradiation for inactivating airborne mycobacteria. *Environmental Science Technology, 39*(24), 9656-9664. doi:10.1021/es0504892

Yao, Q., Yang, Z., Li, H., Buser, M. D., Wanjura, J. D., Downey, P. M., . . . McConnell, L. L. (2018). Assessment of particulate matter and ammonia emission concentrations and respective plume profiles from a commercial poultry house. *Environmental Pollution, 238*, 10-16. doi:https://doi.org/10.1016/j.envpol.2018.02.039

Yu, G., Wang, Y., Wang, S., Duan, C., Wei, L., Gao, J., . . . Cai, Y. (2016). Effects of Microbial Aerosol in Poultry House on Meat Ducks' Immune Function. *Front Microbiol, 7*, 1245. doi: https://doi.org/10.3389/fmicb.2016.01245

Zhang, Y. (2004). *Indoor air quality engineering*: CRC press.

Zhao, Y., Aarnink, A., De Jong, M., Groot Koerkamp, P. W. G. (2014). Airborne Microorganisms From Livestock Production Systems and Their Relation to Dust. *Critical Reviews in Environmental Science and Technology, 44*. doi:https://doi.org/10.1080/10643389.2012.746064

Zhao, Y., Aarnink, A. J. A., De Jong, M. C. M., Ogink, N. W. M., Koerkamp, P. W. G. G. (2011). Effectiveness of multi-stage scrubbers in reducing emissions of air pollutants from pig houses. *Transactions of the ASABE, 54*(1), 285-293. doi:10.13031/2013.36256

Zhao, Y., Aarnink, A. J. A., Doornenbal, P., Huynh, T. T. T., Koerkamp, P. W. G. G., de Jong, M. C. M., Landman, W. J. M. (2011). Investigation of the efficiencies of bioaerosol samplers for collecting aerosolized bacteria using a fluorescent tracer. II: sampling efficiency and half-life time. *Aerosol Science and Technology, 45*(3), 432-442. doi:https://doi.org/10.1080/02786826.2010.543197

Zhao, Y., Aarnink, A. J. A., Hofschreuder, P., Koerkamp, P. W. G. G. (2009). Evaluation of an impaction and a cyclone pre-separator for sampling high PM10 and PM2. 5 concentrations in livestock houses. *Journal of Aerosol Science, 40*(10), 868-878.

Zhao, Y., Aarnink, A. J. A., Xin, H. (2014). Inactivation of airborne Enterococcus faecalis and infectious bursal disease virus using a pilot-scale ultraviolet photocatalytic oxidation scrubber. *Journal of the Air Waste Management Association, 64*(1), 38-46. doi:https://doi.org/10.1080/10962247.2013.831800

Zhao, Y., Richardson, B., Takle, E., Chai, L., Schmitt, D., Xin, H. (2019). Airborne transmission may have played a role in the spread of 2015 highly pathogenic avian influenza outbreaks in the United States. *Scientific Reports, 9*(1), 1-10. doi:https://doi.org/10.1038/s41598-019-47788-z

Zhao, Y., Shepherd, T. A., Li, H., Xin, H. (2015). Environmental assessment of three egg production systems–Part I: Monitoring system and indoor air quality. *Poultry Science, 94*(3), 518-533.

Zhen, H., Han, T., Fennell, D. E., Mainelis, G. (2014). A systematic comparison of four bioaerosol generators: Affect on culturability and cell membrane integrity when aerosolizing *Escherichia coli* bacteria. *Journal of Aerosol Science, 70*, 67-79. doi:https://doi.org/10.1016/j.jaerosci.2014.01.002

Zheng, W., Zhao, Y., Xin, H., Li, B., Gates, R. S., Zhang, Y., Soupir, M. (2013). Concentrations and size distributions of airborne particulate matter and bacteria in an experimental aviary laying-hen chamber. *Transactions of the ASABE, 56*(6), 1493-1501. doi:10.13031/trans.56.10273

Zhou, J., Wu, J., Zeng, X., Huang, G., Zou, L., Song, Y., . . . Yen, H. L. (2016). Isolation of H5N6, H7N9 and H9N2 avian influenza A viruses from air sampled at live poultry markets in China, 2014 and 2015. *Euro Surveill, 21*(35). doi:10.2807/1560-7917.Es.2016.21.35.30331

Zucker, B. A., Müller, W. (2000). [Investigations on airborne microorganisms in animal stables. 3: Relationship between inhalable endotoxin, inhalable dust and airborne bacteria in a hen house]. *Berl Munch Tierarztl Wochenschr, 113*(7-8), 279-283.

Zucker, B. A., Trojan, S., Müller, W. (2000). Airborne gram-negative bacterial flora in animal houses. *Journal of Veterinary Medicine, Series B, 47*(1), 37-46. doi:10.1046/j.1439-0450.2000.00308.x

Zuidhof, M. J., Feddes, J. J. R., Robinson, F. E., Riddell, C. (1993). Effect of ventilation rate and stocking density on turkey health and performance. *Journal of Applied Poultry Research, 2*(2), 123-129.

Zuo, Z., Kuehn, T. H., Verma, H., Kumar, S., Goyal, S. M., Appert, J., . . . Pui, D. Y. H. (2013). Association of airborne virus infectivity and survivability with its carrier particle size. *Aerosol Science Technology, 47*(4), 373-382. doi:https://doi.org/10.1080/02786826.2012.754841

# VITA

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