AIRBORNE TRANSMISSION OF *SALMONELLA* AND ITS ASSOCIATION WITH DUST IN POULTRY HOUSES

By

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ABSTRACT

Poultry house dust has been reported to contain pathogenic bacteria. Previously, the presence of *Salmonella* has been observed in airborne and settled dust in poultry houses. Therefore, it is important to investigate the sources of dust contamination with *Salmonella* in poultry houses and the likelihood of *Salmonella* colonization in chickens through airborne dust. We conducted three studies with objectives to explore the role of litter properties in transfer of *Salmonella* from litter to dust, the changes in *Salmonella* prevalence in dust deposited at different surfaces during broiler growout, and the likelihood of *Salmonella* colonization in broilers through *Salmonella* contaminated aerosol. Briefly, to accomplish these objectives, an *in vitro* setup was designed to assess the role of litter *Salmonella* and moisture levels in transfer of *Salmonella* from litter to dust, dust samples were collected from two broiler flocks (Flocks A and B), where birds in Flock B were inoculated with *S.* Enteritidis, to analyze the changes of *Salmonella* prevalence in dust during growout, and day-old broilers were exposed to *S.* Enteritidis aerosol to analyze *Salmonella* colonization in ceca, trachea, and liver/spleen over time. We observed that increasing litter *Salmonella* (10^2 to 10^9 CFU/mL) levels led to increased occurrence of *Salmonella* positive dust and increasing moisture (12.1 to 34.8%) levels decreased the occurrence of *Salmonella* in subsequent dust samples. Our second study reported the presence of *Salmonella* in dust deposited at different surfaces, however; *Salmonella* prevalence did not vary in cumulatively and non-cumulatively settled dust during growout in both Flocks A and B. In our last study, we observed *Salmonella* colonization and persistence in different tissues of broilers after exposure to different levels of *Salmonella* aerosol. Overall, *Salmonella* presence in poultry house dust and the potential risk of high levels of airborne *Salmonella* to poultry has been confirmed.

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PM_{10}	Particulate matter having aerodynamic diameter of ≤ 10 µm
$PM_{2.5}$	Particulate matter having aerodynamic diameter of ≤ 2.5 µm
APC	Aerobic plate counts
BPW	Buffered peptone water
XLT4	Xylose Lysine Tergitol-4

CHAPTER 1: LITERATURE REVIEW

1.1 INTRODUCTION

Several concerns regarding the air pollutants, which reside inside the poultry production house environment, have been raised for multiple reasons in many published studies. In particular to the prospective of poultry health, the air quality inside poultry production systems become one of the major concerns (Almuhanna et al., 2011). The main air pollutants inside modern poultry houses are gases (ammonia and carbon dioxide), dust, microorganisms, and their toxins, and these can be generated from variety of sources present inside poultry houses (Wathes, 1998; Hartung and Schulz, 2007). Knowledge of these air pollutants is important to understand and improve existing poultry production system air quality. The following review scope is limited to integrate and discuss the existing knowledge of airborne dust and microorganisms that are associated with poultry houses.

Dust present in poultry production facilities is entirely organic in nature and is comprised of both non-viable particles, that arise from feces, litter, feed, and feathers, and viable particles (also referred as bioaerosols) (Al Homidan et al., 2003; Oppliger et al., 2008). Bioaerosol components of dust are composed of airborne microorganisms (bacteria, fungi), viruses, endotoxins, and mycotoxins (Oppliger et al., 2008). Moreover, it has also been reported that airborne dust of poultry houses may contain ammonia and odorants (Takai et al., 2002; Lacey et al., 2004). Dust has a fine particulate characteristic and the capability to disperse in air, therefore, it is also referred as aerosol or airborne particulate matter (Al Homidan et al., 2003; Cambra-López et al., 2010).

Airborne dust can be a hazard in poultry production houses by many ways and one of these are its capability to act as a disseminator of pathogens in poultry houses (David et al.,

2015). Although the role of dust is not yet clear for airborne transmission of microorganisms, it has been recognized that dust acts as a carrier of many microorganisms (Cambra-López et al., 2010). Moreover, Mitchell et al. (2004) reported that airborne dust is one of the main routes responsible for the spread of disease-causing microorganisms in poultry houses. Already, multiple studies have confirmed the presence of pathogenic microorganisms (e.g., *Salmonella* and *Campylobacter*) from the air inside poultry productions units (Chinivasagam et al., 2009; Fallschissel et al., 2009). In addition to this, the fate of airborne transmission of microorganisms and the role of airborne transmission in cross-infection of microorganisms between poultry animals has been confirmed experimentally when challenged and non-challenged animals were physically separated but shared common air circulation (Lever and Williams, 1996). Therefore, airborne dust is an important control point to prevent animal infections through their carried microorganisms and to prevent food safety issues later on final products.

In addition to the animals, the high levels of dust inside the poultry houses may negatively affect the health of poultry workers by affecting their respiratory system or by inducing diseases like allergic and non-allergic rhinitis, organic dust toxic syndrome, bronchitis, asthma, and asthma-like syndrome (Mostafa and Buescher, 2011). Furthermore, the detrimental effects of airborne dust and microorganisms are not only confined inside the poultry production houses. Previously, airborne dust and microorganism emission from livestock facilities into the outside environment has been observed and has the potential to cause health issues to people living near to the poultry production houses (Davis and Morishita, 2005; Chinivasagam et al., 2009; Mostafa and Buescher, 2011). Overall, airborne particulate matter or airborne dust of livestock systems has the potential to cause serious health implications both in animals and farm

workers and may occur directly due to physical properties of inhaled dust or indirectly by compounds and microorganisms carried on inhaled dust (Cambra-López et al., 2010).

Based on the above-mentioned concerns of airborne dust and their associated microorganisms, it is important to understand the generation and distribution of airborne dust and the microorganisms inside the poultry production facilities. This will help to design the future research needs to tackle airborne dust related problems. The objective of this review was to compile the existing knowledge of key aspects of airborne dust and microorganisms in poultry production houses:

1) Originative sources of airborne dust and microorganisms.
2) Common airborne microorganisms identified in poultry houses.
3) Categorization of airborne dust and microorganisms.
4) Factors influencing the levels of airborne dust and microorganisms in poultry production houses.
5) Potential hazards of airborne dust to poultry animals through their associated microorganisms and viruses.
6) Control measures against airborne dust and microorganisms.
7) Knowledge of airborne spread of *Salmonella* spp. in poultry

1.2 KEY ASPECTS OF AIRBORNE DUST AND MICRORGANISMS IN POULTRY PRODUCTION HOUSES

1.2.1 Originative sources of airborne dust and microorganisms

Identification and quantification of originative sources of dust at the livestock production facilities has important role in development of strategies to reduce dust emission (Cambra-López et al., 2011). In poultry houses, organic dust can be produced from feathers, animal skin, feed,

dried fecal material, and microorganisms, and inorganic dust can be generated from building materials including metal surfaces and fiberglass insulation material (Aarnink et al., 1999; David et al., 2015). These originative sources contribute to airborne dust in different proportions depending upon several factors including animal types and housing infrastructure (Zhao et al., 2014). For example, Müller and Wieser (1987) have found that in layers reared on bedding, the major contribution in total dust production was from bedding material (55 to 68 %), followed by feathers (2 to 21%), and then by excreta (2 to 8%). In the same study, observed in layers reared in battery housing, the major proportion of total dust was produced from feed (80 to 90%), followed by feathers (4 to 12%) and lastly by excreta (2 to 8%). However, in the case of broilers reared on bedding material, the major proportion of total dust was generated from feathers and urine constituents (Aarnink et al., 1999). Additionally, when Cambra-López et al. (2011) examined a total of 8 poultry houses, they reported that the contribution of feathers and manure was high in generation of fine (4 to 43% and 9 to 85%, respectively) and coarse (6 to 35% and 30 to 94%, respectively) particulate matter as compared to the other originative sources of airborne dust. Recently, Ahaduzzaman et al. (2021) reported the changes of contributive percentages of different sources in settled dust production over the growth of broilers. They found that excreta was the main source of dust production and its contribution was 60% at d 7 and 95% at d 25. The contribution of bedding material and feed in dust generation was decreased with the age of broilers while feather contribution remained low and almost constant throughout. However, there is still lack of information regarding the concerns of originative sources of airborne microorganism. Generally, all the originative sources of airborne dust are accepted as the sources of airborne microorganisms in animal houses and both airborne dust and microorganisms are hypothesized to be generated together from these sources (Zhao et al., 2014).

1.2.2 Common airborne microorganisms identified in poultry houses

Bacteria and fungi have been identified in the air of poultry houses by many researchers. Table 1.1 lists the genus of the main airborne microorganisms that have been observed in poultry houses. Most published studies quantify the culturable airborne microorganisms in poultry houses and less often measure the counts of the airborne microorganisms based on their species or subspecies levels. The microbial concentration in the air of poultry houses differs greatly in the literature which can be due to several factors including animal types, animal numbers, animal age, sampling seasons, and housing infrastructure, etc. Based on few published studies, the levels of airborne bacteria ranged between 1.7×10^4 to 2.2×10^5 CFU/m^3, 1.1×10^7 to 9.6×10^7 CFU/m^3, and 2.5×10^5 to 6.9×10^5 CFU/m^3 inside the broiler houses, layer houses, and turkey houses, respectively (Vučemilo et al., 2007; Nimmermark et al., 2009; Ostović et al., 2017). Airborne fungi levels were observed to vary between ranges of 4.8×10^3 to 9.2×10^3 CFU/m^3, 1.3×10^3 to 2.3×10^3 CFU/m^3, and 3.27×10^3 CFU/m^3 to 1.06×10^5 CFU/m^3 inside the broiler houses, layer houses, and turkey houses, respectively (Nichita et al., 2010; Ostović et al., 2017; Yang et al., 2018).

1.2.3 Categorization of airborne dust and microorganisms

Airborne dust and microorganisms are generally categorized based on their sizes. The size of airborne particles governs their behavior in air stream and their penetration and deposition in respiratory tracts of poultry workers and animals. Moreover, airborne dust can be categorized based on their origin.

Airborne dust. Before discussing size-based categories of airborne dust, it is important to understand the basic scientific term, aerodynamic equivalent diameter, that is generally used to define particulate matter (airborne dust and microorganisms) size and their behavior in

environment or in the human respiratory system (Cambra-López et al., 2010). Aerodynamic equivalent diameter is described as the hypothetical diameter of an asymmetrical shaped particle (particle in question) that has similar behavior in air as of spherical particle of unit specific gravity with the same diameter (Jacobson and Jordan, 1978). It is also defined as the diameter of spherical particle of unit density (1 g cm^{-3}) whose settling velocity in air is similar to the particle of interest that having irregular shape, size, and density (Melse et al., 2012a).

For occupational health concerns, airborne dust particles, based on their behavior and penetration depth in the human respiratory system, can be classified into three categories: respirable particles (≤ 5 µm), thoracic particles (≤ 10 µm), and inhalable particles (≤ 100 µm) (Millner, 2009; Cambra-López et al., 2010). Due to different sizes, respirable particles may reach the alveoli, thoracic particles may cross the larynx and reach the bronchioles, and inhalable particles usually accumulate in nostrils and the nasal cavity (David et al., 2015). Therefore, the smaller the size of air dust, the deeper the deposition in respiratory tract. For the concern of ambient air quality assessment, particulate matter or dust can be categorized in three types: total suspended particulate, **PM_{10}** (particulate matter having aerodynamic diameter of ≤ 10 µm), and **$PM_{2.5}$** (particulate matter having aerodynamic diameter of ≤ 2.5 µm) (US EPA, 2017; Yao et al., 2018). In some instances, total suspended, PM_{10}, and $PM_{2.5}$ particulates are reported to be comparable to inhalable particles, thoracic particles, and high-risk respirable particles, respectively (Cambra-López et al., 2010; Hoff, 2018). Beyond this, dust particles can also be classified as fine (aerodynamic equivalent diameter of ≤ 2.5 µm) and coarse particles (aerodynamic equivalent diameter = 2.5–10 µm) (Jerez et al., 2014). Dust can also be categorized based on their origin into two types: primary particulates (particulate those are emitted directly into the atmosphere from their sources) and secondary particulates (particulates

those formed from primary particulates within the atmosphere due to chemical reactions) (Melse et al., 2012a).

Categorization of airborne dust particles into different sizes based on their deposition pattern in human respiratory tracts may not be accurate for the poultry animals because of the different morphology of their respiratory system than humans (David et al., 2015). Therefore, it is important to examine the size of airborne dust particles in relation to their deposition in the avian respiratory system. In this regard, Hayter and Besch (1974) examined the deposition of five different size particles (0.091, 0.176, 0.312. 1.1, and 3.7–7 µm) in respiratory systems of anesthetized chickens. After exposure to the particles, they found that the largest particles (3.7–7 µm) were deposited in head and anterior trachea and smaller particles (0.091 µm and 0.176 µm) were deposited in caudal regions. Moreover, the particles of 1.1 µm size were captured from lung and posterior air sacs and particles having 0.312 µm size were noted in upper airways. Similarly, Corbanie et al. (2006) examined the deposition of airborne particles of different sizes (1, 3, 5, 10, and 20 µm) in respiratory and gastro-intestinal tracts of unanesthetized chickens of different ages (1-day-old, 2-week-old, 4-week-old). They found that in 2-week-old and 4-week-old chickens, particles of size 5 and 10 µm were too big to deposit in lungs and air sacs based on the low proportion recovered from these parts. For 1-day-old birds, the particles up to 20 µm in size were able to reach lungs and air sacs and this was attributed to the occurrence of mouth breathing in day old chicks.

Airborne microorganisms. Size based distribution assessment of airborne microorganisms in livestock buildings is generally performed using an Andersen sampler (Anderson, 1958; Adell et al., 2014; Zhao et al., 2014). This sampler was designed to quantify and categorize the airborne microorganism containing particles based on their different sizes. An

Andersen sampler can be used to distinguish airborne microorganism containing particles into six different size ranges including > 7 µm, 4.7 to 7 µm, 3.3 to 4.7 µm, 2.1 to 3.3 µm, 1.1 to 2.1 µm, and 0.65 to 1.1 µm (Adell et al., 2014). However, this sampler does not provide the size-based distribution of individual microorganisms which is more important to examine for occupational health purposes (Zhao et al., 2014). In poultry houses, the airborne microorganism containing particles of all these different sizes (0.65 to > 7 µm) can be present. Previous studies performed in a total of 6 poultry houses (3 broiler houses, 3 layer houses) reported that 17.6 to 49.7%, 29.8 to 51.2%, and 11.4 to 34.3% of airborne bacteria were belonged to 4.7 to > 7 µm, 2.1 to 4.7 µm, and 0.65 to 2.1 µm size range, respectively. The same study observed that 15.6 to 32%, 39.6 to 54%, and 16.8 to 37.5% of airborne fungi had size range of 4.7 to > 7 µm, 2.1 to 4.7 µm, and 0.65 to 2.1 µm, respectively (Yang et al., 2018).

1.2.4 Factors influencing the levels of airborne dust and microorganisms in poultry production houses

The levels of airborne dust and microorganisms in poultry houses are affected by a number of factors related to animal, poultry housing systems, farm management, and environmental conditions (Banhazi et al., 2008; Zhao et al., 2014). These different factors influence airborne dust and microorganism levels jointly in a particular poultry house. However, in most published research, the effects of these factors on airborne dust and/or airborne microorganism concentrations were studied either independently or in a combination of two or more factors.

Among animal factors, Vučemilo et al. (2007) examined the effect of broiler age on concentration of airborne pollutants during the flock growout for 6 weeks in the Spring season. They concluded that airborne dust and fungi levels increased initially and then decreased sharply

during the last 2 weeks of flock growout. The authors mentioned that the decline of dust and fungi levels at end the end of growout was due to the reduction of airborne dust and fungi emission from their sources because of decreased bird activity and increased humidity of litter. However, they observed a "sinusodial rise" in levels of airborne bacteria during the broilers growout. Likewise, Calvet et al. (2009) reported that dust levels increased with the age of the broilers. They found that PM_{10} increased from 0.10 to 2.82 mg/m^3 and 0.05 to 0.79 mg/m^3 during week 1 to week 5 of the growing cycle in light and dark period, respectively. Overall, they found that on average PM_{10} levels in the light period were 4 times higher as compared to the dark period. Moreover, the same study concluded that dust concentration has a strong positive correlation with bird activity and their live weight ($r^2 = 0.89$), and bird activity was found to change with bird age and lighting status of the house. Bird activity was greater in light periods and peaked at week 4 of growout. Therefore, the factors that result in increase of chickens' activities are indirectly responsible for higher levels of airborne dust in poultry production facilities. The direct analyses of animal activities and weight effects on airborne microorganism levels in poultry houses have not yet been extensively studied.

Housing systems also play an important role in regulating the levels of airborne dust and microorganisms. It has been reported that the levels of airborne dust are higher in poultry housing systems where birds have more access to litter such as aviary and on-floor housing systems as compared to cage systems (Le Bouquin et al., 2013). Similarly, de Reu et al. (2005) observed the higher concentration of microorganisms in aviary housing systems compared to cage system for laying hens. Moreover, Madelin and Wathes (1989) observed a higher concentration of airborne respirable dust particles and microorganisms when broilers were reared on "deep litter wood shavings" as compared to when they were reared on a "raised netting

flooring system". Therefore, the presence of litter is associated with more airborne dust and microorganism levels in poultry houses. In addition to this, the type of bedding material can also affect the concentration of airborne particles in poultry houses. Previously, it has been noted that the levels of airborne inhalable particles were higher in houses having straw as a bedding material compared to the wood shavings-bedded houses. However, the same study found higher levels of total airborne bacteria in wood shavings-bedded houses (Banhazi et al., 2008).

House cleaning and feeding operations are two of the main factors related to farm management that influence airborne dust and microorganism levels in poultry houses. Poultry bird activities can be increased while feeding, which can then result in more dust generation (Al Homidan et al., 2003). Banhazi et al., (2008) observed that broiler houses, which were cleaned by replacing bedding material and washing the entire house between two consecutive flocks, had lower concentrations of respirable dust particles and airborne bacteria compared to the non-cleaned broiler houses.

House temperature, relative humidity, and ventilation rate are among the main poultry house environmental parameters that reported to influence airborne pollutant concentration levels. Previously, it has been observed that airborne dust particle levels are positively correlated with indoor temperature up to a certain point, and this correlation turns to negative at higher temperatures. Specifically, Koon et al. (1963) found in a caged layer house that dust levels were low at 10 ℃, high between 16 to 21 ℃, and then declined as the temperature reached to 38 ℃. On the other hand, the airborne dust levels were observed to negatively correlate with relative humidity (Al Homidan et al., 1998; Banhazi et al., 2008). Direct correlation between indoor temperature and relative humidity with airborne microorganism levels in poultry houses is still lacking. Although, both temperature and relative humidity has been reported to influence

airborne microorganism levels in other animal houses (Islam et al., 2020). Moreover, the higher ventilation rate within poultry houses was observed to decrease the levels of airborne microorganisms and dust (Qi et al., 1992; Hinz and Linke, 1998).

1.2.5 Potential hazards of airborne dust to poultry animals through their associated microorganisms and viruses

The detrimental effects of airborne dust on poultry animals' health are one of the main concerns of dust in poultry houses. Inhaled airborne dust can affect the health of livestock animals through their attached pathogenic or non-pathogenic microorganisms and chemical compounds, and itself dust can cause irritation of the respiratory system of the animals which could lower their immune resistance against respiratory diseases (Harry, 1978). Through any one of the above-mentioned means, airborne dust can cause various kinds of health implications in poultry animals via different infection mechanisms.

Dust has been reported to cause infections in poultry through attached microorganisms and viruses in several studies. Specifically, when specific-pathogen-free 4-week-old chickens were inoculated with aerosols of H9N2 avian influenza virus, having concentration of 1.5×10^7 (50% embryo infective dose), the lower respiratory tract infection and cytokine gene interleukin and interferon expressions up-regulation in lung tissues were observed in chickens (Guan et al., 2015). Similarly, Cheng et al. (2020) exposed day-old leghorn chickens to five different concentrations of aerosolized *S.* Pullorum (1.25×10^2 to 1.25×10^9 CFU/m^3) and then examined the dose dependent pattern of colonization and morbidity. They found lung colonization for more than 14 days following aerosol inoculation with $\geq 10^6$ CFU/m^3 of *S.* Pullorum. They observed tachpnoea, depression, and some death in chickens exposed to $\geq 10^8$ CFU/m^3 levels of *S.* Pullorum aerosol. Additionally, they also reported significant up-regulation of inflammatory

cytokine expression and other negative histopathological observations such as lung swelling and lung lesions (tissue injury, inflammatory cell infiltration, and acute hemorrhage) in chickens exposed to high levels of aerosolized *S.* Pullorum. Moreover, when Landman and Feberwee (2004) jointly infected layers with *Mycoplasma synoviae* and infectious bronchitis virus via aerosol and ocular-nasal route, respectively, an enhancing effect of infectious bronchitis virus on *M. synoviae* induced arthritis was observed. Therefore, there is a possibility of interactive effects of different microorganisms and/or viruses, that enter through different routes in chickens, on causing health issues in poultry. Beyond this, dust itself might make the poultry respiratory system more susceptible to non-pathogenic microorganisms. Previously, Oyetunde et al. (1978) observed almost the absence of pathogenic effects on respiratory system of chicks when they were exposed to only harmless *E. coli*. But when chicks were exposed to a mixture of harmless *E. coli* and dust (having concentration between 101 to 104 mg/cm^3) then the presence of pathological lesions on different regions of respiratory tracts of chicks was reported. The authors mentioned that this occurred because of the devitalizing effect on the respiratory system of the chicks by dust.

Dust dangers cannot be restricted only for causing animal infections. Dust also has the potential to spread diseases caused by microorganisms throughout the flock and thereby dust may cause microbial outbreaks and ultimately lead to an economic loss. The possibility of airborne transmission of microorganisms has been confirmed experimentally within poultry houses. Specifically, when a microbial infected group was physically separated from the uninfected group of chickens and both groups were sharing common air circulation, the presence of inoculated microorganisms in the air led to the infection of the uninfected groups of chickens with the same inoculated microorganisms. Based on this phenomenon, the literature has

established the possibility of aerosolization and airborne transmission of inoculated microorganisms and the cross-infection of microorganisms between animals via air (Lever and Williams, 1996; Holt et al., 1998; Li et al., 2009; Gao et al., 2019).

1.2.6 Control measures against airborne dust and microorganisms

The control of airborne dust and microorganisms has upmost importance in animal houses to combat the problems that they can cause such as health and environmental issues (Cambra-López et al., 2010). Airborne dust and microorganism control strategies can be planned to prevent their formation from their originative sources, to prevent their transfer from different sources to the inside air of poultry houses (just to reduce their concentration in air), and to prevent their emission from the poultry houses into the outside environment. Several published studies have investigated different dust removal techniques in poultry buildings and the most common methods are liquid spraying techniques, electrostatic precipitation or air ionization, and air scrubbers. The following review is aimed to discuss these different airborne dust and microorganism control methods.

Liquid spraying techniques. Humidification of bedding material with spraying agents can result in adhering of dust particles with litter and thereby decreasing dust spread in poultry houses (Ogink et al., 2012). Different kinds of liquids have been investigated to reduce airborne dust and/or microorganisms in poultry houses including water, neutral electrolyzed water, rapeseed oil, and slightly acidic electrolyzed water (Aarnink et al., 2009; Ogink et al., 2012; Zheng et al., 2012; Zheng et al., 2014; Winkel et al., 2016). Ogink et al. (2012) reported that spraying of water two times per day at different rates (No water, 75 mL m^{-2}, 150 mL m^{-2}, 300 mL m^{-2}) on top of the bedding material in laying hen houses resulted in reduction of dust particle emission (18 to 64% and 44 to 64% for particles of less than 10 µm and 2.5 µm in size,

respectively). However, they observed an increase in ammonia emission (21 to 64%) linearly with an increase in dosing rates of spraying water. Similarly, Zheng et al. (2014) evaluated the efficacy of spraying slightly acidic electrolyzed water (80 mL m^{-2}/day for 15 min) as compared to the spraying of tap water (80 mL m^{-2}/day for 15 min) and no spraying (control) for the reduction airborne culturable bacteria and particulate matter in an experimental aviary housing chamber. They measured airborne culturable bacteria and particulate matter for 15 min just before spraying different treatments and after 45 min of treatment application from the middle of the room at 1.5 m height from floor. They observed that spraying of slightly acidic electrolyzed water significantly reduced (up to 49%) airborne culturable bacteria of size > 2.1 µm as compared to the control treatment. There was no difference between control and tap water spray treatments for bacteria reduction. However, both slightly acidic electrolyzed water and tap water spraying reduced airborne particulate matter of size > 7.1 µm without showing any significant difference with each other. The authors concluded that the airborne bacteria reduction can also depend on properties of the spraying agent (e.g., bactericidal effects) rather than on the reduction of dust levels.

However, liquid spraying techniques have some drawbacks when used in animal houses. For instance, using oil and water spraying to control dust in animal houses can result in the choking of spraying nozzles. This problem disrupts the distribution of spraying solutions and reduces their efficiency for reduction of dust. This problem is most likely to occur while using this method and yet remains as an unsolved issue (Takai, 2007).

Electrostatic precipitation. Electrostatic precipitation mainly helps to decrease the concentration of airborne dust and microorganisms from air of poultry houses rather than to prevent their generation from different sources. In this technique, an electrostatic space charge

system generates negative charge ions in air that leads to negative charges on airborne dust particles. Then, these negatively charged airborne particles are captured on positive charged surfaces (Zhao et al., 2014). Previously, promising results of this method on reduction of air pollutants have been confirmed in different poultry houses systems (Mitchell et al., 2004; Jerez et al., 2013; Manuzon et al.,2014; Zhao et al., 2018). Specifically, Mitchell et al. (2004) found that an electrostatic space charge system enabled reduction of airborne dust, ammonia, and airborne bacteria on an average of 61%, 56%, and 67%, respectively. Cambra-López et al. (2009) observed that this method reduced PM_{10} and $PM_{2.5}$ emission by 36% and 10%, respectively, on mass basis when examined in pilot-scale broiler rooms. However, they did not find a significant effect on the reduction of microorganisms, ammonia, and odor emission. They also reported that the efficacy of this method to reduce air particulate matter emission differed with respect to the size of particulate matter. Specifically, they observed that the increase in efficacy of this method is proportional to the increase in particulate matter size. Moreover, Zhao et al. (2018) showed that an average reduction efficiency from this method was 68% during spring to summer periods and 45% during fall to spring periods for total suspended particulate matter. Manuzon et al. (2014) reported that the efficiency of this method to reduce air pollution can be varied according to the specification (e.g., voltage) of the electrostatic precipitator.

Overall, this technique was found to have potential in decreasing air pollution in poultry houses. However, the efficacy of this method for the reduction of dust observed varied greatly in the literature. Therefore, a great deal of future work is still required for optimizing this method for commercial poultry production houses. The published research works related to this method are difficult to compare directly with each other for several reasons including different use of dust collection/measuring methods, analysis of different aspects of dust reduction such as

emission and in-house concentration levels, analysis over different kinds of poultry housing systems, and different specification of the electrostatic space charge systems.

Air scrubbers. Air scrubbers come under the "end-of pipe" techniques that are generally equipped at the air outlets of the animal production facilities to reduce the air pollutant emissions to outside of the houses (Zhao et al., 2014). There are different kinds of air scrubbers available to use in animal housing such as acid scrubber, bio scrubber, or the mixed typed scrubber (Aarnink and Ellen, 2007). Originally, these different scrubbers were developed to tackle the problem of ammonia and odor emission from animal house, however, they also have been observed effective to minimize particulate matter emission (Zhao et al., 2014). It has been reported that the average PM_{10} reduction efficiency of acid scrubbers and bio scrubbers ranged between 18 to 67% and 34 to 83%, respectively, at poultry houses (Melse et al., 2011; Mosquera et al., 2011; Melse et al., 2012a; Melse et al., 2012b). Beyond reduction of dust particles, acid scrubbers that had peracetic acid as a circulating solution were able to reduce *E. faecalis* and Gumboro virus in lab-scale study (Aarnink et al., 2011).

Overall, the above discussed methods to control airborne dust and microorganism has been proven effective. However, the great variability in their performance has to be considered as one of their significant limitations. Therefore, there is still work required to develop more optimized and consistent methods to control airborne dust and microorganism at poultry production facilities.

1.2.7 Knowledge of airborne spread of *Salmonella* spp. in poultry

Salmonella spp. cross-infection between chicks can occur through airborne transmission within the poultry production houses (Park et al., 2008). Previously, when *Salmonella* (*S.* Enteritidis or *S.* Typhimurium) challenged and non-challenged chicks were physically separated

from each other but had shared common air circulation, the contamination of air with respective inoculated *Salmonella* serovars was observed and that contaminated air resulted in *Salmonella* infection/colonization in non-challenged chicks (Lever and Williams, 1996; Gast et al., 1998; Holt et al., 1998; Leach et al., 1999). Moreover, it has been reported that eggs hatching can generate *Salmonella* contaminated dust and fluff, which may circulate within the hatcher and potentially colonize the chicks if placed in the same hatcher (Davies and Wray, 1994). These experimentally performed studies demonstrated the possibility of air contamination with *Salmonella*, airborne transmission of *Salmonella*, and cross-infection of *Salmonella* between birds through the air route. Additionally, the chances of airborne cross-infection of *Salmonella* in poultry can be dependent on the stage of growth of birds. Specifically, Holt et al. (1998) reported that the airborne *Salmonella* cross-infection cases were greater in molted hens compared to non-molted hens.

Furthermore, there are few studies that more concisely establish *Salmonella* colonization or infection in chicks can be possible by aerosolized *Salmonella* and the respiratory route can serve as an entry point for *Salmonella* in poultry. Specifically, when Cheng et al. (2020) inoculated day-old leghorn chicks with different levels of aerosolized *S.* Pullorum (1.25×10^2 to 1.25×10^9 CFU/m^3), a dose dependent pattern of *Salmonella* colonization and morbidity was observed. Moreover, when chicks were intratracheal inoculated, simulating the inhaling of fomites with *Salmonella*, the recovery of *Salmonella* in tissues (ceca-cecal tonsils or cecum, trachea, crop, liver/spleen) and cloacal swab samples was observed (Kallapura et al., 2014; Chadwick et al., 2020).

Although, the risks of airborne *Salmonella* in poultry have been speculated to correlate with experimental studies, there is still a need to investigate the fate of airborne transmission of

Salmonella, sources of airborne *Salmonella*, and colonization in chickens with airborne *Salmonella* at commercial poultry houses. Only limited information specific to airborne *Salmonella* or settled dust *Salmonella* is available in literature and is presented in Table 1.2.

1.3 SUMMARY

- Airborne dust and microorganisms can be generated from different sources such as feathers, animal skin, feed, and dried fecal material.
- The contribution of these different sources in production of airborne dust can be varied according to animal type and housing infrastructure.
- Various type of genera of both airborne bacteria and fungi has been identified in poultry houses, and their potential hazards on health of poultry animal have also been noticed.
- Airborne dust can be categorized based on their behavior and penetration depth in the human respiratory system (respirable particles, thoracic particles, and inhalable particles), their origin (primary particulates, secondary particulates), and their sizes that important for ambient air quality assessment (suspended particulate, $PM_{10,}$ $PM_{2.5}$). Moreover, airborne microorganisms can be quantified into different sizes (> 7 µm, 4.7 to 7 µm, 3.3 to 4.7 µm, 2.1 to 3.3 µm, 1.1 to 2.1 µm, and 0.65 to 1.1 µm) using Andersen sampler.
- The deposition of airborne dust particles in the avian respiratory tract can be varied according to their size.
- The levels of airborne dust and microorganisms has been found to be influenced by animal age, animal activity, animal weight, house cleaning conditions, feeding operation, house temperature, house humidity, and ventilation rate.

- Different methods have provided promising results for the abatement of airborne dust and microorganisms levels. However, there is still a need to develop more optimized and consistent methods to control airborne dust and microorganisms in poultry houses.

1.4 KNOWLEDGE GAP IN LITERATURE SPECIFICALLY FOR AIRBORNE *SALMONELLA* SPREAD

It has been demonstrated by experimental studies that airborne transmission of *Salmonella* can be possible in poultry houses and that this transmission may result in cross-infection of *Salmonella* among birds. Moreover, several studies have found the presence of *Salmonella* in air and settled dust in different kinds of commercial poultry facilities, indicating the threat of airborne or settled dust *Salmonella* for poultry health and food safety. Therefore, it is important to explore the mechanism of airborne transmission of *Salmonella* in poultry facilities in order to tackle this problem. Based on the knowledge gaps present in the literature, there is still a need to investigate the major sources of *Salmonella* dust in poultry houses, the spread of *Salmonella* in dust at poultry house and the effect of broiler growout on dust *Salmonella*, and the colonization of *Salmonella* in broilers via aerosol. By keeping these knowledge gaps in mind, we conducted three studies entitled:

1. Impact of poultry litter *Salmonella* levels and moisture on transfer of *Salmonella* through associated *in vitro* generated dust. (Chapter 2)
2. Bacterial composition of settled dust during growout of broiler chickens. (Chapter 3).
3. Investigation of the potential of aerosolized *Salmonella* Enteritidis on internal organ colonization in broilers between age of D 3 to D 21. (Chapter 4)

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Table 1.1 The common airborne microorganisms identified in poultry houses.

Airborne microorganisms	Genus	References
Bacteria	*Streptococcus*, *Staphylococcus*, *Micrococcus*, *Enterococcus*, *Aerococcus*, *Faecalibacterium*, *Bacteroides*, *Corynebacterium*, *Brevibacterium*, *Cellulomonas*, *Bacillus*, *Escherichia*, *Enterobacter*, *Klebsiella*, *Shigella*, *Proteus*, *Citrobacter*, *Pasteurella*, *Pantoea*, *Moraxella*, *Oscillospira*, *Pseudomonas*, *Xanthomonas*, *Lactobacillus*, *Megamonas*, *Ruminococcus*, *Kocuria*, *Acinetobacter*, *Microbacterium*	Baykov and Stoyanov, 1999; Vučemilo et al., 2007; Bródka et al., 2012; Lawniczek-Walczyk et al., 2013; Plewa-Tutaj et al., 2014; Yang et al., 2018; Zhang et al., 2019
Fungi	*Penicillium*, *Aspergillus*, *Fusarium*, *Geotrichum*, *Scopulariopsis*, *Alternaria*, *Trichoderma*, *Drechslera*, *Mucor*, *Rhizopus*, *Cladosporium*, *Candida*, *Cryptococcus*, *Acremonium*, *Trichopyton*, *Verticilum*, *Scedosporium*, *Mycelia*, *Rhodotorula*, *Chaetomum*, *Chrysosporium*	Vučemilo et al., 2007; Witkowska et al., 2010; Wójcik et al., 2010; Pelwa-Tulaj and Lonc, 2011; Sowiak et al., 2012; Lawniczek-Walczyk et al., 2013; Zhang et al., 2019; Horvatek Tomić et al., 2021

Table 1.2 Summary of published studies that reported the information of airborne or settled dust *Salmonella* in poultry houses.

Animal Type	Housing information	Parameters evaluated	Main findings	References
Layers	Automated layer battery house with full controlled climate, n[1]=1	*Salmonella* spp. levels in inside air of the house	On average 7.4×10^1 CFU/m^3	Venter et al., 2004
	Automated layer battery house without full controlled climate, n=1	*Salmonella* spp. levels in inside air of the house	On average 6.6×10^1 CFU/m^3	
Broilers and Layers	Intensive broilers production house, n= 8	SP[2] in settled dust of the houses	5/34	Pieskus et al., 2008
	Conventional and furnished caged house for layers, n=6	SP in settled dust of the houses	6/15 (Conventional cages); 12/38 (Enriched cages)	
	Aviary house for layers, n=2	SP in settled dust of the houses	8/38	
Layers	n=203	Overall *Salmonella* presence and characterization in airborne dust from all the sampled houses	48/203 farms were *Salmonella* positive; A total of 34 *Salmonella* serovars were noticed in airborne dust; Major serovars were: *S.* Infantis, *S.* Agona, *S.* Mbandaka, *S.* Cerro, *S.* Thompson, *S.* Braenderup	Iwabuchi et al., 2010
Broilers	Mechanically ventilated broiler house, n=4	*Salmonella* spp. levels in inside air of the houses	Ranged from 0.65 to 4.4 MPN/m^3, when present	Chinivasagam et al., 2009
Broilers	n=9	Overall *Salmonella* presence and characterization in settled dust from all the sampled houses before chick's placement and after house disinfection	6/9 farms were *Salmonella* positive; Overall 11/90 dust samples were *Salmonella* positive; Four *Salmonella* serovars were observed in dust: *S.* Albany, *S.* Anatum, *S.* Blockley, *S.* Heidelberg	Higgins et al., 1982
Broilers	Floor housing, n=2	*Salmonella* spp. levels in inside air of the houses	On average 3.3×10^2 CFU/m^3	Fallschissel et al., 2009

Broilers and Layers	Broilers placed on deep litter, n=10	*Salmonella* spp. levels in settled dust of the houses	Ranged from 1.1×10^5 to 6.3×10^5 CFU/g, when present	Skóra et al., 2016

[1]n = Number of sampled houses.
[2]SP = *Salmonella* prevalence (positive samples/total samples)

CHAPTER 2: IMPACT OF POULTRY LITTER *SALMONELLA* LEVELS AND MOISTURE ON TRANSFER OF *SALMONELLA* THROUGH ASSOCIATED *IN VITRO* GENERATED DUST

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2.1 ABSTRACT

Dust present in poultry houses can contain high concentrations of microorganisms and have the potential to include pathogens from the litter. The objective of this study was to examine *in vitro* the potential for litter to dust transfer of aerobic bacteria, *Salmonella*, *E. coli*, and coliforms, and the role of the litter moisture on this process. Poultry litter was inoculated with 10^2 to 10^9 CFU/mL of *Salmonella* Typhimurium to evaluate litter to dust transfer of bacteria (Experiment 1). To evaluate the effect of litter moisture on litter to dust microbial transfer (Experiment 2), litter was inoculated with 10^9 *S.* Typhimurium with increasing amounts of sterilized water added for moisture adjustment. Dust was generated by blowing air in a direct stream onto inoculated litter while simultaneously collecting dust through impingement. Following litter and dust sample collection, microbial analyses for aerobic plate counts **(APC),** *Salmonella*, *E. coli*, and coliforms were conducted. Both experiments were repeated 5 times and

their data analyzed by one-way ANOVA and simple logistic regression. In Experiment 1, APC of litter (log_{10} CFU/g) and dust samples (log_{10} CFU/L) were 10.55 and 4.92, respectively. *Salmonella* ranged from 1.70 to 6.16 log_{10} CFU/g in litter and only one dust sample had 1.10 log_{10} CFU/L of *Salmonella*. As *Salmonella* levels in litter increased, the probability of obtaining a dust *Salmonella* positive result also increased. In Experiment 2, attained moisture percentage were 13.0, 18.2, 23.0, 28.2, and 33.3%. Litter recovery for APC, *Salmonella*, *E. coli*, and coliforms counts did not differ ($P > 0.05$) with increasing moisture levels. Dust sample bacterial counts significantly decreased with increasing moisture levels ($P < 0.0001$). Results from this *in vitro* study indicate that there is potential for *Salmonella* to be present in generated dust and the higher levels of *Salmonella* in litter increase the likelihood of detecting *Salmonella* in dust. Additionally, with higher litter moisture percentage, prevalence of *Salmonella* in generated dust was decreased.

Key words: *Salmonella*, litter, dust, impingement, moisture

2.2 INTRODUCTION

Salmonella is a foodborne pathogen of great concern, and often, *Salmonella* outbreaks are found to originate from poultry (CDC, 2018; CDC, 2019). Every year in the United States, *Salmonella* is responsible for causing 1.35 million infections, > 25,000 hospitalizations, and > 400 deaths, and the majority of these illnesses are attributed to food (CDC, 2020). *Salmonella's* major risk factor in poultry meat and table eggs is its presence in live birds (Hugas and Beloeil, 2014). Poultry intestinal tracts colonized with *Salmonella* can potentially contaminate poultry meat during processing and cut-up, which could then result in foodborne salmonellosis in humans after consuming improperly cooked and/or handled contaminated product (Nayak et al., 2004). *Salmonella* is a commensal microorganism in poultry and has the potential to disseminate

to the whole flock without any visible sign (Hugas and Beloeil, 2014). *Salmonella* can spread to poultry farms by both vertical and horizontal transmission. Potential sources for *Salmonella* spread within the poultry production chain include breeders, hatcheries, chicks, the poultry house environment, feed, insects, rodents, and wild birds (Liljebjelke et al., 2005).

Airborne transmission of *Salmonella* is an indirect horizontal method of spread and has previously been observed in several studies. *Salmonella* was transmitted from challenged hens in cages to non-challenged molted hens when they were physically apart (1 m) from each other (Holt et al., 1998). Aerosolized *S.* Pullorum was demonstrated to infect one-day-old chicks (Cheng et al., 2020). Zhao et al. (2014) reported that on livestock farms, airborne microorganisms and dust have an interrelation indicating dust can carry microorganisms in the air, and the origin of dust and airborne microorganisms are usually from the same sources. Reduction of airborne dust concentration has been linked with a decrease in airborne bacteria (Mitchell et al., 2004). Moreover, it was reported that reducing airborne dust levels using negative air ionization may also help to limit the spread of *S.* Enteritidis in poultry flocks (Gast et al., 1999). Therefore, the identification of sources of microorganisms and dust in poultry houses can help to understand the mechanism of airborne transmission and to develop and implement new control strategies to prevent this transfer (Zhao et al., 2014).

Poultry house dust is comprised of feathers, skin debris, feed, litter, and feces, and all of these components may carry microorganisms (Madelin and Wathes, 1989). Occasionally, dust is also termed as an aerosol because of its ability to disperse in the environment and its fine particulate characteristics (Al Homidan et al., 2003). Litter is the main source for the large proportion of dust in floor housing systems (David et al., 2015). Similarly, broiler houses with litter have been associated with higher dust and airborne microorganism levels compared to

litterless houses (Madelin and Wathes, 1989). Moreover, litter properties such as fresh bedding or used litter, as well as litter moisture levels, are also recognized to influence dust density and emission rate in tunnel-ventilated broiler poultry houses (Modini et al., 2010). Specifically, the increasing litter moisture content may reduce the dust emission; however, the higher litter moisture levels support adverse effects such as ammonia and odorant production and their emission in poultry facilities (Al Homidan et al., 2003; Ogink et al., 2012; Dunlop et al., 2016).

The presence of dust in poultry houses, its linkage with litter, and its capability to carry microorganisms in the air have been reported previously in the literature. However, the levels of *Salmonella* transfer to dust generated from contaminated litter and the effect of litter moisture levels on this transmission is still not well defined. Therefore, the objectives of this work were 1) To examine the potential for transfer of aerobic bacteria, *Salmonella, E. coli*, and coliforms to *in vitro* generated dust (settled or airborne) produced from poultry litter and 2) To examine the role of litter moisture content in litter to *in vitro* generated dust transfer of these specified microorganisms.

2.3 MATERIALS AND METHODS

Experimental design

Experiment 1. To assess litter to air transfer of microorganisms, an *in vitro* method was developed. Approximately 3.5 kg of used litter was collected from one source (a single pen) to run all replications of this experiment. The litter collected for this experiment consisted of pine shavings that had hosted 2 previous broiler flocks and a third flock in the rearing stage. For each of 5 replications of this experiment, conducted on separate days, litter was mixed and then four batches of 110 g of litter were taken and individually delivered into 4 separate 2 L Erlenmeyer flasks. The fifth flask served as a negative (no litter) control and remained empty. Four flasks

containing litter were separately inoculated with 10 mL of 4 different levels of a nalidixic acid-resistant strain of *Salmonella* Typhimurium. Four levels of inoculation were 10^2, 10^4, 10^6, or 10^8 CFU/mL for first replication and 10^3, 10^5, 10^7, or 10^9 CFU/mL for the subsequent replications. *Salmonella* inocula were applied in a dropwise manner onto the litter while simultaneously shaking the flask by hand. Dripping of *Salmonella* inoculums was done using transfer pipets (5 mL, VWR International, LLC, Radnor Corporate Center, PA, USA). Following inoculation, each flask was covered with aluminum foil and acclimated for 24 h at room temperature. After 24 h, 20 g of litter from each flask was removed for litter moisture percentage determination and microbial analysis. The remaining litter (90 g) was used for *in vitro* dust generation that was further collected in buffered peptone water **(BPW)** (BBL™, Becton Dickinson and Company, Sparks, MD, USA) by using an impingement system (Figure 2.1). Following dust collection, BPW was used for microbial analysis. The total number of litter and dust samples used for microbial analyses was n = 20 and n = 25, respectively.

Experiment 2. The *in vitro* setup developed for Experiment 1 was used to assess litter moisture effect on the litter to air transfer of microorganisms. Approximately 3.5 kg of litter was gathered from one source (a single pen) to run all replications of this experiment. The litter collected for this experiment consisted of pine shavings that had a single broiler flock in rearing stage. For each of the 5 replications of this experiment, conducted on separate days, 5 batches of litter weighing 110 g were prepared and individually added to 5 separate 2 L flasks for each replication. The sixth flask did not contain litter (negative control). Four flasks containing litter were separately inoculated with 10 mL of a nalidixic acid-resistant strain of *S.* Typhimurium (10^9 CFU/mL). To adjust different litter moisture levels, increasing amounts of sterilized water (10 mL, 15 mL, and 20 mL) were added to 3 of the 4 inoculated flasks. The fifth flask containing

litter was not inoculated. The flasks receiving dropwise inoculum or sterilized water were simultaneously shaken by hand. After inoculation or moisture addition, each flask was covered with aluminum foil and then held 24 h at room temperature. After 24 h, 20 g of litter from each flask was removed for litter moisture percentage determination and microbial analysis. The remaining litter (90 g) was used for dust generation that was further collected into BPW through the impingement system. Following impingement, collected dust samples in BPW were used for microbial analysis. The total number of litter and dust samples used for Experiment 2 microbial analyses was n = 25 and n = 30, respectively.

Mechanism of impingement system for each replication of experiments 1 and 2

An *in vitro* dust generation and collection system was designed and used for experimental testing (Figure 2.1). For sampling, each flask was covered by 4 layers of folded cheesecloth (VWR®, Cheesecloth Wipes™, CAT. NO. 21910-105), which had 2 holes for tubing connection to allow for equalization of pressure. For dust generation, air was blown onto the litter at 143-155 m/s, measured by flow meter (SKC Inc., Eighty Four, PA, USA), using a 3.8 m tube (Tygon S3™, OD= 9.5 mm, ID= 6.4 mm, Akron, OH, USA) with one end connected to a laboratory air port and the other end equipped with a pipet (VWR® 10 mL Serological Pipet, VWR International, LLC, Radnor, PA, USA) as a tip. The air speed of 143 to155 m/s was selected based on the speed of air necessary to generate visible dust within the flask that could simulate high levels of dust production in poultry houses, which are likely to be produced during times of increased bird activity such as during catching for harvest. The tip was continuously swirled by hand while blowing air onto the litter. Simultaneously, the generated dust was collected through a 0.5 m long tube, with the collection end equipped with a 12.7 cm long pipet tip (VWR® 5 mL Serological Pipet, VWR International, LLC, Radnor, PA, USA), by impingement (ACE Glass

Incorporated, 7531 – 10 Midget Impinger Comp., Vineland, NJ, USA) into 10 mL BPW (0.8 L/min for 5 min). The pipet end of the dust collection tube was covered with 2 layers of cheesecloth, except for the first replication of Experiment 1 in which the tip was covered with 4 layers of cheesecloth, to prevent large particles from entering and clogging the impingement system.

Salmonella Typhimurium inoculum preparation

Salmonella enterica serovar Typhimurium used for Experiments 1 and 2 was developed at the US National Poultry Research Center in Athens, GA, and had naturally induced resistance to nalidixic acid. This marker strain, stored in glycerol stock at -80 °C, was first grown on plate count agar for 24 h at 37°C. The colonies were then collected and suspended into sterile saline solution to achieve an optical density for 8 $\log_{10}$ CFU/mL (for the first replication of Experiment 1) or 9 $\log_{10}$ CFU/mL (for the remainder of replications of Experiment 1 and Experiment 2). The actual $\log_{10}$ CFU/mL was further confirmed by plating appropriate inoculum dilutions on Xylose Lysine Tergitol-4 **(XLT4)** (Criterion, Hardy Diagnostics, Santa Maria, CA, USA) agar plates containing 100 μg/mL nalidixic acid and counting the presumptive colonies of *Salmonella* after the incubation period (24 h at 37 °C). The actual $\log_{10}$ CFU/mL levels of prepared inocula were (7.98, 8.68, 8.40, 8.70, 8.88) and (9.04, 9.04, 8.93, 8.40, 8.62) for the 5 replications of Experiments 1 and 2, respectively. The prepared inocula were serially diluted or not to obtain planned different levels of *Salmonella* respective to the replication and experiment.

Moisture analysis of litter samples

To determine moisture percentages, 10 g of litter from each flask was placed into an aluminum dish and dried in a drying oven for 48 h at 90°C. After drying, the litter was weighed, and the following equation was used to calculate moisture percentage:

[(Initial weight of litter before drying - Final weight of litter after drying)/ Initial weight of litter before drying] ×100

Microbial analysis of litter and dust Samples

Litter sample. Litter samples were analyzed for aerobic plate counts **(APC)**, *Salmonella*, *E. coli*, and coliforms. Each litter sample weighing 10 g was mixed with 90 mL of sterile saline solution and stomached for 1 min. Following serial dilution, in a duplicate manner, 1 mL from an appropriate dilution was plated onto 3M Petrifilm™ aerobic count plates (3M Health Care, Convey Ave, MN, USA) and 3M Petrifilm™ rapid *E. coli*/coliform count plates (3M Health care, Convey Ave, MN, USA) and 0.1 mL was spread onto XLT4 agar plates containing 100 µg/mL nalidixic acid. *E. coli*/coliform count petrifilm plates and XLT4 agar plates were incubated for 24 h at 37 °C and aerobic count petrifilm plates for 48 h at 37 °C. The counts were recorded for respective microbes after the incubation period. In the case of getting no detectable *Salmonella* or *E. coli* from litter samples, the original saline diluted samples were further incubated for 24 h at 37 °C and followed by streaking onto XLT4 and MacConkey (BBL™, Becton Dickinson and Company, Sparks, MD, USA) agar plates for Experiment 1 and onto CHROMAagar™ *Salmonella* plus base (CHROMagar, Paris, France) agar plates for Experiment 2. The streaked plates were incubated for 24 h at 37 °C before recording of final results.

Generated dust sample. Dust samples were analyzed for APC, *Salmonella*, *E. coli*, and coliforms as described for litter sampling. Firstly, 5 mL of BPW containing collected dust was serially diluted and used for direct microbial analysis, and the remaining BPW (5 mL) was incubated for 24 h at 37 °C and used for determination of microbial prevalence. The microbial counting of the sample was done by following the same procedure as described for the litter sampling.

Statistical analyses

The microbial counts from litter and generated dust samples were log transformed to $\log_{10}$ CFU/g and $\log_{10}$ CFU/L, respectively. For Experiment 1, one-way ANOVA was used to analyze treatment effect (*Salmonella* inoculum levels) on litter and dust bacterial levels. Means were separated using Tukey's HSD test, and level of significance set at $P \leq 0.05$. Simple logistic regression was used to analyze the relationship between litter *Salmonella* and *Salmonella* in dust. In Experiment 2, data from litter and dust samples were analyzed using one-way ANOVA with the different litter moisture ranges as the treatment. Means were separated using Tukey's HSD test, and the level of significance was set at $P \leq 0.05$. Simple logistic regression was used to analyze the relationship between litter moisture levels and *Salmonella* in dust samples. Prevalence data were statistically analyzed using Fisher's Exact test with significance at $P \leq 0.05$. All data were analyzed using SAS Studio, release 3.8 Enterprise Edition.

2.4 RESULTS AND DISCUSSION

Experiment 1

Using the developed *in vitro* system, bacteria were transferred from poultry litter to generated dust. Litter and dust APC are presented in Table 2.1. Overall average APC of litter and dust samples were 10.55 ± 0.03 $\log_{10}$ CFU/g and 4.92 ± 0.07 $\log_{10}$ CFU/L, respectively. The mean APC contribution by air that was collected from the flask with no litter was 2.28 ± 0.07 $\log_{10}$ CFU/L. APC of litter did not differ between replications ($P = 0.0699$). Homogeneity in APC in litter samples between replications was likely due to collecting litter from one pen to run all replications of Experiment 1, therefore, minimizing litter source or house location as a potential confounding factor. APC from generated dust did not differ except for the first replication which had significantly lower APC (4.42 ± 0.15 $\log_{10}$ CFU/L, $P = 0.0036$). A possible

explanation for the lower APC of dust samples during the first replication could be the increased restriction of dust entering the impingement system due to 4 layers of cheesecloth covering the dust intake tube of designed setup. In replications 2 through 5 only 2 layers of cheesecloth were used. APC of litter in this study are comparable with literature where wood shaving litter and straw litter had APC of 9.89 $\log_{10}$ CFU/g and 9.76 $\log_{10}$ CFU/g, respectively, during flock growout (Fries et al., 2005).

In the present study, litter was collected from one pen of a research broiler house to assess microorganism transfer from litter to dust in a simulation of maximum dust production. Litter moisture content did not vary between replications ($P = 0.7854$) and averaged 25.04% ± 0.09. Within the scope of this study, dust generated from the litter was not weighed or distinguished into settled dust versus airborne dust. The dust sampled in this study contained APC (4.92 $\log_{10}$ CFU/L) that could potentially settle or remain airborne. Additionally, particle sizes were not measured. Covering the pipet tip with 2 layers of cheesecloth allowed for particles of a small enough size to avoid clogging the impingement system, which allows for passage of particles up to 1 mm. Therefore, this study data cannot be directly compared with literature studies where bacteria were generally assessed separately from air and settled dust (Skóra et al., 2016; Yang et al., 2018). Even the differences in sampling procedures and instruments for air sampling can translate a significant variation in the measurement of microorganisms (Adell et al., 2014). More specifically, studies have shown that airborne aerobic bacteria can range from 0.385 to 4.484 × 10^4 CFU/m^3 in broiler houses, and culturable bacteria can be high as 2.9 × 10^6 CFU/m^3 in the air of poultry farms (Lawniczek-Walczyk et al., 2013; Yang et al., 2018). Settled dust, collected by locating 3 metal plates (2 at the ends and 1 at the middle for each sampled house) at 1.6 m height, from 10 broiler houses had an average level of 3.2 × 10^9 CFU/g of total

number of bacteria (Skóra et al., 2016). These results provide support to the conclusion of this study that poultry dust can carry a significant APC if the poultry houses offer the conditions for high levels of dust production.

Results of *E. coli* and coliforms of litter and generated dust samples are presented in Table 2.1. The overall average counts ($\log_{10}$ CFU/g) of *E. coli* and coliforms of litter samples in this experiment were 3.74 ± 0.22 and 4.48 ± 0.27, respectively. Only one dust sample was confirmed positive for *E. coli* with counts equivalent to 0.57 $\log_{10}$ CFU/L. This dust sample was generated from litter having 6.14 $\log_{10}$ CFU/g of *E. coli*. Four of the dust samples were confirmed positive for coliforms (0.57, 0.10, 0.10. 0.40 $\log_{10}$ CFU/L). The respective litter samples used to generate these 4 coliform positive dust samples had 6.52, 6.19, 5.86, 5.78 $\log_{10}$ CFU/g counts of coliforms, respectively. No *E. coli* or coliforms were detected from the air that was collected from the flask with no litter. *E. coli* has previously been recovered in the air of broiler sheds with levels ranging from 10^2 to 10^4 CFU/m^3, when the typical counts of *E. coli* in the litter were around 8 $\log_{10}$ CFU/g (Chinivasagam et al., 2009). With weekly analysis (total sampling for 7 wk growout period) of the environmental condition of broiler houses it was reported that *E. coli* and coliforms in air ranged from 0 to 0.89 and 0.77 to 2.96 $\log_{10}$ CFU/m^3, respectively. The same study found *E. coli* and coliforms in litter ranged between 0 to 2.85 and 1.39 to 4.73 $\log_{10}$ CFU/g, respectively (Hassan and Gherbawy, 2009). The discrepancy of present study results of *E. coli* and coliforms with literature might be explained by the relatively small volume of air sampled, the small volume of litter used in this study, the differences in litter types and age, the intentional generation of dust, or attachment of *E. coli* and coliforms with large particles which could not be transferred to the impinger collection media.

Salmonella recovery from litter and generated dust samples are shown in Table 2.2. *Salmonella* levels in litter ranged from 1.70 to 6.16 $\log_{10}$ CFU/g. Litter *Salmonella* levels were below the detection level (1.65 $\log_{10}$ CFU/g) when litter was inoculated with 10 mL of 10^2 to 10^3 $\log_{10}$ CFU/mL of *Salmonella* and allowed to equilibrate for 24 h. However, *Salmonella* prevalence was 100% from all inoculated litter samples (20/20). *Salmonella* enumeration results of 19 dust samples were below the level of detection (1.10 $\log_{10}$ CFU/L). One dust sample, generated from a litter sample having 5.54 $\log_{10}$ CFU/g of *Salmonella*, had 1.10 $\log_{10}$ CFU/L of *Salmonella*. Overall, *Salmonella* prevalence in dust samples obtained from the inoculated litter was 30% (6/20). No *Salmonella* were detected from the air that was collected from the flask with no litter. Significantly more *Salmonella* positive dust samples were obtained from litter samples with the highest levels of *Salmonella* (5.17 to 6.16 $\log_{10}$ CFU/g, $P = 0.0015$) than the lower inoculum levels. A scatterplot of the relationship between *Salmonella* levels of litter and generated dust samples is shown in Figure 2.2. Using logistic regression, increasing *Salmonella* levels found in litter were significantly related to *Salmonella* positive results in subsequent dust samples ($P = <0.0001$, $R^2 = 0.549$, n = 20).

Multiple studies have detected diverse bacteria from the air and settled dust in poultry houses, including *Salmonella* (Chinivasagam et al., 2009; Skóra et al., 2016). *Salmonella* has been found to remain viable at least for 2 h in laboratory generated aerosols (McDermid and Lever, 1996). However, investigating aerosolized *Salmonella* viability over time in commercial poultry houses may better elucidate its hazards for poultry. It has been observed that airborne *Salmonella* can attach to dust with particle size ranges from 0.65 to > 7 µm in diameter (Adell et al., 2014). Moreover, airborne transmission of *Salmonella* spp. between poultry can occur (Holt et al., 1998; Harbaugh et al., 2006). Previously, Chinivasagam et al. (2009) explained the

interrelationship between litter and aerosolized microorganisms in broiler sheds. They reported that the distribution of *E. coli* in broiler sheds demonstrates the litter-aerosol relationship. The microorganism transfer process occurred via the litter-dust-air interface. They consistently found that higher levels of *E. coli* in litter (~10^8 CFU/g) led to higher levels in the air (10^2 to 10^4 CFU/m^3). However, the same study did not find this relationship with *Salmonella* and *Campylobacter* due to their intermittent presence in litter and air. However, in this study, we observed that litter *Salmonella* levels play a significant role in dust being a carrier of *Salmonella*. Previously, the levels of *Salmonella* in settled dust and air have been reported as being varied between 1.1×10^5 to 6.3×10^5 CFU/g and 4.4 MPN/m^3 to 3.3×10^2 CFU/m^3, respectively, in poultry houses (Chinivasagam et al., 2009; Fallschissel et al., 2009; Skóra et al., 2016). However, generated dust samples (19/20) in this study did not contain a countable number of *Salmonella* even though some were *Salmonella* positive after enrichment. This might be due to a potentially lower quantity of dust collected in 20 L of air, collection of only smaller particle sizes, or competition with other bacteria inside the collection medium (Adell et al., 2014).

It is important to note that the levels and prevalence of bacteria of dust in this study are results of an *in vitro* system where dust production was maximized, to represent "worst case" scenario of dust levels in poultry facilities, by blowing air onto litter to form visible dust. It is anticipated that this degree of dust production would occur during an event of significant litter disruption, such as during harvest for processing, at the commercial poultry farms. Quantities of dust in the air during normal production would not be as high, therefore, expected to pose a lower risk of *Salmonella* transmission. It would be valuable to extend this work to evaluate the transmission of *Salmonella* and other bacteria both over the course of time as well as during the times of elevated dust production, such as harvest.

Experiment 2

The objective of Experiment 2 was to assess the effect of different levels of litter moisture content on transfer of bacteria to dust from litter, and the results of this study are given in Table 2.3. The range of 5 different litter moisture percentages (%) achieved in this study was:12.1 to 13.5, 17.0 to 19.0, 22.2 to 23.7, 27.2 to 29.0, and 32.3 to 34.8. Generally, the litter moisture content in broiler houses can vary from 15 to 57% based on several factors including litter type, ventilation, excreta, water leakage from drinkers, bird age, and growout season (Avcılar et al., 2018). The counts of aerobic bacteria, *Salmonella*, *E. coli*, and coliforms in the litter did not differ ($P > 0.05$) with moisture adjustment of litter. The counts ranged from 10.16 to 10.33, 5.92 to 5.95, 5.93 to 6.00, and 5.94 to 6.02 $\log_{10}$ CFU/g, for APC, *Salmonella*, *E. coli*, and coliforms, respectively. The mean APC contribution by air used to generate dust from litter, as determined with the flask without litter, was 1.90 ± 0.07 $\log_{10}$ CFU/L. No *E. coli*, coliforms, or *Salmonella* were detected from the air that was collected from the flask with no litter. For the dust samples, the increase in litter moisture content resulted in a significant decrease of enumerated counts of APC, *Salmonella*, *E. coli*, and coliforms for each attained moisture range. This may be because an increase in litter moisture content offered more closely binding of dust particles with litter that reduced dust generation from litter and, consequently, decreased bacterial counts in dust (Ogink et al., 2012). However, the quantity of dust generated at different litter moisture content was not measured within the scope of this study. APC of generated dust samples differed by 2.01 $\log_{10}$ CFU/L at the lowest and highest litter moisture range. It is interesting to note that *Salmonella*, *E. coli*, and coliforms in dust samples were below the detection level at the 2 highest ranges of litter moisture (27.2 to 34.8%). Moreover, dust samples obtained from the highest moisture litter samples were negative for *Salmonella*, *E. coli*, and

coliforms. This might be due to a decrease in dust generation with higher moisture levels. APC may have been more likely to be detected in dust due to the much higher levels present in the litter. Overall, litter moisture ranging from 12 to 24% offered an opportunity for a higher contamination of dust with litter microorganisms. The relationship between moisture levels of litter and *Salmonella* presence in generated dust is shown in Figure 2.3. Using logistic regression, increasing moisture levels found in litter led to *Salmonella* negative results in subsequent dust samples (P = <0.0001, R^2 = 0.651, n = 20).

Generally, airborne bacteria can be reduced by limiting dust production (Mitchell et al., 2004). This may be because of the attachment of airborne bacteria with fine dust particles (Zhao et al., 2014). Different approaches can be used to reduce dust levels in animal houses. Spraying techniques that prevent suspension or resuspension of dust particles in the air reduce dust levels (Zhao et al., 2014). Several studies assessed the effect of spraying agents, i.e., water, slightly acidic electrolyzed water, acidic electrolyzed water, to reduce airborne dust, and/or bacteria in poultry houses (Ogink et al., 2012; Zheng et al., 2014; Chai et al., 2018). Typically, humidification of bedding material with spraying agents can result in adhering of dust particles with litter, thereby decreasing dust generation (Ogink et al., 2012).

Previously, Ogink et al. (2012) reported that spraying of water 2 times per day at different rates (No water, 75 mL m^{-2}, 150 mL m^{-2}, 300 mL m^{-2}) on top of bedding material in laying hen houses resulted in reduction of dust particle emission (18 to 64% and 44 to 64% for particles of less than 10 µm and 2.5 µm in size, respectively). However, they observed an increase in ammonia emission (21 to 64%) linearly with an increase in dosing rates of spraying water. Although the high litter moisture content may reduce particulate matter emission from litter, it has also been observed in poultry facilities that high litter moisture levels are correlated

to severe footpad dermatitis, reduce bird's performance, negatively affect other animal welfare aspects (breast cleanliness, breast irritation, hock burn, and gait), and decrease carcass yield (Mayne et al., 2007; De Jong et al., 2014). High litter moisture content also can cause indirect adverse effects in poultry production houses by increasing ammonia production and emission, which is reported to negatively affect poultry health, by affecting the bird's respiratory system and decreasing the bird's ability to fight against infections, and impacting bird performance (Al Homidan et al., 2003; David et al., 2015). Similarly, Kim et al., (2006), while comparing 7 kinds of spraying additives to reduce dust levels and airborne microorganisms in slatted-floor swine houses, reported that the average reduction of all treatments after spraying was 30%, 53%, and 51% for dust, airborne bacteria, and airborne fungi, respectively, compared to their initial levels before spraying. The same study observed that the fluctuation of airborne bacteria and fungi with time after the spray treatments was "somewhat identical" to dust, because of the conjoint movement of airborne microorganisms and dust in the air. However, it has also been noted that airborne bacteria reduction can depend on properties of spraying agent (e.g., bactericidal effects) rather than on reduction of dust levels (Zheng et al. 2014).

Overall, the results of this *in vitro* work confirm that litter can be a source for aerobic bacteria, *Salmonella, E. coli*, and coliform transfer to dust that can be settled or aerosolized. Therefore, the assessment of litter *Salmonella* counts can be an indicator of potential dust *Salmonella* contamination. Litter moisture content plays a significant role in this transmission. Increasing levels of moisture tended towards lower dust contamination with bacteria. However, the drawbacks of increasing litter moisture content need to be considered. Based on this study, the development of mitigation methods to control dust contamination is recommended due its potential role in *Salmonella* transmission.

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Table 2.1 Bacteria recovery from litter and dust samples from an *in vitro* dust production system, Experiment 1.

Replication	Litter Samples[3] $\log_{10}$ CFU/g ± SE[2]			Dust Samples[3] $\log_{10}$ CFU/L ± SE	Dust Samples[3] Prevalence ($\log_{10}$ CFU/L)	
	APC[1]	*E. coli*	Coliforms	APC	*E. coli*	Coliforms
1	10.55 ± 0.04	3.50 ± 0.06	4.07 ± 0.48	4.42 ± 0.15 [b]	0/4	0/4
2	10.68 ± 0.05	3.84 ± 0.58	4.75 ± 0.74	5.16 ± 0.09 [a]	0/4	1/4 (0.57)
3	10.58 ± 0.05	3.36 ± 0.53	4.26 ± 0.82	4.97 ± 0.07 [a]	0/4	1/4 (0.10)
4	10.41 ± 0.09	4.54 ± 0.54	5.08 ± 0.53	5.06 ± 0.09 [a]	1/4 (0.57)	1/4 (0.10)
5	10.54 ± 0.04	3.31 ± 0.33	4.24 ± 0.58	5.01 ± 0.15 [a]	0/4	1/4 (0.40)
Means	10.55 ± 0.03	3.74 ± 0.22	4.48 ± 0.27	4.92 ± 0.07	1/20 (0.57)	4/20 (0.29)
P-value	0.0699	0.3601	0.7854	0.0036	1.000	1.000

[1]APC = Aerobic plate counts.
[2]SE = Standard error.
[3]Litter and dust samples used n = 4/replication/bacteria (or total n = 20/bacteria) for their respective microbial analyses.
[a-b]Means within a column with different superscripts differ significantly $P \leq 0.05$.

Table 2.2 *Salmonella* recovery from litter and dust samples from an *in vitro* dust production system, Experiment 1.

S.[1] Inoculation (CFU/mL)	Litter Samples[3]		Dust Samples	
	S. counts $\log_{10}$ CFU/g	*S.* Prevalence # positive/# sampled	*S.* counts $\log_{10}$ CFU/L	*S.* Prevalence # positive/# sampled
10^2,10^3	ND[2]	5/5	ND	0/5 [b]
10^4,10^5	1.70–2.18	5/5	ND	0/5 [b]
10^6,10^7	3.24–4.46	5/5	ND	1/5 [b]
10^8,10^9	5.17–6.16	5/5	ND–1.10	5/5 [a]
P-value	-	-	-	0.0015

[1]*S.* = *Salmonella*.
[2]ND = *Salmonella* counts of litter or dust samples were below the level of detection (1.65 $\log_{10}$ CFU/g for litter and 1.10 $\log_{10}$ CFU/L for dust).
[3]Litter and dust samples used n = 5/*S.* inoculation (or total n = 20) for their respective *S.* counts assessment.
[a-b]Values within a column with different superscripts differ significantly $P \leq 0.05$.

Table 2.3 Effect of litter moisture contents on transfer of aerobic bacteria, *Salmonella*, *E. coli*, and coliforms from litter to dust samples, Experiment 2.

Moisture range	Litter counts[4] $\log_{10}$ CFU/g				Dust counts[4] and Prevalence $\log_{10}$ CFU/L (# positive / # sampled)			
	APC[1]	*Salmonella*	*E. coli*	Coliforms	APC	*Salmonella*	*E. coli*	Coliforms
12.1–13.5[2]	10.16 ± 0.12	ND[3]	6.00 ± 0.01	6.02 ± 0.01	6.15 ± 0.05 [a]	ND (0/5) [z]	3.51 ± 0.07 [a] (5/5) [y]	3.51 ± 0.07 [a] (5/5) [y]
17.0–19.0	10.33 ± 0.05	5.92 ± 0.08	5.99 ± 0.01	6.00 ± 0.01	5.55 ± 0.06 [b]	2.83 ± 0.03 [a] (5/5) [y]	2.59 ± 0.06 [b] (5/5) [y]	2.59 ± 0.06 [b] (5/5) [y]
22.2–23.7	10.19 ± 0.05	5.95 ± 0.06	5.99 ± 0.02	6.01 ± 0.02	5.25 ± 0.05 [c]	1.85 ± 0.08 [b] (5/5) [y]	1.78 ± 0.04 [c] (5/5) [y]	1.78 ± 0.04 [c] (5/5) [y]
27.2–29.0	10.32 ± 0.04	5.92 ± 0.06	5.93 ± 0.06	5.94 ± 0.06	4.59 ± 0.08 [d]	ND (2/5) [yz]	ND (3/5) [yz]	ND (3/5) [yz]
32.3–34.8	10.31 ± 0.07	5.93 ± 0.06	5.95 ± 0.02	6.00 ± 0.03	4.14 ± 0.03 [e]	ND (0/5) [z]	ND (0/5) [z]	ND (0/5) [z]
P-value	0.2827	0.9875	0.3488	0.4412	<0.0001	<0.0001 (<0.0001)	<0.0001 (0.0004)	<0.0001 (0.0004)

[1]APC = Aerobic plate counts.
[2]12.1–13.5 = Litter samples of this moisture range were not inoculated with *Salmonella*.
[3]ND = Bacteria counts were below the level of detection. These data observations were not included in means.
[4]Litter and dust counts assessment used n = 5/moisture range/bacteria (or total n = 25/bacteria).
[a-c]Means within a column corresponding to log CFU/L count data with different superscripts differ significantly $P \leq 0.05$.
[y-z]Values within a column corresponding to prevalence data with different superscripts differ significantly $P \leq 0.05$.

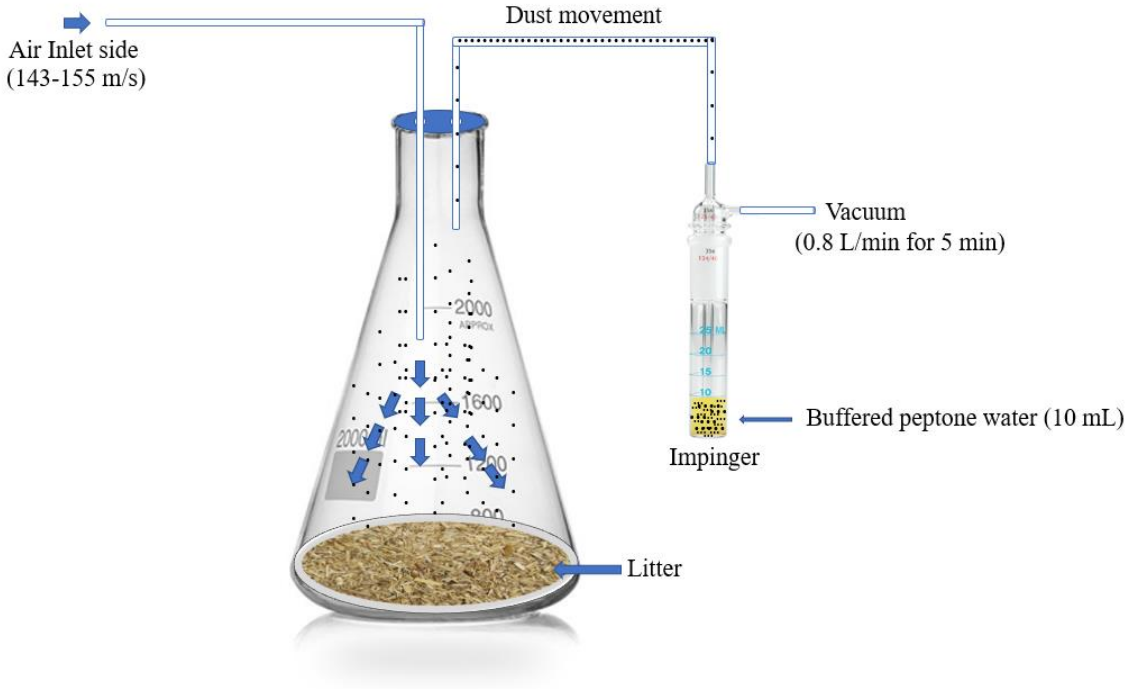

Figure 2.1 Schematic view of mechanism of impingement for experiment 1 and 2.

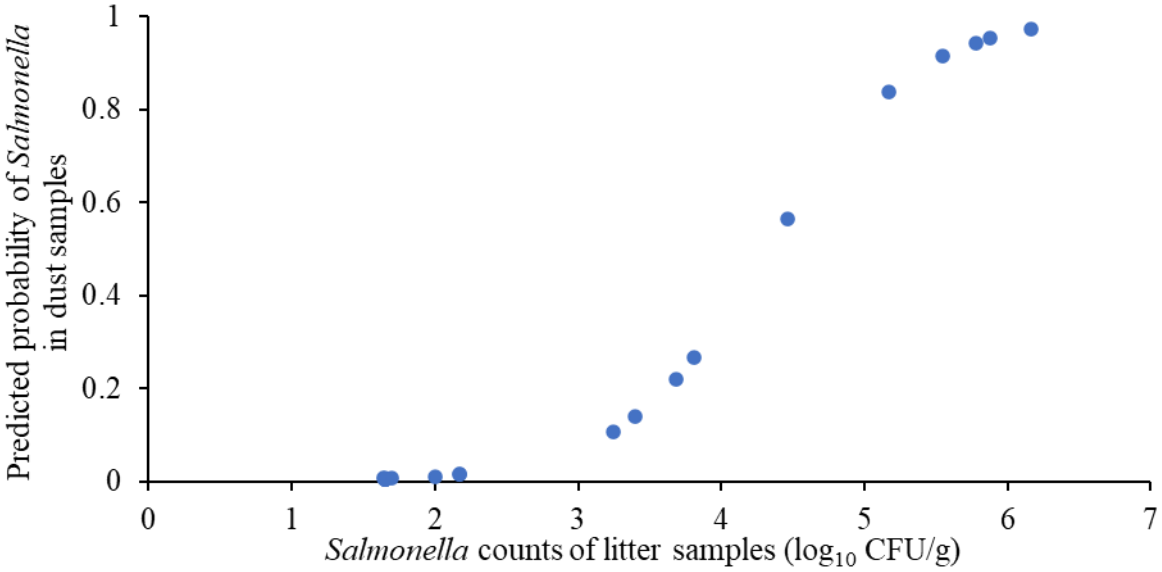

Figure 2.2 Scatterplot of predicted probability from logistic regression model of the presence of *Salmonella* in dust samples in relation *Salmonella* counts in litter samples. The graph equation is [ln (y/1-y) = -8.4434+1.9505 (*Salmonella* counts of litter)]. $R^2 = 0.549$.

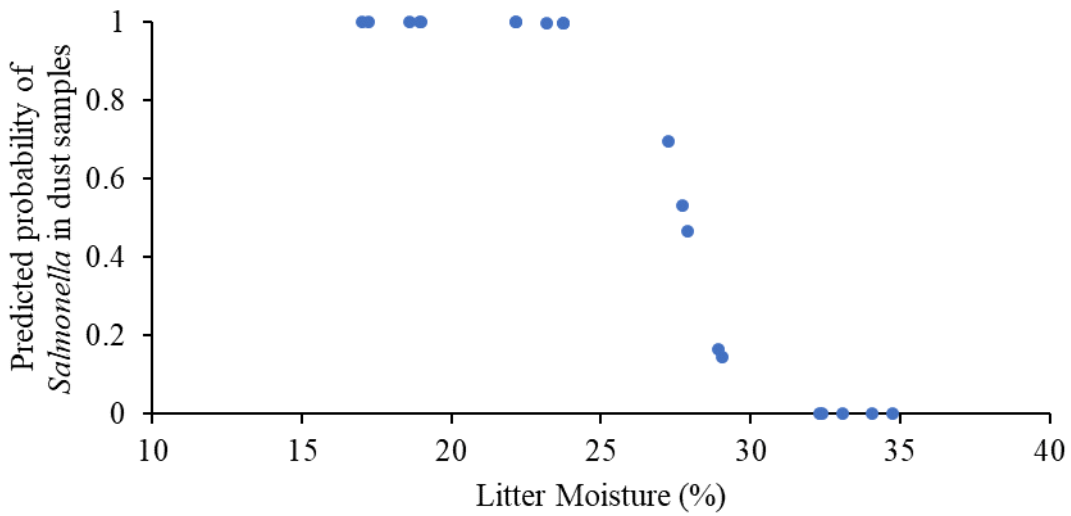

Figure 2.3 Scatterplot of predicted probability from logistic regression model of the presence of *Salmonella* in dust samples in relation litter moisture contents. The graph equation is [ln (y/1-y) = 40.3163 -1.4492 (litter moisture %)]. R^2 = 0.651.

CHAPTER 3: BACTERIAL COMPOSITION OF SETTLED DUST DURING GROWOUT OF BROILER CHICKENS

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3.1 ABSTRACT

Dust present in poultry houses can disseminate bacteria in air and deposit them on surfaces. This study evaluated bacteria in settled dust during growout of broilers. Dust bacteria were analyzed from two flocks (Flocks A and B). Birds in Flock B were inoculated with *Salmonella* Enteritidis. Dust samples for bacteria analyses were obtained during 6 wks of growout (Flocks A and B) and 1 wk after bird harvest (Flock B) by environmental swabbing and collecting dust in petri dishes from different locations inside the poultry house. For weekly swabbing, dust deposited during each wk of sampling period (non-cumulatively, n=12/wk) and cumulatively (n=12/wk) throughout the sampling period was sampled from 6 duplicate locations. Swabbed dust samples were analyzed for counts ($\log_{10}$ CFU/28 cm^2) of aerobic bacteria, *E. coli*, coliforms, and *Salmonella* prevalence. For petri dish dust collection, dust was collected in weekly and bi-weekly time spans during sampling period from 3 duplicate locations and then analyzed for *Salmonella* prevalence. Data were analyzed by one-way ANOVA and Fisher's Exact Test and means were separated using LSD. Only aerobic plate counts were changed over time in dust during growout (Flocks A and B; $P < 0.0001$, $P < 0.0001$). In non-cumulatively

settled dust, aerobic bacteria (Flocks A and B; $P < 0.0001$, $P < 0.0001$), *E. coli* (Flock A; $P = 0.0432$), and coliforms (Flock B; $P = 0.0303$) varied during growout with peak counts on wk 5 or wk 6, wk 4, and wk 4, respectively, after bird placement. *Salmonella* prevalence did not vary in cumulatively (3/72, 10/84) and non-cumulatively (0/12, 10/84) settled dust during growout in both flocks. In dust sampled by bi-weekly collection in petri dishes, *Salmonella* prevalence was highest (5/6) between wk 2 to wk 4 for Flock B ($P = 0.0118$). Overall, this study displayed that settled dust bacteria numbers fluctuated during broiler growout, and dust can contain *Salmonella*.

Key Words: Dust, *Salmonella*, Broiler, Poultry house, bacteria

3.2 INTRODUCTION

Dust in poultry houses is comprised of various constituents including feathers, skin debris, feed, litter, and fecal matter and all of these can be carriers of bacteria, fungi, and viruses (Madelin and Wathes,1989). In animal houses, dust generated from different sources (feed, animals, feces, urine, bedding) can deposit on surfaces (settled dust) or become airborne (airborne dust) due to different activities or forces. Moreover, airborne dust can settle on surfaces again, and vice versa (Aarnink and Ellen, 2007). Generally, litter is a main source of dust in broiler houses, where animal activities and air movements disturb litter that leads to the generation of airborne dust particles (Al Homidan et al., 2003).

Bacteria can enter poultry houses through multiple sources. The potential spreading routes and sources of bacteria can be prioritized to investigate strategies to decrease live bird and poultry product contamination (Kwon et al., 2000). Poultry dust can also be an area of control to prevent contamination of birds during production. Dust plays a role in the transportation of microorganisms in the air by acting as their carrier (Zhao et al., 2014). Previously, the presence of potentially pathogenic microorganisms (*Klebsiella pneumoniae, Enterococcus* spp., *E. coli*,

Salmonella spp., etc.) has been noted in poultry settled dust and air (Skóra et al., 2016; Yang et al., 2018**)**. Moreover, dust can play a role in airborne transmission of pathogenic bacteria in poultry houses. It has been reported that eggs hatching can generate *Salmonella* contaminated dust and fluff, which may circulate within the hatcher and potentially colonize the healthy chicks if placed in the same hatcher (Davies and Wray, 1994). Airborne dust may act as a one of primary means to spread disease causing microorganisms in poultry houses and reduction in concentration of airborne dust can help to decrease airborne microorganism levels (Mitchell et al., 2004).

Levels of airborne microorganisms and dust in animal houses are influenced by different factors such as animals (age, weight, activity, and density), housing types (aviary vs cage system, natural vs ventilation system), and management system (feed management, ventilation management, and hygienic conditions) (Zhao et al., 2014). However, these factors and their influences in relation to settled dust and its microorganism levels have not been well defined. Chinivasagam et al. (2009) suggested that microorganism aerosolization process occurs via the litter-dust-aerosol interface, therefore, the pathogens present in settled dust can enter into the air. Airborne dust levels can be decreased by preventing the generation of dust from their sources (Takai, 2007). Therefore, changes in settled dust levels and microflora during growout is important to take into consideration as they can dictate the levels and generation mechanisms of airborne microorganisms. Moreover, the settled dust microorganisms, present on the floor, are in closer proximity to the birds than airborne microorganisms and thus can be a potential hazard for poultry health. Apart from this, the dosage of spraying disinfectant, one of the mitigation methods for airborne microorganisms and dust in poultry houses, was studied to determine its capability to reduce bacteria from air and litter (Chai et al., 2018). However, settled dust,

deposited on walls and ceilings of poultry houses, can resuspend in the air, and thereby may influence airborne bacteria levels (Aarnink and Ellen, 2007; Banhazi et al., 2008). Therefore, the study of settled dust also has importance in devising control measures for dust contamination at the poultry production house.

Evaluating settled dust has an important role in understanding microorganism distribution in the poultry house environment and devising strategies to prevent contamination from dust carrying microorganisms at poultry production houses. Therefore, the objective of this study was to assess the changes in levels of aerobic plate counts **(APC)**, *E. coli*, coliforms, and *Salmonella* in settled dust during growout of broilers.

3.3 MATERIALS AND METHODS

This study was performed in an experimental broiler house at the Miller Center of Auburn University. All procedures used in this study were approved by the Auburn University Institutional Animal Care and Use Committee (IACUC) (PRN #2019-3621). Environments from 2 broiler flocks, July to August, 2020 (Flock A); October to December, 2020 (Flock B), were sampled for microbiological analyses of litter and settled dust. In each flock, a total of 1,200 birds (25 per pen) were harvested at 42 days of age. The litter, in the sampled house of this study, had been seeded with nalidixic acid resistant *Salmonella* Enteritidis by bird inoculation in the first flock that was placed on fresh bedding. In this study, Flock A and Flock B were the third and fifth flock reared on the same litter in the same house, respectively. In Flock B, all chicks were administrated with an oral gavage of nalidixic acid resistant *Salmonella* Enteritidis (10^7 CFU/bird) at d 7 of age. Litter and settled dust sampling was conducted on the day of bird placement (litter only), after 1, 2, 3, 4, and 5 wks of growout with birds present, 1 d following

bird harvest at 6 wk, and 1 wk following harvest (wk 7 for Flock B only). Humidity and temperature data from Flocks A and B are provided in Table 3.1.

Litter sampling methods

Litter grab method. Litter grab samples were collected from two pens on each sampling day in both flocks. A total of four (two samples/pen) litter samples were taken on each sampling day. For each sample, litter was collected from multiple locations inside the pen (adjacent to the feeder, underneath the water lines, and from the middle area between feeder and water lines) into a clean bag (Ziploc, Chicago, IL) and then transferred immediately to ice. Upon arrival to the laboratory (within 30 min after sampling), for each sample, bag was hand shaken to mix litter and then 10 g of litter was transferred into a sterile sampling bag (VWR International, LLC, Radnor, PA, USA). Next, 90 mL of buffered peptone water **(BPW)** (Becton Dickinson and Company, Sparks, MD, USA) was added and then stomached for 1 min. After that, 10 ml of the mixed sample was removed and stored at 4 °C for later use. From the remaining litter sample, aliquots were serially diluted in sterile saline and used for enumeration of APC, *E. coli*, and coliforms. The diluted samples were duplicate plated onto 3M Petrifilm™ aerobic count plates (3M Health Care, Convey Ave, MN, USA) and 3M Petrifilm™ rapid *E. coli*/coliform count plates (3M Health care, Convey Ave, MN, USA), and then incubated for 48 h at 37 °C or 24 h at 37 °C, respectively. After incubation, colonies were enumerated for APC, *E. coli*, and coliforms (ISO method 4832 was used for coliform enumeration). The remaining litter sample (88 mL) was incubated at 37 °C for 24 h for enrichment. After 24 h, the enriched sample was evaluated for *Salmonella* detection by streaking onto Xylose Lysine Tergitol-4 **(XLT4)** (Criterion, Hardy Diagnostics, Santa Maria, CA, USA) agar plates and using the 3M^{TM} molecular detection system (Saint Paul, MN, USA). The 3M^{TM} molecular detection system was used by following

instrument and *Salmonella* kit instructions. Streaked XLT4 agar plates were incubated at 37 °C for 24 h before confirming presumptive isolated *Salmonella* colonies using *Salmonella* agglutination test (Difco™ *Salmonella* O Antiserum Poly A–I and Vi, Becton Dickinson and Company, Sparks, MD, USA).

Boot swab method. Two boot swabs (one swab per pen) were taken on each sampling day from the same pens in both flocks. For each sample collection, one boot was covered with a pre-moistened shoe cover (Hardy Diagnostics, Santa Maria, CA) upon entering the pen. After jumping on different locations inside the pen, the shoe cover was placed back into its bag (Whirl-Pak® Bag) and then immediately transferred to ice. At the laboratory, 60 mL BPW was added to each sample bag which was then incubated for 24 h at 37 °C for enrichment. Enriched samples were analyzed for *Salmonella* detection as described for litter grab samples.

Settled dust sampling methods

A total of 12 locations, six on each of the two opposite diagonal corners of the house (Light trap, baffle, wall, floor, railing top inside the pen, and bottom ridge of empty pen), were fixed for dust sampling, using environmental swabs, for both flocks (Figure 3.1). The light trap sampling location was a vertical surface, 145 cm in vertical height from the floor and 813 cm in horizontal distance from the nearest pen containing birds. The baffle sampling location was a fixed horizontal surface just beneath the air intake baffle, 224 cm in height from the floor and 203 cm in distance from the nearest pen containing birds. The wall sampling location was a protruded ridge surface with a 45º angled surface on the wall, 155 cm in height from the floor and 203 cm in distance from the nearest pen containing birds. The floor sampling location was a horizontal surface, 196 cm in distance from the nearest pen. The railing top inside the pen sampling location was a horizontal surface, 59 cm in height from the floor. Finally, the bottom

ridge of empty pen sampling location was a horizontal surface, 4 cm in height from the floor and 455 cm in distance from the nearest pen containing birds. Locations sampled on the two opposite diagonal corners were at the same places and distances. These locations were not cleaned during the sampling period although routine sweeping of the floor was performed. Dust swab samples for bacteria analyses were obtained during 6 wks of growout (Flocks A and B) and 1 wk after bird harvest (Flock B). Bacteria in dust deposited during each wk of sampling period (non-cumulatively, n = 12/wk) and cumulatively (n = 12/wk) throughout the sampling period were analyzed on each location. All locations were cleaned and sanitized on the day of bird placement with 70% ethanol. Non-cumulatively settled dust bacteria were analyzed by cleaning and sanitizing the sampled area on each location following every weekly sample collection. Cumulatively settled dust bacteria were analyzed by sampling an area adjacent to the previously sampled area at each location. For settled dust sample collection from each of the 12 locations, a swab moistened in a non-nutrient phosphate buffered neutralizing solution (902C, Copan Diagnostics Inc., Murrieta, CA, USA) was used to swab an area of 28 cm^2. After swabbing, the swab was inserted into a transport tube containing 10 mL of the non-nutrient phosphate buffered neutralizing solution (the handle end snapped off) and then transferred to ice. After reaching the laboratory, each swab sample tube was vortexed and then a 5 mL aliquot of sample was taken out and added to 5 mL double-strength BPW, which was then incubated at 41.5 °C for 24 h. This incubation temperature and time for swab samples was selected based on the enrichment recommendation guidelines for environmental swab samples of 3M™ molecular detection system. Enriched samples were used to assess *Salmonella* prevalence in the same manner as described in the litter grab method. The remaining non-enriched swab containing solution (5 mL)

was used for enumeration of APC, *E. coli*, and coliforms. The plating and incubation of these specified bacteria was performed as described previously.

In addition to swab sampling of dust, the dust deposited within a one wk and two wk time span was collected by placing empty petri dishes (100 ×15 mm) at 6 locations (three on each of the two opposite diagonal corners of the house) at a 25 cm vertical height (Figure 3.1). The locations on both opposite diagonal corners were at same places and distances such as location A (near to light trap and 721 cm in horizontal distance from the nearest pen containing birds), location B (close to the wall and 203 cm in distance from the nearest pen containing birds), and location C (13 cm in distance from the nearest pen containing birds) (Figure 3.1). Two petri dishes were placed next to each other on the day of bird placement at each location (A, B, and C for each house corner, n=6 samples) for both Flocks A and B. After 1 wk, one petri dish was removed from each location for dust sampling and replaced with a sterile petri dish. After 2 wks, both petri dishes were removed for dust sampling and replaced with two sterile petri dishes. Dish collection and placement continued for the entire growout and 1 wk following flock harvest (Flock B). During sample collection, a lid was placed on each petri dish, sealed with parafilm, and then samples were transported to the laboratory. In the laboratory, 20 mL of BPW was added to each petri dish while simultaneously swirling the petri dish by hand in order to suspend dust in BPW. Next, each petri dish was incubated at 41.5 °C for 24 h for enrichment. After 24 h, the enriched sample was used for the analysis of *Salmonella* detection as described previously.

Statistical analyses

The bacterial counts from dust and litter samples were transformed into $\log_{10}$ CFU/28cm^2 and $\log_{10}$ CFU/g, respectively, before statistical analyses. Data for each flock were analyzed separately. One-way ANOVA was used to analyze the week-wise or sample location-wise

variation of bacterial counts and means were separated using LSD at $P \leq 0.05$ level of significance. *Salmonella* prevalence data were analyzed using Fisher's exact test. SAS Studio, release 3.8 Enterprise Edition was used to analyze the data.

3.4 RESULTS

Data for week-wise variation of APC in cumulatively and non-cumulatively settled dust and litter for both flocks are shown in Table 3.2. For both Flocks A and B, aerobic bacteria increased significantly in cumulatively ($P < 0.0001$, $P < 0.0001$) and non-cumulatively ($P < 0.0001$, $P < 0.0001$) collected dust during the growout of broilers. In cumulatively collected dust samples of Flock A, aerobic bacteria increased during the first 3 wks of growout from 5.38 to 7.09 $\log_{10}$ CFU/28 cm^2 and then remained constant between wk 4 to wk 6 (7.73 to 8.25 $\log_{10}$ CFU/28 cm^2). In Flock B, aerobic bacteria in cumulatively collected dust remained constant for the first 5 wks of growout (6.71 to 6.87 $\log_{10}$ CFU/28 cm^2), then increased at wk 6 following flock termination (8.25 $\log_{10}$ CFU/28 cm^2) and followed by a reduction at wk 7 to pre-termination levels. Non-cumulatively collected dust samples from both flocks had a similar trend for aerobic bacteria during growout as observed in their respective cumulatively collected dust samples. APC in non-cumulatively collected dust ranged between 5.38 to 7.92 $\log_{10}$ CFU/28 cm^2 and 6.67 to 7.92 $\log_{10}$ CFU/28 cm^2 for Flocks A and B, respectively. APC in litter varied weekly during growout ($P = 0.0002$) only for Flock A, where litter aerobic bacteria ranged from 8.35 to 10.20 $\log_{10}$ CFU/g with the highest counts occurring between wk 2 to wk 6 of growout. For Flock B, aerobic bacteria ranged from 8.81 to 10.10 $\log_{10}$ CFU/g in litter and did not differ over time during growout.

Results of weekly variation of *E. coli* counts in cumulatively and non-cumulatively settled dust and litter samples for Flocks A and B are presented in Table 3.3. *E. coli* did not vary

during the growout period in cumulatively settled dust for either flock ($P = 0.1298$, $P = 0.1597$). *E. coli* counts in cumulatively settled dust ranged between 1.12 to 2.34 $\log_{10}$ CFU/28 cm^2 and 1.62 to 2.57 $\log_{10}$ CFU/28 cm^2 for Flocks A and B, respectively. Non-cumulatively collected dust *E. coli* levels were significantly different at different sampling days during growout only in Flock A ($P = 0.0432$). In non-cumulative settled dust samples for Flock A, *E. coli* counts were the lowest at wk 1 (1.12 $\log_{10}$ CFU/28 cm^2), highest at wk 4 (2.46 $\log_{10}$ CFU/28 cm^2), and intermediate (1.67 to 2.15 $\log_{10}$ CFU/28 cm^2) at rest of the sampling days during growout. For Flock B, non-cumulatively settled dust *E. coli* levels did not differ over time ($P = 0.2707$) and ranged from 1.47 to 2.28 $\log_{10}$ CFU/28 cm^2. Litter *E. coli* levels for both flocks varied during growout ($P < 0.0001$, $P = 0.0011$). For both flocks, litter *E. coli* counts began low at bird placement (3.01 $\log_{10}$ CFU/g, 5.47 $\log_{10}$ CFU/g), peaked at wk 2 after bird placement (8.68 $\log_{10}$ CFU/g, 8.16 $\log_{10}$ CFU/g), and then gradually declined through wk 6 (Flock A, 5.92 $\log_{10}$ CFU/g), or remained constant from wk 2 to wk 7 of growout (Flock B).

Weekly coliform counts in cumulatively and non-cumulatively settled dust and litter samples for Flocks A and B are shown in Table 3.4. Coliforms in cumulative settled dust did not vary over time during growout in either flock ($P = 0.7919$, $P = 0.3454$). Coliforms counts ranged between 1.85 to 2.40 $\log_{10}$ CFU/28 cm^2 and 1.59 to 2.62 $\log_{10}$ CFU/28 cm^2 in cumulative settled dust for Flocks A and B, respectively. Coliforms in non-cumulative settled dust samples for Flock A did not differ over time and ranged from 1.77 to 2.36 $\log_{10}$ CFU/28 cm^2. Coliforms present in dust deposited during each wk of growout (non-cumulative) were significantly different by weeks in Flock B ($P = 0.0303$), where counts were lowest at wk 5 (1.54 $\log_{10}$ CFU/28 cm^2) and wk 7 (1.42 $\log_{10}$ CFU/28 cm^2) compared to wk 4 (2.50 $\log_{10}$ CFU/28 cm^2). Coliforms counts on the rest of the sampling days were at intermediate levels. Like *E. coli*, litter

coliforms levels changed during growout in both flocks ($P < 0.0001$, $P = 0.0009$). The highest counts of coliforms in litter were obtained at wk 2 after bird placement in both flocks (8.69 $\log_{10}$ CFU/g, 8.19 $\log_{10}$ CFU/g). After the highest peak, litter coliform counts decreased through wk 6 (Flock A) or remained constant (Flock B).

Weekly changes in *Salmonella* prevalence in cumulatively and non-cumulatively settled dust and litter samples for Flocks A and B are shown in Table 3.5. *Salmonella* prevalence in cumulatively and non-cumulatively settled dust did not differ across time points during growout in either flock (Flock 1: $P = 0.4205$, $P = 1.000$, Flock 2: $P = 0.4622$, $P = 0.7656$). In cumulatively settled dust, *Salmonella* prevalence was 3/72 and 10/84 for Flocks A and B, respectively. *Salmonella* prevalence in non-cumulatively settled dust was 0/72 and 10/84 for Flocks A and B, respectively. Overall, *Salmonella* prevalence in dust for Flock A (3/144) was lower than Flock B (20/168) ($P < 0.0001$). For Flock A, there were 3 *Salmonella* positive litter grab samples on wk 3 of growout and all boot swab samples were negative. For Flock B, *Salmonella* detection varied at different sampling weeks in litter grab samples (20/32), where the highest number of *Salmonella* positive samples were observed at wk 3 and wk 5 of growout, and wk 7 following bird termination. *Salmonella* prevalence (12/16) for boot swab samples of Flock B did not differ between weeks but followed a similar trend as was observed for their respective litter grab samples.

Bacterial levels or prevalence at each sampled location in cumulatively settled dust for Flocks A and B are shown in Table 3.6. In cumulatively settled dust samples for Flocks A and B, aerobic bacteria (7.85 $\log_{10}$ CFU/28 cm^2, 7.60 $\log_{10}$ CFU/28 cm^2), *E. coli* (2.32 $\log_{10}$ CFU/28 cm^2 Flock A), and coliform (2.73 $\log_{10}$ CFU/28 cm^2, 2.56 $\log_{10}$ CFU/28 cm^2) counts were highest on the floor. But *E. coli* counts in Flock B were highest in dust deposited on top of pen railing (2.36

$\log_{10}$ CFU/28 cm^2). Dust settled cumulatively on the light trap had lowest bacterial counts in most cases. *Salmonella* prevalence in cumulatively settled dust differed by sampling locations only in Flock B ($P = 0.0311$), where dust on floor and top of railing inside the pen had the highest *Salmonella* prevalence.

Bacterial levels or prevalence at each sampled location in non-cumulatively settled dust for Flocks A and B are presented in Table 3.7. In non-cumulatively collected dust, APC differed at different sampling locations in both flocks ($P = 0.0346$, $P = 0.0046$), however, *E. coli* and coliforms counts differed by sampling locations only in Flock B. For both Flocks A and B, APC (7.66 $\log_{10}$ CFU/28 cm^2, 7.47 $\log_{10}$ CFU/28 cm^2) were highest in dust deposited on the floor. For Flock B, *E. coli* (2.22 $\log_{10}$ CFU/28 cm^2) and coliforms (2.50 $\log_{10}$ CFU/28 cm^2) were also highest in dust settled on floor. Dust settled non-cumulatively on the light trap generally had lowest bacterial counts. *Salmonella* prevalence was not affected by sampling locations in non-cumulatively deposited dust for both flocks.

S*almonella* detection in dust collected, by placing petri dishes, within weekly and biweekly time spans during growout is shown in Table 3.8. *Salmonella* prevalence in dust sampled by weekly collection did not significantly differ during growout for either flock ($P = 1.000$, $P = 0.0678$). Overall, *Salmonella* prevalence in dust settled in petri dishes within a 1 wk time span was 1/36 and 18/42 for Flock A and Flock B, respectively. In dust sampled by bi-weekly collection, *Salmonella* prevalence was highest between wk 2 to wk 4 for Flock B ($P = 0.0118$).

3.5 DISCUSSION

Poultry house dust is a mixture of dander, feed, bedding material, and microorganisms (Lenhart and Olenchock, 1984; Radon et al., 2002; O'Brien et al., 2016). The major hazard of

dust in poultry houses is its capability to spread disease causing microorganisms (Mitchell et al., 2004). Dust producing sources contribute differently to dust production depending on animal types and their house infrastructure, and several factors related to animal, housing, and management practices can influence dust and its associated microorganism levels in animal production facilities (Zhao et al., 2014). In this study, the variation of bacteria levels in settled dust during growout of broilers was analyzed. We observed from non-cumulatively settled dust that bacteria levels deposited during different wks of growout may or may not vary. Aerobic bacteria (Flock A and Flock B), *E. coli* (Flock A), and coliforms (Flock B) were found to vary in dust accumulated during different wks of growout. The possible reason for this fluctuation in bacteria levels could be the variation of dust levels during different stages of rearing. Dust production levels can be affected with animal age, animal weight, and their activities (Zhao et al., 2014). Previously, Calvet et al. (2009) reported that dust levels increased with the age of the poultry birds. They found that particulate matter, with diameter of 10 μm or less, increased from 0.10 to 2.82 mg/m^3 and 0.05 to 0.79 mg/m^3 during wk 1 to wk 5 of the growing cycle in light and dark period, respectively. In the same study, dust concentration had strong positive correlation with bird activities and their live weight ($r^2 = 0.89$), and bird activities found to change with bird age and lighting status of the house. In the current study, we observed similar trends where bacterial levels in dust for a set area tended to increase with increasing bird age. Measurement of the dust sample weight was not within the scope of this study. However, the quantity of dust production is likely to have played a major role in the increases in dust bacterial counts.

We observed that only APC were increased over time during growout in cumulatively settled dust in either flock. Previously, aerobic bacteria in airborne dust were found to have an increasing concentration of 0.91×10^3 CFU/m^3, 6.86×10^3 CFU/m^3, and 13.77 CFU $\times 10^3$

CFU/m^3 at d 3, d 22, and d 40, respectively, following bird placement (Jiang et al., 2018). In terms of settled dust, Skóra et al. (2016) reported an average of 3.2×10^9 CFU of total bacteria and 1.6×10^5 CFU of *E. coli* per gram of the dust when sampled from 10 broiler houses. In this study, *E. coli* and coliforms remained stable in cumulatively settled dust during growout from either flock. This may be due of an inability of *E. coli* and coliforms to persist in dust for longer time periods. Likewise, Wójcik et al. (2010) observed stable variation of fungi counts in air over time during the growing cycle when sampled in summer and winter from inside and outside of 3 rooms.

Salmonella was also detected in cumulatively (Flock A, Flock B) and non-cumulatively (Flock B) settled dust in this study. Previously, Skóra et al. (2016) also observed the presence of *Salmonella* in settled dust and their levels ranged between 1.1 to 6.3×10^5 CFU/g. It is interesting to note that the overall prevalence of *Salmonella* in dust collected non-cumulatively and cumulatively during the growout were not different from each other in Flock B. This finding indicates that *Salmonella* may be continuously transferred in dust each wk during growout but was unable to remain viable over time, and thus failed to increase in an additive manner. Specifically, for *Salmonella*, Chinivasagam et al. (2009) reported that multiple factors can influence *Salmonella* survivability in dust and *Salmonella* resilience to poultry environment conditions can vary according to different serovars. Moreover, *Salmonella* was found in dust one week after Flock B termination despite its absence in swab samples and low prevalence in petri dish samples on the day following birds harvest (wk 6 + 1 d). These results underlay the possibility of dust to act as a horizontal means of *Salmonella* transmission to the new flock entering in the house. Previously, Broennum Pedersen et al. (2008) confirmed the persistence of *Salmonella* Senftenberg for more than 2 years in one of the sampled poultry houses which had

undergone cleaning, disinfections, and desiccation once during the sampling period. Additionally, in Flock B, during the sampling period of 7 wks, the dust collected by the petri dish method had higher overall *Salmonella* prevalence from weekly (18/42 or 43%) and bi-weekly (6/18 or 33%) collected dust than the dust collected by the swab method (20/168 or 12%). This implies that the petri dish dust collection method may be superior for detecting *Salmonella* from poultry house dust. Overall, the bacterial fluctuations during growout in cumulatively and non-cumulatively settled dust of Flock A and Flock B were not the same. This could be due to the growout of studied flocks in different seasons with Flock A in summer and Flock B in winter. Seasonal variation effects moisture content in settled dust and litter due to change in atmospheric humidity and this change in moisture content may further affect the generation of airborne particles from settled dust and litter (Carpenter, 1986). Therefore, the seasonal variation may affect both settled and airborne dust and their bacteria levels.

In the current study, an indirect interrelationship between litter and settled dust bacteria counts or prevalence was observed in some instances. We observed that aerobic bacteria levels in both litter and cumulatively settled dust tended to increase concurrently over time. Additionally, the litter in Flock B had higher *Salmonella* prevalence (20/32 in litter grab samples, 12/16 in boot swabs) due to the inoculation of birds. Consequently, the cumulative and non-cumulative settled dust samples of Flock B had higher *Salmonella* prevalence compared to Flock A. These findings confirm that aerobic bacteria and *Salmonella* can transfer from litter to dust during growout, and their levels in dust are dependent on their respective levels in litter. Previously, Chinivasagam et al. (2009) found the linkage between the levels of bacteria in litter and air. Their study observed that *E. coli* in litter were consistently present at higher levels, around 10^8 CFU/g, and thus in the aerosol, 10^2 to 10^4 CFU/m^3. Moreover, they reported that *Salmonella*

presence was intermittent and at lower levels in litter and therefore detected intermittently and at lower levels in air.

In this study, we observed that dust associated bacteria settled at different levels at different locations, demonstrating their non-homogenous distribution inside the house. Moreover, the settling of dust associated *Salmonella* in this study indicated its possibility to spread by airborne transmission. Previously, the airborne transmission of *Salmonella* Enteritidis from challenged hens to non-challenged hens was observed when both sets of hens were physically separated from each other in the same rearing houses and were sharing same air circulation (Holt et al., 1998). The authors observed the presence of *Salmonella* in air of rearing house and infection of non-challenged birds with *Salmonella*. However, based on the relatively low prevalence of *Salmonella* detected from dust samples in this study, long-term exposure to dust containing *Salmonella* may be required in order to achieve an infectious dose.

Overall, this study displayed that dust associated bacteria can vary with different stages of growout and they may or may not multiply in an additive manner over the time of growout. The distribution of bacteria can be varied within the poultry houses and was found at higher levels near the birds. Moreover, *Salmonella* can transfer through dust onto surfaces. Based on the results of this study, we recommended the control of the dust *Salmonella* in poultry houses using litter recycling management, windrowing, litter treatments, controlling ventilation speed etc.

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Table 3.1 Humidity and temperature data on the days of sampling for Flocks A and B.

Flock A			Flock B		
Sampling time	Humidity (%)	Temperature (°F)	Date	Humidity (%)	Temperature (°F)
Bird Placement	N/A[1]	N/A	Bird Placement	69	89.8
Week 1	63	91.2	Week 1	42	81.8
Week 2	85	83.6	Week 2	58	79.6
Week 3	88	81.8	Week 3	43	73.4
Week 4	83	81.0	Week 4	73	71.1
Week 5	66	83.1	Week 5	47	70.4
Week 6+1d[2]	69	76.2	Week 6+1d	66	69.6
			Week 7	68	68.3

[1]N/A = Data on this sampling day was not collected.
[2]Week 6 + 1d = This sampling was performed on the day after birds harvest that was equivalent to a day after week 6.

Table 3.2 Week wise variation of aerobic plate counts [$\log_{10}$ CFU/28 cm^2 (dust) or $\log_{10}$ CFU/g (litter) ± Standard error] in cumulatively and non-cumulatively settled dust and litter for Flocks A and B.

Sampling Time	Flock A		Flock B		Flock A	Flock B
	Cumulative Dust Samples	Non-cumulative Dust Samples	Cumulative Dust Samples	Non-cumulative Dust Samples	Litter Samples	Litter Samples
BP[1]	-	-	-	-	8.47 ± 0.25 [bc]	8.81 ± 0.81
Week 1	5.38 ± 0.20 [d]	5.38 ± 0.20 [c]	6.71 ± 0.11 [b]	6.71 ± 0.11 [b]	8.35 ± 0.32 [c]	9.68 ± 0.09
Week 2	6.27 ± 0.26 [c]	5.53 ± 0.19 [c]	6.83 ± 0.15 [b]	6.80 ± 0.14 [b]	9.66 ± 0.23 [ab]	9.76 ± 0.19
Week 3	7.09 ± 0.16 [b]	7.12 ± 0.13 [b]	6.87 ± 0.15 [b]	6.67 ± 0.15 [b]	9.84 ± 0.26 [a]	9.88 ± 0.12
Week 4	7.73 ± 0.14 [ab]	7.33 ± 0.23 [ab]	6.79 ± 0.14 [b]	6.86 ± 0.15 [b]	10.20 ± 0.30 [a]	10.10 ± 0.06
Week 5	8.25 ± 0.19 [a]	7.92 ± 0.17 [a]	6.87 ± 0.09 [b]	6.95 ± 0.09 [b]	10.20 ± 0.21 [a]	9.86 ± 0.10
Week 6 + 1d[2]	8.11 ± 0.14 [a]	7.56 ± 0.20 [ab]	8.25 ± 0.19 [a]	7.92 ± 0.17 [a]	9.68 ± 0.33 [ab]	9.96 ± 0.07
Week 7			6.96 ± 0.09 [b]	6.95 ± 0.04 [b]		9.98 ± 0.04
P value	< 0.0001	<0.0001	< 0.0001	<0.0001	0.0002	0.1407

[1]BP = Bird placement.
[2]Week 6 + 1d = This sampling was performed on the day after birds harvest that was equivalent to a day after week 6.
[a–d]Values within a column with different superscripts differ significantly $P \leq 0.05$.

Table 3.3 Week wise variation of *E. coli* counts [$\log_{10}$ CFU/28 cm^2 (dust) or $\log_{10}$ CFU/g (litter) ± Standard error] in cumulatively and non-cumulatively settled dust and litter for Flocks A and B.

Sampling Time	Flock A		Flock B		Flock A	Flock B
	Cumulative Dust Samples	Non-cumulative Dust Samples	Cumulative Dust Samples	Non-cumulative Dust Samples	Litter Samples	Litter Samples
BP[1]	-	-	-	-	3.01 ± 0.30 [d]	5.47 ± 0.14 [c]
Week 1	1.12 ± 0.12	1.12 ± 0.12 [b]	1.93 ± 0.30	1.93 ± 0.30	7.19 ± 0.19 [b]	6.29 ± 0.79 [bc]
Week 2	2.06 ± 0.20	1.67 ± 0.13 [ab]	1.79 ± 0.28	1.59 ± 0.24	8.68 ± 0.10 [a]	8.16 ± 0.15 [a]
Week 3	1.54 ± 0.28	1.83 ± 0.18 [ab]	1.81 ± 0.22	1.86 ± 0.15	7.21 ± 0.22 [b]	6.58 ± 0.26 [abc]
Week 4	1.68 ± 0.56	2.46 ± 0.58 [a]	2.57 ± 0.26	2.28 ± 0.18	6.63 ± 0.15 [bc]	6.76 ± 0.32 [abc]
Week 5	2.19 ± 0.46	1.84 ± 0.50 [ab]	1.62 ± 0.18	1.65 ± 0.26	6.39 ± 0.11 [bc]	6.93 ± 0.32 [abc]
Week 6 + 1d[2]	2.34 ± 0.54	2.15 ± 0.28 [ab]	1.74 ± 0.23	1.62 ± 0.15	5.92 ± 0.11 [c]	7.15 ± 0.09 [ab]
Week 7			2.01 ± 0.33	1.47 ± 0.30		7.21 ± 0.04 [ab]
P value	0.1298	0.0432	0.1597	0.2707	<0.0001	0.0011

[1]BP = Bird placement.
[2]Week 6 + 1d = This sampling was performed on a day after birds harvest that was equivalent to a day after week 6.
[a–d]Values within a column with different superscripts differ significantly $P \leq 0.05$.

Table 3.4 Week wise variation of coliforms counts [$\log_{10}$ CFU/28 cm^2 (dust) or $\log_{10}$ CFU/g (litter) ± Standard error] in cumulatively and non-cumulatively settled dust and litter for Flocks A and B.

Sampling Time	Flock A		Flock B		Flock A	Flock B
	Cumulative Dust Samples	Non-cumulative Dust Samples	Cumulative Dust Samples	Non-cumulative Dust Samples	Litter Samples	Litter Samples
BP[1]	-	-	-	-	3.01 ± 0.30 [d]	5.47 ± 0.14 [c]
Week 1	N/A[3]	N/A	1.86 ± 0.30	1.86 ± 0.30 [ab]	7.19 ± 0.19 [b]	6.34 ± 0.77 [bc]
Week 2	2.07 ± 0.20	1.77 ± 0.13	2.14 ± 0.38	1.80 ± 0.23 [ab]	8.69 ± 0.10 [a]	8.19 ± 0.14 [a]
Week 3	2.11 ± 0.25	1.92 ± 0.16	2.05 ± 0.31	1.92 ± 0.16 [ab]	7.22 ± 0.22 [b]	6.59 ± 0.25 [bc]
Week 4	1.85 ± 0.24	2.20 ± 0.33	2.62 ± 0.28	2.50 ± 0.22 [a]	6.61 ± 0.25 [bc]	6.85 ± 0.34 [abc]
Week 5	1.96 ± 0.46	1.86 ± 0.43	1.59 ± 0.15	1.54 ± 0.23 [b]	6.45 ± 0.09 [bc]	6.95 ± 0.33 [abc]
Week 6 + 1d[2]	2.40 ± 0.48	2.36 ± 0.28	2.06 ± 0.38	1.62 ± 0.14 [ab]	5.96 ± 0.11 [c]	7.19 ± 0.10 [ab]
Week 7			1.85 ± 0.31	1.42 ± 0.24 [b]		7.21 ± 0.04 [ab]
P value	0.7919	0.5435	0.3464	0.0303	<0.0001	0.0009

[1]BP = Bird placement.
[2]Week 6 + 1d = This sampling was performed on a day after birds harvest that was equivalent to a day after week 6.
[3]N/A = Data on this sampling day was not collected.
[a–d]Values within a column with different superscripts differ significantly $P \leq 0.05$.

Table 3.5 Week wise variation of *Salmonella* prevalence in cumulatively and non-cumulatively settled dust and litter samples for Flocks A and B.

Sampling Time	Flock A		Flock B		Flock A		Flock B	
	Cumulative Dust Samples	Non-cumulative Dust Samples	Cumulative Dust Samples	Non-cumulative Dust Samples	LGS[3]	BS[4]	LGS	BS
BP[1]	-	-	-	-	0/4	0/2	0/4 [b]	0/2
Week 1	0/12	0/12	1/12	1/12	0/4	0/2	2/4 [ab]	2/2
Week 2	2/12	0/12	0/12	1/12	0/4	0/2	2/4 [ab]	1/2
Week 3	1/12	0/12	2/12	3/12	3/4	0/2	4/4 [a]	2/2
Week 4	0/12	0/12	2/12	2/12	0/4	0/2	3/4 [ab]	1/2
Week 5	0/12	0/12	2/12	2/12	0/4	0/2	4/4 [a]	2/2
Week 6 + 1d[2]	0/12	0/12	0/12	0/12	0/4	0/2	1/4 [ab]	2/2
Week 7			3/12	1/12			4/4 [a]	2/2
P value	0.4205	-	0.4622	0.7656	0.0085	-	0.0122	0.3846

[1]BP = Bird placement.
[2]Week 6 + 1d = This sampling was performed on a day after birds harvest that was equivalent to a day after week 6.
[3]LGS = Litter grab samples.
[4]BS = Boot swabs.
[a–b]Values within a column with different superscripts differ significantly $P \leq 0.05$.

Table 3.6 Location-wise variation of bacteria levels (log_{10} CFU/28 cm^2 ± Standard error) or prevalence in cumulatively settled dust samples for Flocks A and B.

Sampled Locations	Flock A				Flock B			
	APC[1] levels	*E. coli* levels	Coliforms levels	SP[4]	APC levels	*E. coli* levels	Coliforms levels	SP
Light trap	6.21 ± 0.40 [b]	1.35 ± 0.65 [ab]	1.52 ± 0.28 [bc]	0/12	6.51 ± 0.12 [c]	0.85 ± 0.15	0.92 ± 0.08 [b]	0/14
Baffle	7.16 ± 0.34 [a]	1.09 ± 0.14 [b]	1.59 ± 0.15 [c]	0/12	6.86 ± 0.18 [bc]	1.74 ± 0.21	1.66 ± 0.17 [b]	0/14
Wall	7.24 ± 0.30 [a]	1.14 ± 0.17 [b]	1.91 ± 0.27 [abc]	2/12	6.87 ± 0.18 [bc]	1.43 ± 0.21	1.37 ± 0.18 [b]	1/14
Floor	7.85 ± 0.32 [a]	2.32 ± 0.45 [a]	2.73 ± 0.41 [a]	0/12	7.60 ± 0.18 [a]	2.12 ± 0.18	2.56 ± 0.25 [a]	4/14
Ptop[2]	7.24 ± 0.31 [a]	2.29 ± 0.24 [a]	2.54 ± 0.28 [ab]	0/12	7.09 ± 0.13 [b]	2.36 ± 0.23	2.33 ± 0.24 [a]	4/14
Pbot[3]	7.14 ± 0.30 [ab]	1.39 ± 0.15 [b]	1.58 ± 0.22 [c]	1/12	7.31 ± 0.15 [ab]	2.15 ± 0.22	2.55 ± 0.33 [a]	1/14
P value	0.0360	0.0159	0.0216	0.4205	0.0001	0.0079[5]	0.0002	0.0311[6]

[1]APC = Aerobic plate counts.
[2]Ptop = railing top inside the pen.
[3]Pbot = bottom ridge of last pen.
[4]*Salmonella* prevalence.
[5]0.0079 = Although *P* value was significant, but direct comparisons of *E. coli* levels between different sampled locations were not significant.
[6]0.0311 = Although *P* value was significant, but direct comparisons of *Salmonella* prevalence between different sampled locations were not significant.
[a–c]Values within a column with different superscripts differ significantly $P \leq 0.05$.

Table 3.7 Location-wise variation of bacteria levels (log_{10} CFU/28 cm^2 ± Standard error) or prevalence in non-cumulatively settled dust samples for Flocks A and B.

Sampled Locations	Flock A				Flock B			
	APC[1] levels	*E. coli* levels	Coliforms levels	SP[4]	APC levels	*E. coli* levels	Coliforms levels	SP
Light trap	6.04 ± 0.40 [b]	1.18 ± 0.00	1.51 ± 0.20	0/12	6.70 ± 0.23 [b]	1.62 ± 0.08 [abc]	1.31 ± 0.31 [bc]	2/14
Baffle	6.72 ± 0.30 [b]	1.57 ± 0.31	1.90 ± 0.28	0/12	6.80 ± 0.14 [b]	1.39 ± 0.15 [c]	1.37 ± 0.14 [c]	1/14
Wall	6.73 ± 0.29 [b]	1.35 ± 0.15	1.48 ± 0.17	0/12	6.81 ± 0.14 [b]	1.48 ± 0.20 [bc]	1.51 ± 0.16 [c]	0/14
Floor	7.66 ± 0.40 [a]	2.29 ± 0.40	2.52 ± 0.34	0/12	7.47 ± 0.17 [a]	2.22 ± 0.23 [a]	2.50 ± 0.22 [a]	3/14
Ptop[2]	6.84 ± 0.28 [ab]	2.02 ± 0.29	2.22 ± 0.32	0/12	7.05 ± 0.06 [b]	2.00 ± 0.19 [ab]	2.04 ± 0.18 [ab]	3/14
Pbot[3]	6.84 ± 0.29 [ab]	1.69 ± 0.14	2.04 ± 0.20	0/12	7.07 ± 0.08 [ab]	1.77 ± 0.19 [abc]	1.68 ± 0.17 [bc]	1/14
P value	0.0346	0.1647	0.1039	-	0.0046	0.0246	0.0002	0.5011

[1]APC = Aerobic plate counts.
[2]Ptop = railing top inside the pen.
[3]Pbot = bottom ridge of last pen.
[4]*Salmonella* prevalence.
[a–c]Values within a column with different superscripts differ significantly $P \leq 0.05$.

Table 3.8 *Salmonella* prevalence in weekly and bi-weekly collected dust by placing petri dishes for Flocks A and B.

Weekly Collection	*Salmonella* prevalence		Bi-weekly Collection	*Salmonella* prevalence	
	Flock A	Flock B		Flock A	Flock B
BP[1] – Week 1	0/6	0/6	BP – Week 2	0/6	1/6 [ab]
Week 1– Week 2	0/6	3/6	Week 2 – Week 4	2/6	5/6 [a]
Week 2 – Week 3	0/6	2/6	Week 4 – Week 6	0/6	0/6 [b]
Week 3 – Week 4	1/6	4/6			
Week 4 – Week 5	0/6	3/6			
Week 5 – Week 6	0/6	1/6			
Week 6 – Week 7		5/6			
Total *Salmonella* prevalence	1/36	18/42	Total *Salmonella* prevalence	2/18	6/18
P value	1.000	0.0678	*P* value	0.2941	0.0118

[1]BP = Bird placement.

[a–b]Values within a column with different superscripts differ significantly $P \leq 0.05$.

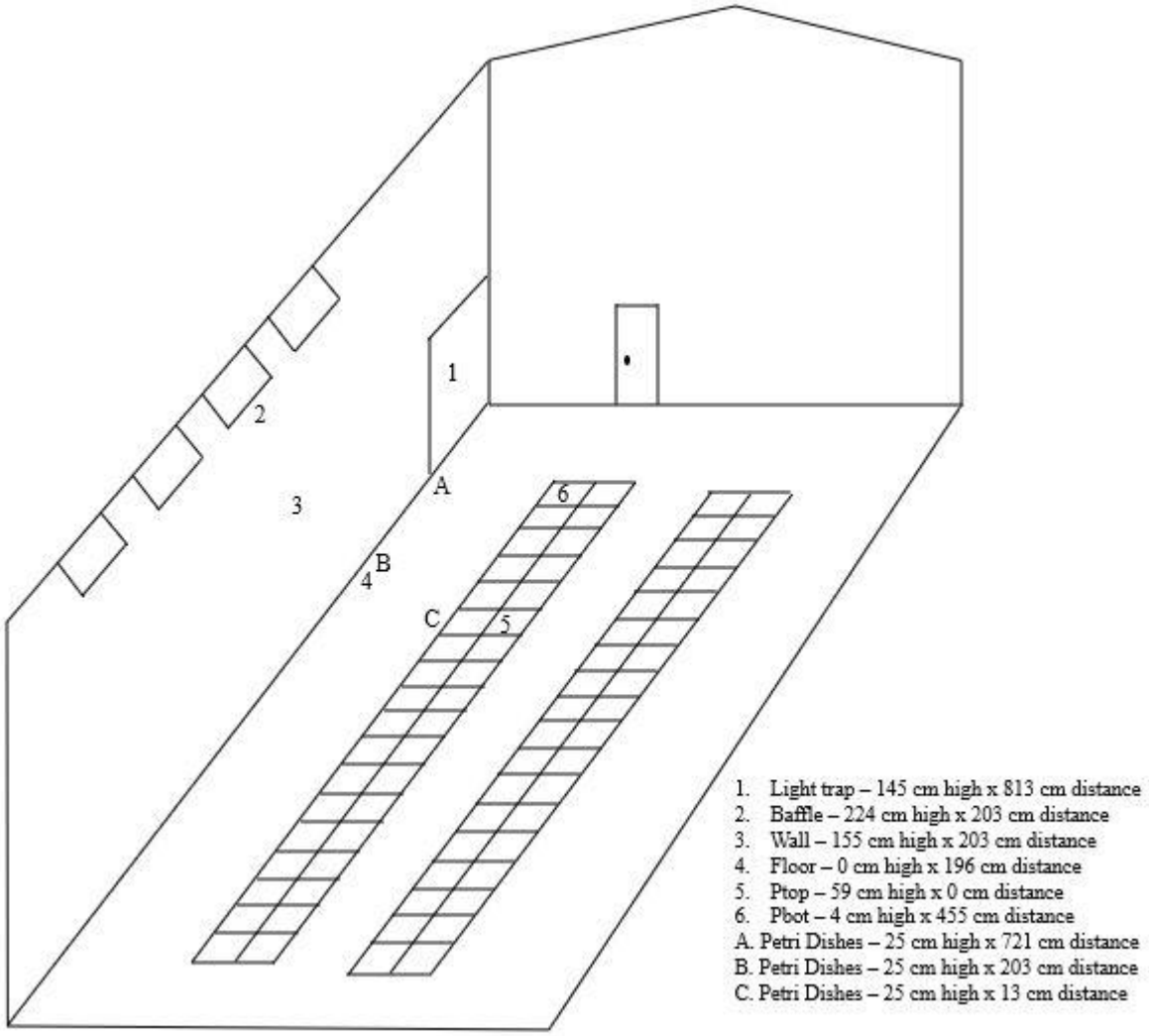

Figure 3.1 Schematic view of the poultry house representing settled dust sampling locations. Dust samples were collected by swabbing 28 cm^2 area at each location 1 through 6, on each of the two opposite diagonal corners of the house. Light trap (1) is 145 cm in vertical height from the floor and 813 cm in horizontal distance from the nearest pen containing birds. Baffle (2) is 224 cm in height from the floor and 203 cm in distance from the nearest pen containing birds. Wall (3) is 155 cm in height from the floor and 203 cm in distance from the nearest pen containing birds. Floor (4) is 0 cm in height from the floor and 196 cm in distance from the nearest pen containing birds. Ptop (5, railing top inside the pens containing birds) is 59 cm in height from the floor and 0 cm in distance from the nearest pen containing birds. Pbot (6, bottom ridge of empty pen) is 4 cm in height from the floor and 455 cm in distance from the nearest pen containing birds. Dust samples were collected by placing petri dishes at 25 cm height from ground level at locations A (721 cm in horizontal distance from the nearest pen containing birds), B (203 cm in distance from the nearest pen containing birds), and C (13 cm in distance from the nearest pen containing birds) at each of the two opposite diagonal corners of the house.

CHAPTER 4: INVESTIGATION OF THE POTENTIAL OF AEROSOLIZED *SALMONELLA* ENTERITIDIS ON INTERNAL ORGAN COLONIZATION IN BROILERS BETWEEN AGE OF D 3 TO D 21

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4.1 ABSTRACT

The presence of *Salmonella* in air of poultry houses has been previously confirmed. Therefore, it is important to investigate the entry of *Salmonella* into broilers through air. The present study aimed to evaluate different levels of *Salmonella* Enteritidis aerosol inoculations in broiler chicks for colonization in their ceca, liver/spleen, and trachea over time. For each of the three independent trials, a total of 112 1-d old birds were randomly divided into four groups (n=28/group). On d 1 of bird age, one group was exposed to an aerosol of sterile saline and the remaining three groups were exposed to an aerosol generated from one of three doses (10^3 CFU/mL, 10^6 CFU/mL, and 10^9 CFU/mL) of *S.* Enteritidis inoculum. Aerosol exposure time was 30 min/group and was performed using a nebulizer. On d 3, 7, 14, and 21 of age, ceca, liver/spleen, and trachea were aseptically removed. Ceca were cultured for *Salmonella* counts ($\log_{10}$ CFU/g) and all tissues were cultured for *Salmonella* prevalence. All sampled tissues from the control group were *Salmonella* negative. On sampling d 3 and 7, ceca *Salmonella* counts

were highest (5.14 and 5.11, respectively) when challenged with 10^9 CFU/mL *S.* Enteritidis ($P \leq$ 0.0281). Ceca *Salmonella* counts increased from d 3 (2.43) to d 7 (4.43) and then remained constant following inoculation at 10^3 CFU/mL *S.* Enteritidis, and counts decreased over time for all other groups. For each tissue type, *Salmonella* prevalence increased with increasing inoculum levels at all sampling timepoints ($P \leq 0.0213$). *Salmonella* prevalence was low (0/18 to 4/18) and did not change over time following 10^3 CFU/mL *S.* Enteritidis inoculation ($P \geq 0.2394$). Prevalence decreased over time in ceca and trachea following 10^6 and 10^9 CFU/mL *Salmonella* inoculation ($P \leq 0.0483$). Liver/spleen *Salmonella* prevalence increased from d 3 (13/18) to d 14 (18/18) and then decreased at d 21 (10/18) in birds exposed to an aerosol of highest inoculum of *S.* Enteritidis but remained constant over time for rest of the *Salmonella* inoculated groups. Overall, this study demonstrated the *Salmonella* colonization and persistence in different tissues in broilers following exposure to aerosolized *Salmonella*.

Key words: *Salmonella*, broilers, aerosol, tissues, poultry.

4.2 INTRODUCTION

More than 2,500 *Salmonella* serotypes have been characterized, and >100 serotypes cause human infections (CDC, 2020). *Salmonella* causes human salmonellosis which is major foodborne illness encountered in the United States. Poultry products have been frequently found to be linked to *Salmonella* outbreaks (CDC, 2018).

Presence of *Salmonella* in live poultry populations is one of the major factors for *Salmonella* contamination of poultry meat and eggs (Hugas and Beloeil, 2014). During integrated broiler production, *Salmonella* spread can be possible by both horizontal and vertical pathways through several possible sources including breeders, hatcheries, feed, production house environment, rodents, and insects (Liljebjelke et al., 2005). In poultry production houses,

Salmonella colonization in birds can be possible through several routes. Previously, Cox et al. (1996) found that *Salmonella* administration in young broiler chicks through mouth, cloaca, eyes, and nasal passages readily results in production of seeder birds which may then spread *Salmonella* throughout the poultry production house. The entry of bacteria from air through the respiratory route in poultry birds has not been deeply explored, although some studies have examined and confirmed this possibility by performing inoculation of poultry birds (broilers, turkeys, and layers) with bacterial contaminated aerosol (Cox et al., 1996; Knab et al., 2018; Cheng et al., 2020).

In livestock houses, there are several sources of airborne microorganisms including litter, feed, animal respiratory tracts, animal skin, and farm workers (Zhao et al., 2014). Diverse kinds of bacteria (including *Salmonella* spp.) have been confirmed from the air in broiler houses (Chinivasagam et al., 2009; Fallschissel et al., 2009). *Salmonella* can travel in air by either being carried on dust particulate or in aerosol (Gast et al., 1998). Some studies have reported the airborne transmission of *Salmonella* in poultry facilities. Specifically, Gast et al. (1998) reported transmission of *S.* Enteritidis through air from challenged to non-challenged groups of layers when both bird groups were physically separated from each other but sharing the same air circulation in a controlled environmental isolation cabinet. They found *Salmonella* positive results both from circulating air and non-challenged birds. Similarly, the observations of *Salmonella* infection in turkeys after exposure to aerosol, containing *Salmonella* contaminated fecal dust particles, confirmed the airborne transmission of *Salmonella* (Harbaugh et al., 2016). Additionally, Kallapura et al. (2014) recovered *Salmonella* from ceca-cecal tonsil, trachea, and liver/spleen after intratracheal administration of *Salmonella* in broiler chicks, and thereby they confirmed the possibility of respiratory route to serve as an entry point for *Salmonella* in poultry

birds. Moreover, when two different *Salmonella* serotypes (*S.* Enteritidis and *S.* Heidelberg) were administered in day-of-hatch broiler chicks via one of several different inoculation routes (oral, intratracheal, subcutaneous, ocular, and cloacal), then the overall highest recovery from the samples (trachea, crop, liver/spleen, cecum, and cloacal swab) of market-age broilers was observed following intratracheal inoculation compared to the other inoculation routes for both *Salmonella* serotypes (Chadwick et al., 2020).

Therefore, published research implicate airborne *Salmonella* as a risk factor for *Salmonella* infections or colonization in chickens by detecting the existence of *Salmonella* in air of poultry houses, airborne transmission of *Salmonella*, and the possibility of respiratory route to serve as an entry portal for *Salmonella*. However, this phenomenon can be explained by inoculation of chickens through *Salmonella* contaminated aerosol, that mimics the natural route of bacterial infection through air, in a more concise way. In this regard, our objective was to evaluate the potential of different levels of *Salmonella* Enteritidis aerosol inoculation in day-old broiler chicks for colonization in their ceca, trachea, and liver/spleen (pooled) over time.

4.3 MATERIALS AND METHODS

All the procedures conducted in this study were approved by the Auburn University Institutional Animal Care and Use Committee (IACUC) (PRN #2021-3841).

Experimental design

For each of the three independent trials, a total of 112 1-d old broilers (trial 1: Ross708, trials 2 and 3: YPMxRoss708) were randomly divided into four groups (n=28/group). On d 1 of bird age, one group was exposed to an aerosol of sterile saline and the remaining three groups were exposed to an aerosol generated from one of three doses (10^3 CFU/mL, 10^6 CFU/mL, and 10^9 CFU/mL) of *S.* Enteritidis inoculum. Aerosol exposure time was 30 min/group. Following

aerosol exposures, all the birds were placed in battery cages at the Auburn University Poultry Research Farm Battery House (2 cages/group, total cages = 8). The amount of allotted space per bird (d 1: 51 in^2/bird, d 3: 66 in^2/bird, d 7: 91 in^2/bird, d 14: 145 in^2/bird, d 21: 364 in^2/bird) exceeded the minimum allowed space for broilers up to 21 d of age. Cages were separated from each other by one empty cage (66 cm). Birds were provided ad-libitum feed and water during growout. On d 3, 7, 14, and 21 of age, ceca, trachea, and liver/spleen of 6 birds/group/trial (or 3 birds/cage/trial) were aseptically removed after euthanizing the birds by CO_2 asphyxiation and placed separately into sterile sampling bags (Nasco whirl-pak® sample bag, Madison, WI). After collecting samples, bags were placed on ice and then transported to the laboratory for microbiological examination. Collected tissues were cultured for *Salmonella* prevalence and ceca for *Salmonella* enumeration (log_{10} CFU/g).

Salmonella inoculum preparation

Salmonella enterica serotype Enteritidis, resistant to 100 µg/mL nalidixic acid, was used for aerosol inoculations of birds. The marker strain, stored in glycerol at -80 °C, was first plated onto plate count agar (Hardy Diagnostics, Santa Maria, CA, USA). The colonies were collected from plate count agar plates after the incubation period of 24 h at 37 °C and then suspended in sterile saline to achieve an optical density approaching 10^9 CFU/mL. The actual counts were confirmed by plating the appropriate inoculum dilutions onto 100 µg/mL nalidixic acid containing Xylose Lysine Tergitol-4 **(XLT4)** (Criterion, Hardy Diagnostics, Santa Maria, CA, USA) agar plates in duplicate. *Salmonella* counts from XLT4 agar plates were reported after an incubation period of 24 h at 37 °C. The actual obtained *Salmonella* counts (log_{10} CFU/mL) were 8.70, 8.54, and 8.48 for trials 1, 2, and 3, respectively. Each prepared inoculum was further

serially diluted in sterile saline to obtain the desired levels of *Salmonella* required for aerosol inoculations.

Procedure of aerosol exposure

Within each trial, for aerosol exposure of each group, 28 birds were first placed into a cleaned and sanitized plastic tub (58.4 cm × 41.3 cm × 31.4 cm, LWH, Sterilite®, Townsend, MA, USA) within a biosafety cabinet (Figure 4.1). The plastic tub was equipped with a disposable nebulizer cup and mouthpiece (Aeromist Compact, Medline Industries, Inc., Northfield, IL, USA) in the middle. The nebulizer cup contained 8 mL of *Salmonella* inoculum dose or sterile saline depending on the assigned group treatment. *Salmonella* or saline was nebulized for 30 min from the nebulizer cup to the birds within the tub through a mouthpiece which had two open ends. The tub was closed with a lid on top during aerosol exposure treatments. The nebulizer compressor (Aeromist Compact, Medline Industries, Inc., Northfield, IL, USA) was attached to the nebulizer cup to generate the *Salmonella* or sterile saline aerosol through the mouthpiece and itself was placed outside the biosafety cabinet. The average rate of *Salmonella* inoculum and sterile saline distribution in air was 0.20 mL/min. Based on the manufacturer's specifications, the nebulized particles size was less than 5 µm. After nebulization for 30 min, the plastic tub remained untouched in the biosafety cabinet for 5 min to allow suspended aerosol to settle. For each treatment group, simultaneously during aerosol exposure, the counts of *Salmonella* in tub air were assessed by collecting air from the tub for 30 min into 10 mL of buffered peptone water **(BPW)** (BBL™, Becton Dickinson and Company, Sparks, MD, USA) using an impinger system that had an air collection rate of 0.75 L/min (ACE Glass Incorporated, 7531 – 10 Midget Impinger Comp., Vineland, NJ, USA). After that, direct or an appropriate serial dilution in BPW was duplicate plated onto XLT4 agar plates, containing 100

μg/mL of nalidixic acid, and then presumptive *Salmonella* counts were recorded after the incubation period of 24 h at 37 °C. The remaining volume of the BPW air sample (8.8 mL) was further incubated for 24 h at 37 °C for enrichment. After 24 h, the enriched BPW air sample was streaked onto XLT4 agar plates (containing 100 μg/mL of nalidixic acid) and then presumptive colonies of *Salmonella* were reported after the incubation period of 24 h at 37 °C. The levels of *Salmonella* in tub air, to which the chicks were exposed, with respect to trial and assigned group treatment are given in Table 4.1. The biosafety cabinet and plastic tubs were sanitized with ethanol each time before and after every aerosol exposure. After completing one group aerosol exposure cycle (30 + 5 min), birds were transferred individually by hand to a cleaned and sanitized plastic tub and then transported to the battery house. During transport, birds remained in the plastic tub and were not handled until present in the room in which they were housed. Aerosol nebulization was performed in the order of control (Group 1), 10^3 CFU/mL (Group 2), 10^6 CFU/mL (Group 3), and then 10^9 CFU/mL (Group 4).

Microbial analyses of collected tissues

Collected ceca, trachea, and liver/spleen were first macerated within their respective sampling bag and then the average weight of each type of tissue was calculated using five random samples. Next, BPW (10 mL when the tissue weight was < 3.3 g or 3 times the weight of tissue when tissue weight was > 3.3g) was added into each sampling bag. Following this, tissues were stomached for 1 min. For *Salmonella* enumeration from ceca, an aliquot from direct BPW homogenates or their appropriate dilutions, in sterile saline, were duplicate plated onto XLT4 agar plates that contained 100 μg/mL of nalidixic acid. The *Salmonella* counts were recorded after the incubation period of 24 h at 37 °C. For *Salmonella* prevalence detection from each type of tissues, the original BPW homogenates were incubated for 24 h at 37 °C for enrichment. After

24 h, each sample was streaked onto 100 μg/mL nalidixic acid containing XLT4 agar plates and the confirmation of *Salmonella* was completed after incubation of 24 h at 37 °C.

Statistical analyses

Salmonella counts were transformed into $\log_{10}$ CFU/g before data analysis. Ceca *Salmonella* count data were analyzed using two-way ANOVA. Means value of *Salmonella* counts were compared among the inoculated groups using Tukey's HSD test and level of significance was set at $P \leq 0.05$. *Salmonella* prevalence data was analyzed using Fisher's exact test. *Salmonella* prevalence data comparisons were performed between all the possible combinations and level of significance was set at $P \leq 0.05$. All data of this study was analyzed using SAS Studio, release 3.8 Enterprise Edition.

4.4 RESULTS

Data of *Salmonella* counts or presence in the air, that was circulating within the tub during exposure of broilers to different aerosol treatments, are given in Table 4.1. All the air samples were *Salmonella* negative when birds were exposed to an aerosol of sterile saline. When birds were exposed to an aerosol of *S.* Enteritidis inoculum of 10^3 CFU/ml levels, 100% *Salmonella* prevalence in air samples was observed and *Salmonella* counts in air were ≤ 3.35 $\log_{10}$ CFU/m^3. *Salmonella* counts in air ($\log_{10}$ CFU/m^3) ranged between 5.25 to 6.05 and 8.05 to 8.32 when air samples were obtained from the tub simultaneously during bird exposure to an aerosol of *S.* Enteritidis inoculum of 10^6 CFU/ml and 10^9 CFU/ml levels, respectively.

Salmonella counts ($\log_{10}$ CFU/g) in ceca obtained at different ages (d 3, d 7, d 14, d 21) from broilers after exposure to different aerosol treatments are presented in Table 4.2. No *Salmonella* counts were observed in ceca for control group birds exposed to an aerosol of sterile saline. Ceca *Salmonella* counts increased ($P = 0.0188$) from d 3 (2.43) to d 7 (4.43) and then

remained constant for birds exposed to an aerosol generated from lowest dosed inoculum of *S.* Enteritidis (10^3 CFU/mL). For bird groups exposed to an aerosol of *S.* Enteritidis inoculum of 10^6 CFU/mL and 10^9 CFU/mL levels, ceca *Salmonella* counts decreased with broiler age ($P = 0.005$ and $P < 0.0001$, respectively) from 4.56 at d 3 to 2.59 at d 21 and 5.14 at d 3 to 2.81 at d 21, respectively. Differences in ceca *Salmonella* counts among *Salmonella* aerosol-inoculated bird groups were observed at d 3 ($P < 0.0001$) and d 7 ($P = 0.0281$). On d 3 and 7, the highest *Salmonella* counts in ceca (5.14 and 5.11, respectively) were observed for the birds challenged with an aerosol of *S.* Enteritidis inoculum of 10^9 CFU/mL levels. The lowest ceca *Salmonella* counts on d 3 (2.43) and 7 (3.85) was observed in the bird groups challenged with an aerosol of *S.* Enteritidis inoculum of 10^3 CFU/mL and 10^6 CFU/ml levels, respectively.

Salmonella prevalence in different types of tissues obtained at different ages (d 3, d 7, d 14, d 21) from broilers after exposure to different aerosol treatments are presented in Table 4.3. All the sampled tissues from control group birds were *Salmonella* negative. *Salmonella* prevalence did not change over time in any of the sampled tissues (ceca, trachea, and liver/spleen) for the bird group exposed to an aerosol generated from lowest dosed inoculum of *S.* Enteritidis (10^3 CFU/mL, $P \geq 0.2394$). For this group of birds, *Salmonella* prevalence ranged between 2/18 to 4/18, 0/18 to 2/18, and 1/18 to 3/18 in ceca, trachea, and liver/spleen, respectively. For birds exposed to an aerosol of *S.* Enteritidis inoculum of 10^6 and 10^9 CFU/mL levels, *Salmonella* prevalence decreased over time in ceca from 17/17 to 8/18 and 18/18 to 12/18, respectively, and in trachea from 17/18 to 5/18 and 18/18 to 14/18, respectively. *Salmonella* prevalence in liver/spleen did not change with increasing broiler age ($P \geq 0.1703$) for bird groups exposed to an aerosol of *S.* Enteritidis inoculum of 10^3 CFU/mL and 10^6 CFU/mL levels. However, *Salmonella* prevalence in liver/spleen increased from d 3 (13/18) to d 14

(18/18) and then decreased at d 21 (10/18) for birds exposed to an aerosol generated from highest dosed inoculum of *S.* Enteritidis (10^9 CFU/mL, $P = 0.0015$). In each kind of sampled tissue, *Salmonella* prevalence increased with increasing *S.* Enteritidis inoculum levels, at all sampling timepoints ($P \leq 0.0213$). Overall, *Salmonella* persisted in both ceca and liver/spleen at all inoculum levels. However, in the trachea, *Salmonella* only persisted through 21 d of age at the higher 10^6 and 10^9 CFU/mL inoculum levels.

4.5 DISCUSSION

Salmonella colonization in internal tissues of broilers through *Salmonella* contaminated aerosol at commercial poultry houses is still an undefined phenomenon. However, there are a few experimentally conducted studies indicating the possibility of spread of *Salmonella* infection among poultry birds via air and *Salmonella* entry in broilers through the respiratory tract (Gast et al., 1998; Kallapura et al., 2014). Moreover, Cheng et al. (2020) observed tissue colonization and significant inflammatory cytokine expressions in leghorn chickens after exposure to *S.* Pullorum contaminated aerosol. Therefore, the objective of this study was to explore the colonization in different tissues (ceca, trachea, and liver/spleen) of broilers over time following their exposure to an aerosol of *S.* Enteritidis.

In this study, the colonization in each of the sampled tissues of birds occurred following bird exposure at d 1 to an aerosol generated from *S.* Enteritidis inoculum at each of the different levels (10^3, 10^6, and 10^9 CFU/mL). The actual counts of *Salmonella* in air during aerosol inoculations of birds ranged between < 3.35 to 8.32 $\log_{10}$ CFU/m^3 (Table 4.1). The lowest infectious dose of airborne *S.* Enteritidis for broilers colonization in this study was less than 3.35 $\log_{10}$ CFU/m^3. Previously, the minimum levels of airborne *S.* Pullorum responsible for colonization in lungs and liver of poultry birds were reported to be 2.10 $\log_{10}$ CFU/m^3 (Cheng et

al., 2020). In commercial poultry production houses, the airborne *Salmonella* levels were reported to range between 1.82 to 2.52 $\log_{10}$ CFU/m^3 (Venter et al., 2004; Fallschissel et al., 2009). Therefore, there is potential for *Salmonella* colonization in broilers at commercial poultry houses through *Salmonella* contaminated aerosol. Moreover, it has also been experimentally examined that the inhalation of 2.46 $\log_{10}$ CFU (or 290 cells) of *S.* Enteritidis by lying hens can cause infection (Chart et al., 1992). However, the ability of chickens to inhale at least this many cells of *Salmonella* through air and the existence of continuous airborne exposure of chickens to aerosolized *Salmonella* in poultry houses still requires investigation. These findings may help to elucidate the threat of airborne *Salmonella* to poultry animals at commercial poultry farms.

In the present study, the *Salmonella* counts ($\log_{10}$ CFU/g) in ceca ranged between 2.43 to 4.43, 2.59 to 4.56, and 2.81 to 5.14 for bird groups exposed to an aerosol of *S.* Enteritidis inoculum of 10^3, 10^6, and 10^9 CFU/mL levels, respectively. Overall, the decreasing trend of ceca *Salmonella* counts was observed with broilers growth. Also, *Salmonella* prevalence in ceca and trachea was diminishing over time during growout. However, *Salmonella* prevalence in liver/spleen changed over time only in one of the bird groups that was exposed to an aerosol generated from *S.* Enteritidis inoculum of 10^9 CFU/mL levels, where *Salmonella* prevalence was increased first up to d 14 and then decreased on d 21. Previously, when broiler chicks were inoculated directly into the crop at 1 d after hatching with 10^7 and 10^6 CFU of *S.* Typhimurium per chick, the ceca *Salmonella* counts ($\log_{10}$ CFU/g) and prevalence decreased with broiler age (Gast and Beard, 1989). Specifically, they observed that ceca *Salmonella* counts (ceca *Salmonella* prevalence) were 8.0 (100%) and 7.4 (100%) at 1 wk, and 3.6 (87.5%) and 3.4 (75.0%) at 7 wk, after inoculation of 10^7 and 10^6 CFU of *S.* Typhimurium per chick, respectively. In the same study, when chicks were inoculated with 10^8 CFU of *S.* Typhimurium

directly into the crops of birds at 1 d after hatching, a decreasing trend of *Salmonella* prevalence in liver and spleen, 100% to 16.7% and 100% to 0.00%, respectively, with broiler age was observed. The reason for initial rise of *Salmonella* prevalence in liver/spleen for bird group, exposed to the highest inoculum level, in this present study is not clear. It may have been due to slow invasion or translocation of *S.* Enteritidis from the aerosol exposure to the liver/spleen or due to an increase in systemic infection over time at this high inoculum dose. It is also important to note that *Salmonella* persisted in all types of sampled tissue at d 21 following 30 min *Salmonella* aerosol exposure of day of hatch chicks. Continued persistence will need to be assessed through to market age of broilers. Recently, when day-of-hatch broiler chicks were administered with *S.* Enteritidis and *S.* Heidelberg via different inoculation routes such as oral, intratracheal, cloacal, ocular, and subcutaneous, the recovery of both *Salmonella* serotypes from trachea, crop, liver/spleen, cecum, and cloacal swab was occurred when broilers reached to market weight (Chadwick et al., 2020).

Overall, the order of *Salmonella* prevalence in sampled tissues was ceca (138/287) > trachea (111/288) > liver/spleen (106/288) in this study. High *Salmonella* prevalence in the trachea indicates that broiler chicks did inhale airborne *S.* Enteritidis. Among 111 birds, which had *Salmonella* in their trachea, 102 and 84 birds had *Salmonella* presence in ceca and liver/spleen, respectively (data not shown). This indicates that *Salmonella* might follow a systemic route of infection after entering the respiratory tract of birds through aerosol. Likewise, when *Salmonella* was administered intratracheally in broiler chicks in a previous study, the recovery of *Salmonella* from liver/spleens and ceca-cecal tonsils along with trachea was observed (Kallapura et al., 2014). The authors suggested that the recovery of *Salmonella* from ceca-cecal tonsils and liver/spleens indicates the systemic pathway of infection of *Salmonella*

following intratracheal challenge in birds. Gast et al. (1998) also pointed out the possibility of the transfer of inhaled bacteria into the gastrointestinal tract by crossing oropharynx. Moreover, we observed that the overall prevalence in ceca (138/287) was greater compared to trachea (111/288), and *Salmonella* was recovered from ceca and liver/spleen but not from trachea in some instances, 36/138 and 22/106, respectively. This finding suggested that *Salmonella* might enter broilers from other body openings (mouth, eyes, cloaca etc.) along with the nasal passage during aerosol inoculation. This could explain why a higher ceca *Salmonella* prevalence was observed. Previously, Cox et al. (1996) observed a higher level of *Salmonella* colonization in ceca compared to lungs when broiler chicks were inoculated with *Salmonella* through aerosol. Moreover, the same study also demonstrated that, using different methods of *Salmonella* inoculations in chicks, the entry of *Salmonella* in chickens can be possible through multiple body openings such as mouth, nasal passage, cloaca, navel, and eyes, and passage of *Salmonella* through all these different pathways resulted in ceca colonization. Additionally, the possibility of mouth breathing in 1-day-old broilers was speculated when they were being exposed to microsphere aerosols of different sizes (Corbanie et al., 2006). Furthermore, during aerosol exposure of birds, *Salmonella* could be deposited on external surfaces of birds from where it later entered in birds during growout via oral ingestion during instances like preening or picking, and thereby increased intestinal colonization.

We observed the absence of *Salmonella* in each sampled tissue, on all sampling days, from the control group of birds that were exposed to an aerosol of sterile saline. This indicates that there was an absence of airborne spread of *Salmonella* among bird groups, which were housed within the same room. However, the previously conducted experimental studies observed the airborne transmission of *S.* Enteritidis from infected to uninfected chicks (control) when both

sets of chicks were reared in the same house (Lever and Williams, 1996; Gast et al., 1998). This contrast in findings might be because of very low levels of *Salmonella* in air during growout that were not enough to colonize the control groups of chicks or might be due to failure of aerosolization of *Salmonella* during growout, in the present study. However, the counts or presence of *Salmonella* in air were not assessed in this study during growout.

Overall, the findings of this study indicate that airborne *Salmonella* may enter broiler chicks through aerosol, acquire systematic route of infection, and colonized multiple tissues. We observed the persistence of *Salmonella* colonization in tissues up to 21 d of birds age after *Salmonella* aerosol exposures of chicks at d 1, and the persistence of *Salmonella* colonization needs to be further assessed through market age of birds. Based on this experimental study, we recommended the control the of airborne *Salmonella* levels in poultry houses and specifically during the entry of the flock into the house. Further investigation regarding the likelihood of inhalation of airborne *Salmonella* by chickens in commercial poultry houses is still needed and would provide more knowledge about the aerosol route of *Salmonella* colonization in poultry.

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Table 4.1 *Salmonella* counts or presence in air (within the tub), during aerosol exposures of broilers, with respect to trial number and assigned group treatment.

Aerosol exposure treatments	*Salmonella* counts (log_{10} CFU/m^3) or presence (positive or negative)		
	Trial 1	Trial 2	Trial 3
Sterile saline	ND[1] (negative)	ND (negative)	ND (negative)
10^3 CFU/mL SE[2]	ND (positive)	3.35	ND (positive)
10^6 CFU/mL SE	5.25	6.04	6.05
10^9 CFU/mL SE	8.32	8.25	8.05

[1]ND = Not Detected by direct plating for *Salmonella* counts. Minimum detection limit was 3.35 log_{10} CFU/m^3.
[2]SE = *Salmonella* Enteritidis.

Table 4.2 *Salmonella* counts in ceca obtained at different ages (d 3, d 7, d 14, d 21) from broilers following exposure to aerosol of different levels of *S.* Enteritidis inoculum or sterile saline for 30 min at d 1 of age. (n = 18/group/sampling day)

Aerosol exposure treatments	*Salmonella* counts (log_{10} CFU/g ± Standard error)[5]				*P* value
	d 3	d 7	d 14	d 21	
Sterile saline[1]	ND[4]	ND	ND	ND	-
10^3 CFU/mL SE[2]	2.43 ± 0.33 ^{b,y}	4.43 ± 0.42 ^{a,xy}	3.68 ± 0.04 ^{ab}	3.10 ± 0.67 ^{ab}	0.0188
10^6 CFU/mL SE[3]	4.56 ± 0.22 ^{a,x}	3.85 ± 0.40 ^{ab,y}	2.84 ± 0.26 ^{b}	2.59 ± 0.32 ^{b}	0.0005
10^9 CFU/mL SE	5.14 ± 0.21 ^{a,x}	5.11 ± 0.22 ^{a,x}	2.94 ± 0.36 ^{b}	2.81 ± 0.29 ^{b}	<0.0001
P value	<0.0001	0.0281	0.5666	0.7781	-

[1]Sterile saline = The data of this group were not used for statistical analysis.
[2]SE = *Salmonella* Enteritidis.
[3]10^6 CFU/mL (SE) = One of the ceca samples of this treatment group was lost at d 3.
[4]ND = Not Detected by either direct plating or enrichment for *Salmonella*.
[5]*Salmonella* counts (log_{10} CFU/g ± Standard error) = Only *Salmonella* positive samples were included for statistical analysis.
[a-b]Values within a row with different superscripts are significant different ($P \leq 0.05$).
[x-y]Values within a column with different superscripts are significant different ($P \leq 0.05$).

Table 4.3 *Salmonella* prevalence in ceca, trachea, and liver/spleen detected at different ages (d 3, d 7, d 14, d 21) from broilers following exposure to aerosol of different levels of *S.* Enteritidis inoculum or sterile saline for 30 min at d 1 of age. (n = 18/group/sampling day)

Sampled tissues	Aerosol exposure treatments	*Salmonella* Prevalence (Positive samples/Total samples)				*P* value
		d 3	d 7	d 14	d 21	
Ceca	Sterile saline[1]	0/18	0/18	0/18	0/18	-
	10^3 CFU/mL (SE[2])	4/18 ^{y}	4/18 ^{y}	4/18 ^{y}	2/18 ^{y}	0.8125
	10^6 CFU/mL (SE)[3]	17/17 ^{a,x}	18/18 ^{a,x}	15/18 ^{a,x}	8/18 ^{b,xy}	<0.0001
	10^9 CFU/mL (SE)	18/18 ^{a,x}	18/18 ^{a,x}	18/18 ^{a,x}	12/18 ^{b,x}	0.0005
	P value	<0.0001	<0.0001	<0.0001	0.0020	-
Trachea	Sterile saline[1]	0/18	0/18	0/18	0/18	-
	10^3 CFU/mL (SE)	0/18 ^{y}	2/18 ^{z}	0/18 ^{y}	0/18 ^{y}	0.2394
	10^6 CFU/mL (SE)	17/18 ^{a,x}	10/18 ^{b,y}	11/18 ^{b,x}	5/18 ^{b,x}	0.0004
	10^9 CFU/mL (SE)	18/18 ^{x}	18/18 ^{x}	16/18 ^{x}	14/18 ^{x}	0.0483[4]
	P value	<0.0001	<0.0001	<0.0001	<0.0001	-
Liver/spleen	Sterile saline[1]	0/18	0/18	0/18	0/18	-
	10^3 CFU/mL (SE)	1/18 ^{y}	2/18 ^{y}	3/18 ^{z}	2/18 ^{y}	0.9542
	10^6 CFU/mL (SE)	12/18 ^{x}	12/18 ^{x}	10/18 ^{y}	6/18 ^{xy}	0.1703
	10^9 CFU/mL (SE)	13/18 ^{bc,x}	17/18 ^{ab,x}	18/18 ^{a,x}	10/18 ^{c,x}	0.0015
	P value	<0.0001	<0.0001	<0.0001	0.0213	-

[1]Sterile saline = The data of this group were not used for statistical analysis.
[2]SE = *Salmonella* Enteritidis.
[3]10^6 CFU/mL (SE) = One of the ceca samples of this treatment group was lost at d 3.
[4]0.0483 = Although the overall *P* value was significant, but direct comparisons between days were not significant.
[a-b]Values within a respective tissue type and within a row with different superscripts are significant different ($P \leq 0.05$).
[x-y]Values within a respective tissue type and within a column with different superscripts are significant different ($P \leq 0.05$).

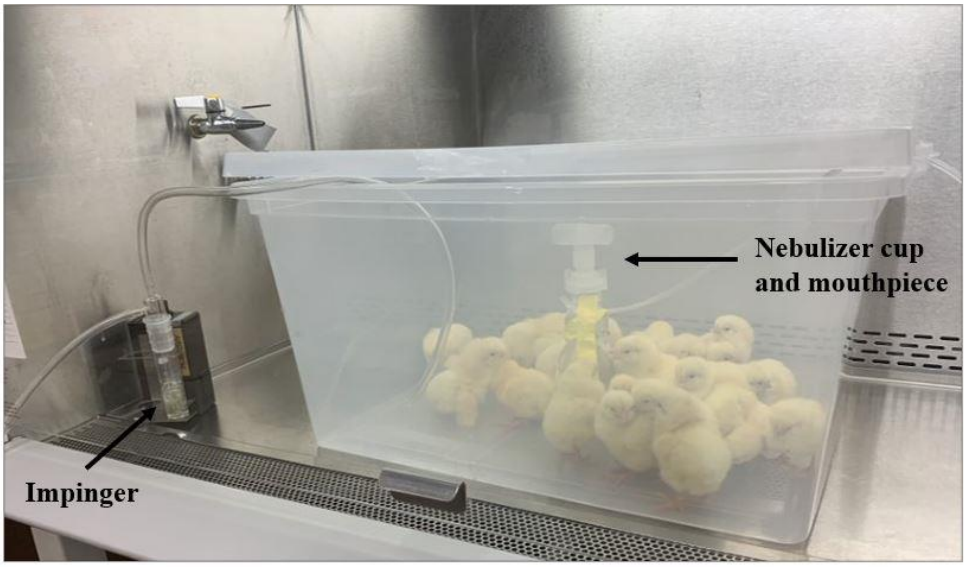

Figure 4.1 Experiment setup for exposure of broiler chicks to different aerosol treatments.

CHAPTER 5: CONCLUSION AND FUTURE IMPLICATIONS

Based on the findings of Chapter 2, we confirmed that litter can be a source of aerobic bacteria, *E. coli*, coliforms, and *Salmonella* contamination of dust in poultry houses. The levels of *Salmonella* contamination of dust were dependent on litter *Salmonella* counts and litter moisture content. We reported that increasing levels of litter moisture content can decrease transfer of *Salmonella* from litter to dust. However, an optimum level of litter moisture content that would decrease dust contamination with *Salmonella* without negatively effecting the animal welfare aspects (breast cleanliness, breast irritation, hock burn, and gait) and ammonia content in commercial poultry houses still needs to be investigated. The results of Chapter 3 suggested that the levels of dust associated bacteria can be dependent on stage of broiler growout. Moreover, we observed the non-homogenous distribution of dust associated bacteria during the broiler rearing period. Therefore, future research evaluating new methods to control bacterial dust contamination should consider these results in order to obtain efficient outcomes throughout the rearing period and the poultry house environment. Our final experimental study demonstrated the likelihood of *Salmonella* colonization in internal tissues of broilers after exposure to *Salmonella* contaminated aerosol. We observed *Salmonella* persistence in ceca, trachea, and liver/spleen up to 21 d of bird age. The future studies can be designed to evaluate *Salmonella* persistence in broilers up to their market age following exposure to *Salmonella* aerosol. That would indicate the hazard of airborne *Salmonella* colonization in chickens to food safety more precisely. Moreover, the future studies can also be designed to assess the viability time period of *Salmonella* in air in commercial poultry facilities, which would help to elucidate the threat of airborne *Salmonella* to poultry.