Research Note: Bacterial composition of settled dust during growout of broiler chickens

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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 from 2 flocks (Flocks A and B). Dust samples for bacteria analyses were obtained during 6 wk of growout (Flocks A and B) and 1 wk after flock termination (Flock B) by environmental swabbing and collecting dust in petri dishes from multiple locations inside the poultry house. For weekly swabbing, dust deposited during each wk of the sampling period (noncumulatively, n = 12/wk) and cumulatively (n = 12/wk) throughout the sampling period was collected. Swabbed dust samples were analyzed for counts (log10 CFU/28 cm2) of aerobic bacteria, E. coli, coliforms, and Salmonella recovery. For petri dish dust collection, dust was collected in weekly and bi-weekly time spans during the sampling period and then analyzed for Salmonella recovery. Data were analyzed by one-way ANOVA and Fisher’s Exact Test and means were separated using LSD. Only aerobic plate counts changed over time in dust during growout (Flocks A and B; P < 0.0001). In noncumulatively settled dust, aerobic bacteria (Flocks A and B; 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 recovery did not vary in cumulatively (3/72, 10/84) and noncumulatively (0/12, 10/84) settled dust during growout in both flocks. In dust sampled by bi-weekly collection in petri dishes, Salmonella recovery was highest (5/6) between wk 2 to wk 4 for Flock B (P = 0.0118). Overall, this study displayed that settled dust bacteria levels can fluctuate during broiler growout, and dust can contain Salmonella.

Key words: dust, Salmonella, broiler, poultry house, bacteria

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 or become airborne due to different house activities or disruptive forces. Moreover, airborne dust can settle on surfaces, and vice versa (Aarnink and Ellen, 2007).

Poultry dust can be an area of control to prevent bacterial contamination of birds during production.

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Generally, 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 (E. coli, Salmonella spp., etc.) has been noted in poultry settled dust and air (Chinivasagam et al., 2009; Skóra et al., 2016). Moreover, dust can play a role in airborne transmission of pathogenic bacteria in poultry houses. Specifically, it has been reported that eggs hatching can generate Salmonella contaminated dust and fluff, which may circulate within the hatcher and potentially colonize other chicks in the same hatcher (Davies and Wray, 1994).

Levels of airborne microorganisms and dust in animal houses are influenced by different factors such as the animals (age, weight, activity, gender, and stocking density), housing types (aviary vs. cage system, natural curtain vs. mechanical ventilation system), and management system (feed distribution 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 the microorganism aerosolization process occurs via the litter-dust-aerosol interface; therefore, the microorganisms present in settled dust can enter into the air. Changes in settled dust levels and microflora during growout are 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.

Overall, evaluating settled dust has an important role in understanding microorganism distribution in the poultry house environment. 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.

MATERIALS AND METHODS

This study was performed in an experimental broiler house at the Miller Center of Auburn University and was approved by the Auburn University Institutional Animal Care and Use Committee (IACUC) (PRN #2019-3621). Two 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/pen) were terminated at 42 d of age. Litter in the sampled house of this study had been seeded with nalidixic acid resistant Salmonella Enteritidis by bird inoculation in a previous flock that was placed on fresh bedding. Flock A and 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 (107 CFU/bird) at d 7 of age to increase the level of Salmonella within the house. Litter and settled dust sampling was conducted on the day of bird placement (litter only), after 1, 2, 3, 4, and 5 wk of growout with birds present, 1 d following flock termination at 6 wk, and 1 wk following flock termination (wk 7 for Flock B only). Humidity and temperature values ranged between 63 and 88% and 24.6°C and 32.9°C, respectively, for Flock A and 42 and 73% and 20.2°C and 32.1°C, respectively, for Flock B.

Litter Sampling Methods

Litter Grab Method. Four litter grab samples were collected from 2 pens (2 samples/pen) on each sampling day in both flocks. For each composite sample, litter was collected from multiple locations inside the pen into a clean bag (Ziploc, Chicago, IL) and then transferred immediately to ice. For each sample, 10 g of litter was transferred into a sterile sampling bag (VWR International, Radnor, PA). Next, 90 mL of buffered peptone water (BPW) (Becton Dickinson and Company, Sparks, MD) was added and stomached for 1 min.

Aliquots were serially diluted in sterile saline for enumeration of APC, E. coli, and coliforms. Samples from appropriate dilutions were duplicate plated onto 3M Petrifilm aerobic count plates and 3M Petrifilm rapid E. coli/coliform count plates (3M Health Care, St. Paul, MN), and incubated 48 h at 37°C or 24 h at 37°C, respectively. Colonies were enumerated for APC, E. coli, and coliforms (ISO method 4832). The remaining litter sample (88 mL) was enriched at 37°C for 24 h. Enriched samples were evaluated for Salmonella detection by streaking onto Xylose Lysine Tergitol-4 (XLT4) (Hardy Diagnostics, Santa Maria, CA) agar plates and using the 3M molecular detection system (Saint Paul, MN) to avoid false negatives from plating. Plates were incubated at 37°C for 24 h before confirming presumptive isolated Salmonella colonies using Salmonella agglutination test (Salmonella O Antisemum Poly A–I and Vi, Becton Dickinson, Sparks, MD).

Settled Dust Sampling Methods

A total of 12 samples, 6 on each of the 2 opposite diagonal corners of the house (Light trap, baffle, wall, floor, pen top, and pen bottom ridge), were fixed for dust sampling for both flocks. Sampling locations were not cleaned during the sampling period. Dust swab samples for bacteria analyses were obtained during 6 wk of growout (Flocks A and B) and 1 wk after flock termination (Flock B). Bacteria in dust deposited during each wk of sampling period (noncumulatively, n = 12/wk) and cumulatively (n = 12/wk) throughout the sampling period were analyzed. All locations were cleaned and sanitized at bird placement with 70% ethanol. Noncumulatively settled dust bacteria were analyzed by cleaning and sanitizing the sampled area following every weekly sample collection. Cumulatively settled dust bacteria were analyzed by sampling an area adjacent to the previously sampled area. For dust sample collection, a swab moistened in a non-nutrient phosphate buffered neutralizing solution (902C, Copan Diagnostics Inc., Murrieta, CA) was used to swab an area of 28 cm2. The swab was inserted into a transport tube containing 10 mL of non-nutrient phosphate buffered neutralizing solution (the handle end snapped off) and then transferred to ice. Each swab sample tube was vortexed and a 5 mL aliquot was added to 5 mL double-strength BPW and incubated at 41.5°C for 24 h. Enriched samples were used to assess Salmonella recovery in the same manner as described previously. The remaining nonenriched swab containing solution (5 mL) was used for enumeration of APC, E. coli, and coliforms as described previously.

Moreover, the dust deposited within a 1 wk and 2 wk time span was collected by placing 24 empty petri dishes (100 × 15 mm) at 12 locations at a 25 cm vertical height. After 1 wk, 12 petri dishes were removed for dust sampling and replaced with a sterile petri dish. After 2 wk, all petri dishes were removed for dust sampling and replaced with 24 sterile petri dishes. Dish collection and
Statistical Analyses

The bacterial counts from dust and litter samples were transformed into $\log_{10}$ CFU/28 cm$^2$ and $\log_{10}$ CFU/g, respectively, before statistical analyses using SAS Studio, release 3.8 Enterprise Edition. Data for each flock were analyzed separately. One-way ANOVA was used to analyze the week-wise variation of bacterial counts and means were separated using LSD at $P \leq 0.05$ level of significance. Salmonella recovery data were analyzed using Fisher’s exact test.

RESULTS

Data for week-wise variation of aerobic bacteria, *E. coli*, and coliforms counts in settled dust and litter for both flocks are shown in Table 1. For Flocks A and B, aerobic bacteria increased in cumulatively ($P < 0.0001$) and noncumulatively ($P < 0.0001$) collected dust during broiler growout. In cumulatively collected dust samples from Flock A, aerobic bacteria increased during the first 3 wk of growout and then remained constant between wk 4 to wk 6. In Flock B, aerobic bacteria in cumulatively collected dust remained constant for the first 5 wk of growout, increased at wk 6 following flock termination, and then decreased at wk 7 to pretermination levels. Noncumulatively 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 litter varied weekly during growout ($P = 0.0002$) only for Flock A where the highest counts occurred between wk 2 to wk 6 of growout. *E. coli* counts did not vary during the growout period in cumulatively settled dust for either flock ($P = 0.1597$). *E. coli* levels in noncumulatively collected dust differed during the sampling period only in Flock A ($P = 0.0432$) where counts were the lowest at wk 1, the highest at wk 4, and intermediate at rest of the sampling days. Litter *E. coli* levels for both flocks varied during growout ($P < 0.0001, P = 0.0011$). Likewise *E. coli*, coliform counts in cumulative settled dust did not vary over time during growout in either flock ($P = 0.7919, P = 0.3454$). In noncumulative settled dust samples, coliforms were different by weeks only in Flock B ($P =$

| Sampling time | Flock A | Flock B | Flock A | Flock B |
|---------------|---------|---------|---------|---------|
|               | Cumulative dust samples | Noncumulative dust samples | Cumulative dust samples | Noncumulative dust samples |
| APC           |         |         |         |         |
| BP            |         |         |         |         |
| Wk 1          | 5.38 ± 0.20$^a$ | 5.38 ± 0.20$^a$ | 6.71 ± 0.11$^b$ | 6.71 ± 0.11$^b$ |
| Wk 2          | 6.27 ± 0.26$^a$ | 5.53 ± 0.19$^a$ | 6.83 ± 0.15$^b$ | 6.80 ± 0.14$^b$ |
| Wk 3          | 7.09 ± 0.16$^a$ | 7.12 ± 0.13$^a$ | 6.87 ± 0.15$^b$ | 6.67 ± 0.15$^b$ |
| Wk 4          | 7.73 ± 0.14$^a$ | 7.33 ± 0.23$^a$ | 6.79 ± 0.14$^b$ | 6.86 ± 0.15$^b$ |
| Wk 5          | 8.25 ± 0.19$^a$ | 7.92 ± 0.17$^a$ | 6.87 ± 0.09$^b$ | 6.95 ± 0.09$^b$ |
| Wk 6 + 1d$^b$ | 8.11 ± 0.14$^a$ | 7.56 ± 0.20$^a$ | 8.25 ± 0.19$^b$ | 7.92 ± 0.17$^b$ |
| Wk 7          | 6.96 ± 0.09$^b$ | 6.95 ± 0.04$^b$ | 7.01 ± 0.09$^b$ | 6.95 ± 0.04$^b$ |
| P value       | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0010 |
| $E. coli$     |         |         |         |         |
| BP            |         |         |         |         |
| Wk 1          | 1.12 ± 0.12 | 1.12 ± 0.12$^a$ | 1.93 ± 0.30 | 1.93 ± 0.30 |
| Wk 2          | 2.06 ± 0.20 | 1.67 ± 0.13$^a$ | 1.79 ± 0.28 | 1.59 ± 0.24 |
| Wk 3          | 1.54 ± 0.28 | 1.83 ± 0.18$^a$ | 1.81 ± 0.22 | 1.86 ± 0.15 |
| Wk 4          | 1.68 ± 0.56 | 2.46 ± 0.58$^a$ | 2.57 ± 0.26 | 2.28 ± 0.18 |
| Wk 5          | 2.19 ± 0.46 | 1.84 ± 0.50$^a$ | 1.62 ± 0.18 | 1.65 ± 0.26 |
| Wk 6 + 1d$^b$ | 2.34 ± 0.54 | 2.15 ± 0.28$^a$ | 1.74 ± 0.23 | 1.62 ± 0.15 |
| Wk 7          | 2.01 ± 0.33 | 1.47 ± 0.30 | 2.01 ± 0.33 | 1.47 ± 0.30 |
| P value       | 0.1298   | 0.0432   | 0.1597   | 0.2707   |

| Coliforms    |         |         |         |         |
| BP$^c$       |         |         |         |         |
| Wk 1          | N/A$^d$ | N/A$^d$ | 1.86 ± 0.30 | 1.86 ± 0.30$^b$ |
| Wk 2          | 2.07 ± 0.20 | 1.77 ± 0.13 | 2.14 ± 0.38 | 1.80 ± 0.23$^a$ |
| Wk 3          | 2.11 ± 0.25 | 1.92 ± 0.16 | 2.05 ± 0.31 | 1.92 ± 0.16$^a$ |
| Wk 4          | 1.85 ± 0.24 | 2.20 ± 0.33 | 2.62 ± 0.28 | 2.50 ± 0.22$^a$ |
| Wk 5          | 1.96 ± 0.46 | 1.80 ± 0.43 | 1.59 ± 0.15 | 1.54 ± 0.23$^b$ |
| Wk 6 + 1d$^b$ | 2.40 ± 0.48 | 2.36 ± 0.28 | 2.06 ± 0.38 | 1.62 ± 0.14$^a$ |
| Wk 7          | 1.85 ± 0.31 | 1.42 ± 0.24$^b$ | 1.85 ± 0.31 | 1.42 ± 0.24$^b$ |
| P value       | 0.7919   | 0.5435   | 0.3464   | 0.0303   |

1APC: Aerobic plate counts.
2BP: Bird placement.
3Wk 6 + 1d: This sampling was performed on a day after flock termination that was equivalent to a day after wk 6.
4N/A: Data on this sampling day was not collected.

$^a$, $^b$, $^c$, $^d$, $^e$, $^f$, $^g$, $^h$, $^i$, $^j$, $^k$, $^l$, $^m$, $^n$, $^o$, $^p$, $^q$, $^r$, $^s$, $^t$, $^u$, $^v$, $^w$, $^x$, $^y$, $^z$, $^{|}$, $^~$, $^\$
Dust producing sources contribute differently to dust production depending on animal types and their house infrastructure, and several factors related to animal, housing, and management practices can influence dust levels and its associated microorganism levels in animal production facilities (Zhao et al., 2014). We observed from noncumulatively settled dust that bacteria levels deposited during different wk of growout may or may not vary. A possible reason for fluctuation in bacteria levels could be variation of dust levels during different stages of rearing. Dust production levels can be affected with animal age, animal weight, and activities (Zhao et al., 2014). Previously, Calvet et al. (2009) reported that dust levels increased with broiler age. They found that particulate matter, with diameter of 10 μm or less, increased from 0.10 to 2.82 mg/m³ and 0.05 to 0.79 mg/m³ during wk 1 to wk 5 of the growing cycle in the light and dark period, respectively. In the same study, dust concentration had a strong positive correlation with bird activities and their live weight ($r^2 = 0.89$), and bird activities were 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. In cumulatively settled dust, we observed that only APC were increased over time during growout in either flock. Previously, aerobic bacteria in airborne dust were found to have an increasing concentration of $0.91 \times 10^3$ CFU/m³, $6.86 \times 10^3$ CFU/m³, and $13.77 \times 10^3$ CFU/m³ at d 3, d 22, and d 40, respectively, following bird placement (Jiang et al., 2018). For settled dust, Skóra et al. (2016) reported an average of $3.2 \times 10^3$ CFU of total bacteria and $1.6 \times 10^3$ CFU of E. coli per gram of 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 recovery in dust and litter samples for Flocks A and B are shown in Table 2. Salmonella recovery in cumulatively and noncumulatively settled dust did not differ across time points during growout in either flock (Flock 1: $P = 0.4205$, $P=1.000$, Flock 2: $P = 0.4622$, $P = 0.7656$). Overall, Salmonella recovery 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 samples on wk 3 of growout. For Flock B, Salmonella detection varied at different sampling weeks in litter samples ($P = 0.0122$). Salmonella recovery (Flock A: 1/36; Flock B: 18/42) in dust sampled by weekly collection, using petri dishes, did not differ during growout for either flock ($P = 1.000$, $P = 0.0678$). For Flock A, the single positive petri dish sample was detected at wk 3. For Flock 2/6, 0/6, 3/6, 2/6, 4/6, 3/6, 1/6, or 5/6 positive samples were detected from wk 1 to 7, respectively. In dust sampled by bi-weekly collection, Salmonella recovery was highest at wk 4 (5/6 positive) and lowest at wk 2 (1/6) and wk 6 (0/6) for Flock B ($P = 0.0118$) and was not changed over time for Flock A ($P = 0.2941$, 0/6, 2/6, or 0/6 for wk 2, 4, or 6, respectively). Overall, Salmonella recovery in dust settled in petri dishes within a 2 wk time span was 2/18 and 6/18 for Flock A and B, respectively.

In this study, we observed the presence of Salmonella in cumulatively (Flock A, Flock B) and noncumulatively (Flock B) settled dust. Previously, Skóra et al. (2016) also observed that Salmonella in settled dust ranged between 1.1 and $6.3 \times 10^5$ CFU/g. It is interesting to note that the overall recovery of Salmonella in dust collected noncumulatively 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 consistently be detected. Chinivasagam et al. (2009) reported that multiple factors can influence Salmonella recovery in cumulatively and noncumulatively settled dust and litter samples for Flocks A and B.

### Table 2. Week wise variation of Salmonella recovery in cumulatively and noncumulatively settled dust and litter samples for Flocks A and B.

| Sampling time | Flock A | Flock B | Flock A | Flock B |
|---------------|---------|---------|---------|---------|
|               | Cumulative dust samples | Noncumulative dust samples | Cumulative dust samples | Noncumulative dust samples | LGS | LGS |
| BP$^1$        | -       | -       | -       | -       | 0/4 | 0/4$^a$ |
| Wk 1          | 0/12    | 0/12    | 1/12    | 1/12    | 0/4 | 2/4$^{ab}$ |
| Wk 2          | 2/12    | 0/12    | 0/12    | 1/12    | 0/4 | 2/4$^{ab}$ |
| Wk 3          | 1/12    | 0/12    | 2/12    | 3/12    | 0/4 | 4/4$^a$ |
| Wk 4          | 0/12    | 0/12    | 2/12    | 2/12    | 0/4 | 3/4$^{ab}$ |
| Wk 5          | 0/12    | 0/12    | 2/12    | 2/12    | 0/4 | 4/4$^a$ |
| Wk 6 + 1d$^2$ | 0/12    | 0/12    | 0/12    | 0/12    | 0/4 | 1/4$^{ab}$ |
| Wk 7          | 3/12    | 1/12    | 0/12    | 0/12    | 0/4 | 4/4$^a$ |
| $P$ value     | 0.4205  | -       | 0.4022  | 0.7656  | 0.0085 | 0.0122 |

$^1$BP: Bird placement.
$^2$Wk 6 + 1d: This sampling was performed on a day after flock termination that was equivalent to a day after wk 6.
$^3$LGS: Litter grab samples.

$^{a,b}$Values within a column with different superscripts differ significantly $P \leq 0.05$. 


survivability in dust and *Salmonella* resilience to poultry environment conditions can vary according to different serovars. Moreover, *Salmonella* was found in dust one wk after Flock B termination indicating the possibility of dust to act as a horizontal means of *Salmonella* transmission to the chicks in the new flock placed in the house. Additionally, in Flock B, during the sampling period of 7 wk, the dust collected by the petri dish method had higher overall *Salmonella* recovery 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 noncumulatively 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 should be considered when evaluating settled and airborne dust and their bacteria levels. Moreover, the difference between 2 flocks in terms of operating conditions of the poultry house such as relative humidity or temperature might cause the differences in the bacterial fluctuations during growout of Flock A and B.

In the current study, an indirect interrelationship between litter and settled dust bacteria counts or *Salmonella* recovery 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* recovery (20/32) due to the inoculation of birds. Consequently, the cumulative and noncumulative settled dust samples of Flock B had higher *Salmonella* recovery 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.

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. Moreover, dust can be contaminated with *Salmonella*. Based on the results of this work, the control of dust in poultry houses should be considered for the reduction of transmission of airborne bacteria and potentially foodborne pathogens.

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**DISCLOSURES**

All authors have participated in (a) conception and design, or analysis and interpretation of the data; (b) drafting the article or revising it critically for important intellectual content; and (c) approval of the final version. This manuscript has not been submitted to, nor is under review at, another journal or other publishing venue. The authors have no affiliation with any organization with a direct or indirect financial interest in the subject matter discussed in the manuscript.

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