Environmental sampling methods' influence on detection of pathogens in cage-free aviary housing

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ABSTRACT The environmental sampling of layer housing systems is essential to identifying potential pathogens that are of concern to human health. To identify the natural occurrence of pathogens (*Listeria, Campylobacter*, and *Salmonella*) at various locations in a cage-free aviary housing system, swabs were collected when hens were 22 to 39 wks of age. Duplicate environmental swabs were taken and inoculated with a low dose (10^1 cfu) *Salmonella* Enteritidis (**SE**) and examined for the recovery of SE from environmental samples. Detection of *Listeria* ($P < 0.0001$) and *Campylobacter* ($P < 0.0001$) varied between the environmental sample types taken: concrete dust, drag swabs, egg belt dust, manure belt scraper swabs, and wall dust. Detection of *Listeria* ($P < 0.0001$) was the highest (70.0%) at the beginning of the study (22 wk) and decreased over time. Detection of *Campylobacter* ($P < 0.001$) was also the highest at 22 wk, however the decrease over time was more gradual. Interestingly, detection of *Campylobacter* ($P < 0.0001$) was the greatest in concrete dust samples (96.25%), which can be attributed to the presence of rodent excreta in the samples. Drag swabs and manure belt scraper swabs were the best sampling types for high detection of *Listeria* and *Campylobacter*. It should be noted that Listeria recovered was not of human health concern. No naturally occurring *Salmonella* was identified in this study. The recovery of the SE inoculum increased over time, reaching the greatest recovery in drag (81.25%; $P < 0.0001$), egg belt dust (100.00%; $P < 0.0001$) and wall dust swabs (100.00%; $P < 0.0001$) by 39 wk. This high rate of SE recovery occurred just before US mandatory SE environmental monitoring at 40 to 45 wks of age. Based on this study, the use of drag and manure belt scraper swabs are effective in detecting *Listeria* and *Campylobacter* in cage-free aviary housing. Along with good pest management, the occurrence of pathogens could be monitored and reduced in laying hen flocks.

Key words: cage-free aviary, environmental sampling, campylobacter, listeria, salmonella

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INTRODUCTION

Foodborne pathogens such as *Campylobacter, Listeria* and *Salmonella* are important microorganisms to the poultry and egg industries since the organisms can cause illness in humans and have been detected in the production environment. In 2019, *Campylobacter, Listeria* and *Salmonella* were among the top 10 causes of bacterial foodborne illness in the United States (CDC, 2020).

Environmental sampling is a useful tool to identify the possible presence of pathogens in poultry flocks. Previous studies have detected *Salmonella* and *Campylobacter* spp. in environmental and eggshell samples from a variety of layer housing systems (Jones et al., 2015; Jones et al., 2016). Live birds have also been found to be important vectors for *Listeria* contamination of processing equipment (Rothrock et al., 2017).

Salmonella Enteritidis (**SE**) is of concern to the egg industry due to its ability to be vertically transferred to eggs from infected hens. Under the Food and Drug Administration (**FDA**) Egg Rule, U.S. shell egg producers with ≥3,000 hens are required to monitor for SE by environmental swabbing at designated times during the life of a production flock (FDA, 2009). Drag swabs and manure belt scraper swabs are the identified means to conduct the sampling. Most of the research utilized in the development of the FDA Egg Rule was from conventional housing systems. Much of the world is transitioning to extensive hen housing systems for egg laying hens. In recent years other *Salmonella* serotypes have been linked to foodborne outbreaks (CDC, 2016, 2018). Additionally, other organisms beyond SE can be important when developing egg safety programs. However, it is not known which environmental sampling methods

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are best for detecting *Listeria, Campylobacter*, and *Salmonella* in cage-free aviary housing systems.

The objectives of this study were to 1) determine the natural occurrence of pathogens (*Campylobacter, Listeria*, and *Salmonella*) by collecting environmental samples from various locations in a cage-free aviary housing system and 2) to examine the recovery of *Salmonella* from duplicate environmental swab samples collected from the cage-free aviary housing system, following a low dose (10^1 cfu) *Salmonella* Enteritidis inoculation into the environmental sample.

MATERIALS AND METHODS

Laying Hen Flock Management

Laying hen chicks (Lohmann Brown, n = 3,200) were obtained from a commercial hatchery. From 1 d to 16 wk of age, chicks were reared in floor pens in 8 rooms (10.26 m long × 37.85 m wide; 400 chicks/room) within the grower house at the Purdue Animal Sciences Research and Education Center (**ASREC**) Poultry Unit. Chicks were randomly assigned to rooms and initially brooded in brood rings on a central platform (7.98 m long × 1.63 m wide × 0.58 m high) in each room for the first 4 weeks, and then given access to the entire room. Birds could access the platform using ramps. Four rooms had AstroTurf on the floor and 4 rooms had wood shavings on the floor. At 17 wk, pullets were transferred to 4 aviary-style rooms within the layer house at the ASREC poultry unit; 2 rooms had AstroTurf on the floor area and 2 rooms had wood shavings on the floor area.

The aviary rooms were originally enriched colony cage systems that had been converted into aviaries by removing the cage fronts and installing ramps to provide birds with access to the 2 cage tiers located above the floor area. Each room contained a bank of 36 enriched colony cages (73.66 cm (width) × 121.92 cm (length) × 45.72 cm high at the back of the cage and 58.42 cm high at the front of the cage) with 2 adjacent rows of 9 cages each comprising the bottom tier and 2 rows of 9 cages each comprising the top tier. Within each cage, 6 nipple waterers provided water ad libitum and there was a nesting area (38.10 cm (width) × 55.22 cm (length)) with an AstroTurf Poultry Nest Pad secured to the wire floor and orange vinyl curtain flaps at the front and sides of the nest. Eggs were collected from egg belts that ran in front of the cages and were situated underneath the chain feeding system that ran throughout the room on each tier. Within each tier, birds could freely move between each set of 3 cages, which were separated from the next set of 3 cages by a wire partition. A clear acrylic platform (23.18 cm wide) installed in front of the feeder enabled birds to move around the wire partition and access the next set of cages. Each tier also had a round metal perch (3.80 cm in diameter) located in front of the plastic platform, approximately 25.00 cm from the feeder. Manure was collected on a manure belt located under each tier.

Each room was divided into 2 larger (6.10 m in length × 1.98 m in width) and 2 smaller (4.88 m in length × 1.98 m in width) sections; wire mesh running longitudinally underneath the cage system prevented hens from crossing over into adjacent sections, and metal gates prevented birds from accessing sections but enabled observers and farm staff to move between sections. The two rooms that had AstroTurf flooring also had a custom-built false floor that enabled a manure scraper to move underneath the floor and collect manure that fell through the AstroTurf flooring. The false floor and manure removal system was designed and installed by GrassWorx LLC who also provided the AstroTurf used in the study. The two remaining rooms that had wood shavings on the floor did not have a false floor or manure scraper.

Pullets were assigned to rooms such that 218 to 219 birds were placed in each of the larger sections and 171 to 172 birds were placed in each of the smaller sections. Within each room, one large and one small section had birds that had been raised on the same type of flooring in the grower room, and the other two sections had birds that had been raised on the other type of flooring. Therefore, half of the birds in each room had been raised on AstroTurf and the other half had been raised on wood shavings, with equal numbers of birds being randomly selected from each of the grower rooms. Within a week after moving birds to the aviary rooms, locking mechanisms on some of the gates failed, resulting in birds from different sections within each aviary room becoming mixed.

Hens were housed at a density of about 0.09 m^2 per hen. Diets were commercial formulations and prepared according to breeder's recommendations. Hens were provided with ad libitum access to water and feed. Lighting was provided in accordance with the Lohmann management guide (Lohmann Tierzucht, 2019). All hen management protocols were approved by the Purdue University Institutional Animal Care and Use Committee (protocol number 1901001848).

Environmental Sample Collection

Environmental sampling took place about every 4 to 5 wks between August (22 wks of age) and December (39 wks of age) and a duplicate of each environmental sample was taken. Drag swabs were conducted by walking the substrate area of each treatment replicate within a housing room with a single commercially prepared drag swab (Solar Biological, Romer Labs, Newark, DE; n = 32 swabs each hen age). Manure scraper swabs were conducted by removing the string from a prepared drag swab and wiping across the manure scraper for each treatment replicate within a room (n = 32 swabs each hen age). Egg belt, wall and concrete floor dust swabs were conducted with premoistened sterile sponges (B01422, Whirl-pak, Madison, WI; n = 32 swabs of each type for each hen age). Approximately a 30 cm length of area was swabbed utilizing both sides of the sample

sponge. Aseptic techniques were utilized to collect all environmental samples. After collection, swabs were shipped overnight to the laboratory on ice.

Naturally Occurring Microbial Level Determinations

Upon arrival at the laboratory, half of each type of sample had 20 mL of sterile phosphate-buffered saline (**PBS**) added and stomacher-blended (Stomacher 400 Circulator, Seward Ltd., London, UK) for 1 min at normal speed. Detection of *Listeria, Campylobacter*, and *Salmonella* was conducted according to the methods described in Jones et al. (2006). Briefly, a 5 mL aliquot of sample was introduced into 45 mL of UVM modified *Listeria* enrichment broth (Acumedia Manufacturers) and incubated at 37°C for 24 h to initiate *Listeria* pre-enrichment. Afterwards, 100 μL of pre-enrichment was introduced into 10 mL of Fraser broth enrichment and incubated at 30°C for 24 h to 48 h (broth, Acumedia Manufacturers; supplements, Becton Dickinson, Sparks, MD). Positive Fraser tubes were streaked onto modified Oxford agar and incubated at 37°C for 24 h (Becton Dickinson). Presumptive positive colonies were introduced into motility agar (Acumedia Manufacturers) and incubated at room temperature for 48 h. Presumptive positive colonies were then identified biochemically (Microgen *Listeria* ID kit, Microbiology International).

For *Campylobacter* determination, a 5 mL aliquot of sample was enriched in 45 mL of Bolton's broth (*Campylobacter* enrichment broth, Acumedia Manufacturers, Lansing, MI; Bolton broth selective supplement, Oxoid Limited, Basingstoke, UK; defibrinated horse blood, Lampire Biological Laboratories, Pipersville, PA) under modified atmosphere and incubated at 42°C for 24 h. One-tenth of a milliliter was plated onto Campy Cefex plates (Stern et al., 1995; agar, Acumedia Manufacturers; defibrinated horse blood, Lampire Biological Laboratories; cefoperazone sodium salt, Sigma-Aldrich, St. Louis, MO) and incubated at 42°C for 48 h under modified atmosphere. Typical colonies were confirmed via latex agglutination (Microbiology International, Frederick, MD).

The remaining sample, including the swab or sponge, was pre-enriched with 10 mL of buffered peptone water (**BPW**; Acumedia Manufacturers), stomacher-blended for 1 min at normal speed and incubated at 37°C for 24 h to initiate *Salmonella* detection. Aliquots (0.1 mL) were then enriched in both Rappaport-Vassiliadis (RV; Becton Dickinson) and Tetrathionate Hajna (Becton Dickinson) broths and incubated at 42°C for 24 h. Each enriched sample was streaked on both brilliant green sulfa (**BGS**; Acumedia Manufacturers) and XLT4 (agar, Acumedia Manufacturers; supplement, Becton Dickinson) agars and incubated at 37°C for 24 h. Presumptive positive colonies were stabbed on both lysine iron agar (Becton Dickinson) and triple sugar iron agar (Becton Dickinson) slants and incubated at 37°C for 24 h. Colonies presenting *Salmonella* properties were subjected to latex agglutination (Microbiology International).

Salmonella Inoculation of Environmental Samples and Detection Procedures

The remaining environmental sample duplicates (n = 16/sampling) were used to determine the recovery of *Salmonella* Enteritidis (**SE**) after being inoculated with a low dose of SE. A SE isolate collected from the egg production environment in a previous study, trained to be resistant to 200 ppm Nalidixic acid, was used. The isolate was resuscitated from double nutrient agar by streaking onto a BGS plate containing 200 ppm of nalidixic acid (**BGS-NAL;** Sigma-Aldrich) and incubated at 37°C for 24 h. The following day, one colony from the BGS-NAL plate was transfer into tryptic soy broth (Acumedia Manufacturers) and incubated at 37°C for 24 h.

Approximate concentration of the overnight culture was determined by optical density at 600 nm. Serial dilutions were made with BPW to achieve a final mean cell level of 10^2 CFU/mL inoculum. From the inoculum, 0.10 mL was plated onto duplicate BGS-NAL plates and incubated for 24 h at 37°C to determine final challenge concentration. The diluted culture of NAL-resistant SE was used to inoculate the remaining environmental swabs. Each swab or sponge was inoculated by placing 0.10 mL of the diluted culture directly onto the swab or sponge surface (approximately 10^1 CFU). Each contaminated swab or sponge then received 10 mL BPW, stomacher-blended for 1 min at normal speed and incubated for 24 h at 37°C.

A 0.10 mL aliquot was then transferred into 10 mL RV and incubated at 42°C for 24 h. The enriched sample was struck on BGS-NAL and incubated at 37°C for 24 h to determine the rate of inoculum recovery.

Statistical Analysis

The prevalence of detection for *Listeria, Campylobacter*, and *Salmonella* from the non-inoculated environmental swabs was determined by chi-square analysis (SAS Institute, Cary, NC). The main effects for analysis were substrate type, hen age and sample type. Additionally, prevalence of *Salmonella* Enteritidis within the inoculated environmental samples was also determined by chi-square analysis (SAS Institute, Cary, NC). The main effects for analysis were hen age and sample type.

RESULTS AND DISCUSSION

Substrate type did not impact the detection of *Listeria, Campylobacter*, and *Salmonella*. Overall detection of *Listeria* in environmental samples from the cage-free aviary in this study varied between sample type (Table 1). Detection ($P < 0.0001$) of *Listeria* was

Table 1. Overall detection of *Listeria* and *Campylobacter* in environmental samples at 22, 26, 30, 34, and 39 wks of age from a cage-free aviary housing system.

Organism	Sample type	Detection % (Prevalence)
Listeria	Concrete dust	48.75% (39/80)
	Drag swabs	75.00% (57/79)
	Egg belt dust	28.75% (23/79)
	Manure belt scraper	50.00% (40/80)
	Wall dust	16.25% (13/79)
	P-value	<0.0001
	Hen age	Detection % (Prevalence)
	22 wk	70.00% (56/80)
	26 wk	38.75% (31/80)
	30 wk	50.00% (40/80)
	34 wk	13.92% (11/79)
	39 wk	44.16% (34/77)
	P-value	<0.0001
	Sample type	Detection % (Prevalence)
Campylobacter	Concrete dust	96.25% (77/80)
	Drag swabs	49.37% (39/76)
	Egg belt dust	34.18% (27/80)
	Manure belt scraper	73.75% (59/80)
	Wall dust	11.25% (9/79)
	P-value	<0.0001
	Hen age	Detection % (Prevalence)
	22 wk	65.00% (52/80)
	26 wk	62.50% (50/80)
	30 wk	53.16% (42/79)
	34 wk	35.44% (28/79)
	39 wk	48.75% (39/80)
	P-value	0.001

Table 2. Detection of *Listeria* within an environmental sample at 22, 26, 30, 34, and 39 wks of age from a cage-free aviary housing system.

Sample type	Hen age	Detection % (Prevalence)
Concrete dust	22 wk	62.50% (10/16)
	26 wk	68.75% (11/16)
	30 wk	31.25% (5/16)
	34 wk	0.00% (0/16)
	39 wk	81.25% (13/16)
	P-value	<0.0001
Drag swabs	22 wk	100.00% (16/16)
	26 wk	50.00% (8/16)
	30 wk	100.00% (16/16)
	34 wk	40.00% (6/15)
	39 wk	84.62% (11/13)
	P-value	<0.0001
Egg belt dust	22 wk	68.75% (11/16)
	26 wk	37.50% (6/16)
	30 wk	18.75% (3/16)
	34 wk	0.00% (0/16)
	39 wk	18.75% (3/16)
	P-value	0.0003
Manure belt scraper	22 wk	56.25% (9/16)
	26 wk	18.75% (3/16)
	30 wk	100.00% (16/16)
	34 wk	31.25% (5/16)
	39 wk	43.75 (7/16)
	P-value	<0.0001
Wall dust	22 wk	62.50% (10/16)
	26 wk	18.75% (3/16)
	30 wk	0.00% (0/16)
	34 wk	0.00% (0/16)
	39 wk	0.00% (0/16)
	P-value	<0.0001

greatest in drag swabs (75.00%), followed by manure scrapers (50.00%) and concrete dust (48.75%), and finally egg belt dust (28.75%) and wall dust (16.25%). Of the *Listeria* positive samples (172/397 total samples; 43.3% positive), 91.9% were identified as *L. innocua*, 7.6% were *L. welshimeri* and 0.5% were *L. grayi*, all of which are commonly found in the environment and are not generally considered pathogenic to humans or animals (Jones et al., 2012; Milillo et al., 2012; Rothrock et al., 2017). Chemaly et al. (2008) reported that in *L. monocytogenes*-positive flocks, detection was greater in fecal samples than in dust samples. Although *L. monocytogenes* was not detected in the current study, this trend was also observed as detection of *Listeria* spp. was greater in drag and manure belt scraper swabs compared to the concrete, egg belt, and wall dust swabs.

Detection of *Listeria* (Table 1) varied between the age of the flocks when the samples were taken. Detection ($P < 0.0001$) of *Listeria* was greatest at 22 wk of age (70.00%) and varied in detection (13.92−50.00%) thereafter. *Listeria* spp. has been isolated in younger birds, which may indicate that as the bird's intestinal microbiota develops, *Listeria* spp. levels decline (Milillo et al., 2012). Upon further analysis, the detection of *Listeria* (Table 2) within the environmental samples varied as the flocks aged (late summer to late fall). In concrete dust, *Listeria* detection ($P < 0.0001$) was high at 22 wk and 26 wk (62.50% and 68.75%, respectively), low at 30 wk (31.25%), no detection at 34 wk (0%) and increasing to 81.25% by 39 wk. Egg belt dust started with a similar trend in the detection of *Listeria* ($P < 0.0003$), 68.75% at 22 wk, 37.50% at 26 wk, 18.75 % at 30 wk, 0% at 34 wk and but did not exhibit the increased detection at 39 wk (18.75%). However, this is not observed in the wall dust ($P < 0.0001$) as detection of *Listeria* was high at 22 wk (62.50%), decreases to 18.75% at 26 wk and remained at 0% for the remainder of the study. For both concrete dust and egg belt dust, fecal matter could also be present in the dust sample as opposed to the wall dust samples. Drag swabs and manure scraper ($P < 0.0001$) samples had same trends with high detection of *Listeria* at 22 wk, 30 wk, and 39 wk and low detection of *Listeria* at 26 wk and 34 wk. Chemaly et al. (2008) found that dust samples from floor-reared hens were more frequently contaminated with *Listeria* due to the increased likelihood of contact with feces. Drag swabs and manure belt scraper swabs had the best overall detection for *Listeria* and would be the best environment samples to take for the detection of *Listeria* in cage-free aviary housing.

Overall detection of *Campylobacter* in environmental samples from a cage-free aviary varied between sample type (Table 1). Detection ($P < 0.0001$) of *Campylobacter* was greatest for concrete dust (96.25%), followed by manure scrapers (73.75%), drag swabs (49.37%), egg belt dust (34.18%), and wall dust (11.25%). The high detection of *Campylobacter* in concrete dust is counter to previous studies which have found that dry environments tend to be unfavorable for the growth and recovery of *Campylobacter* (Jones et al., 2015; Smith et al., 2016). However, there was a noticeable amount of rodent excreta on the concrete dust sample sponges, which could have contributed to the higher detection of *Campylobacter* in the concrete dust. Previous studies

have found that the presence of rodents increased the risk of flocks becoming infected with *Campylobacter* (Meerburg and Kijlstra, 2007; McDowell et al., 2008; Meerburg, 2010). The concrete dust swabs in the current study were collected from the concrete floor of the entry area of the cage-free aviary housing rooms. The laying hens did not have access to the room entry area. Furthermore, it was noted that rodent activity appeared to be limited to areas outside of the cage-free aviary systems. Rodent excreta was not obvious in the other sample types throughout the study.

Detection ($P < 0.001$) of *Campylobacter* was also the greatest early in sampling (22 wk: 65.00%), as seen in the detection of *Listeria*. However, detection of *Campylobacter* ($P < 0.001$) remained high through 30 wk (65.00%−53.16%), with a decrease at 34 wk (35.44%) and an increase at 39 wk (48.75%). Higher prevalence of *Campylobacter* has been reported in more intensive housing systems, where >90% of the hens were found to be colonized with *Campylobacter* compared to 85% infection rate in cage-free aviary hens (Jones et al., 2016).

Upon further analysis, the detection of *Campylobacter* (Table 3) within the environmental samples varied as the flocks aged (late summer to late fall). In manure scraper, detection of *Campylobacter* ($P < 0.01$) is high at 22 (75.00%), 26 (93.75%), and 30 (87.50%) wks, decreases at 34 (43.75%) wk and increases at 39 (68.75%) wk. In drag swabs, detection of *Campylobacter* ($P < 0.0004$) was high at 22 (87.50%) wk and decreases at 26 (56.25%), 30 (50.00%), and 34 (6.67%) wks and increases again at 39 (43.75%) wk. The rate of *Campylobacter* detection for manure scraper and drag swab samples are different from those previously reported for cage-free aviaries (Jones et al., 2015). The design of cage-free aviaries is unique to each model of equipment on the market with the two studies utilizing different cage-free aviary designs which could contribute to the difference in pathogen detection. Previous research has found a higher level of *Campylobacter* detection during the summer than in the winter (Kers et al., 2018; Marmion et al., 2021). The high levels of *Campylobacter* detection during 22 wk (late summer) and 26 wk (early fall) might be linked to seasonal impacts. However, it is unclear why increases in detection of *Campylobacter* in drag swabs and manure scraper were observed at later sample times. The presence of rodents could have played a role as the high detection of *Campylobacter* in concrete dust occurred in the presence of rodent excreta and stresses the importance of a good pest control program.

Table 3. Detection of *Campylobacter* within an environmental sample at 22, 26, 30, 34, and 39 wks of age from a cage-free aviary housing system.

Sample type	Hen age	Detection % (Prevalence)
Concrete dust	22 wk	93.75% (15/16)
	26 wk	100.00% (16/16)
	30 wk	93.75% (15/16)
	34 wk	93.75% (15/16)
	39 wk	100.00% (16/16)
	P-value	0.72
Drag swabs	22 wk	87.50% (14/16)
	26 wk	56.25% (9/16)
	30 wk	50.00% (8/16)
	34 wk	6.67% (1/15)
	39 wk	43.75% (7/16)
	P-value	0.0004
Egg belt dust	22 wk	56.25% (9/16)
	26 wk	43.75% (7/16)
	30 wk	20.00% (3/16)
	34 wk	25.00% (4/16)
	39 wk	25.00% (4/16)
	P-value	0.16
Manure belt scraper	22 wk	75.00% (12/16)
	26 wk	93.75% (15/16)
	30 wk	87.50% (14/16)
	34 wk	43.75% (7/16)
	39 wk	68.75% (11/16)
	P-value	0.01
Wall dust	22 wk	12.50% (2/16)
	26 wk	18.75% (3/16)
	30 wk	12.50% (2/16)
	34 wk	6.25% (1/16)
	39 wk	6.25% (1/16)
	P-value	0.78

Naturally occurring *Salmonella* was not detected in any of the environmental samples. Detection of SE in the laying house environment can be a useful predictor of possible egg contamination in commercial flocks (Henzler et al., 1998; Arnold et al., 2010; 2015). The sample inoculation model allowed for determining if naturally occurring flora within sample types would impede SE detection. The inoculation dose for environmental samples at 22, 26, 30, 34, and 39 wk were as follows: 2.0×10^1 CFU/mL, 1.7×10^1 CFU/mL, 8.2×10^1 CFU/mL, 9.9×10^1 CFU/mL, and 4.1×10^1 CFU/mL, respectively. No differences between challenge dosages were observed ($P < 0.09$). Overall recovery of inoculated SE from environmental samples (Table 4) varied as the flocks aged but followed similar trends. In the concrete dust ($P < 0.0001$), the SE inoculum was not recovered (0%) at 22 and 26 wks, was low (37.50%) at 30 wk and high at 34 (81.25%) and 39 (68.75%) wks. At 22 and 26, *Listeria* and *Campylobacter* detection in the concrete dust was high, and the SE inoculation concentration was low, which could have deterred SE growth. Furthermore, 22 and 26 wks had the lowest dose of SE inoculated into the samples. Other intestinal bacteria found in the gastrointestinal tract of birds can be harvested by environmental swabs and can provide a competitive environment for *Salmonella* during transport (Rolfe et al., 2000). In drag swabs, recovery ($P < 0.0001$) of SE inoculum was low (6.25%) at 22 and 26 wks, high at 30 (56.25%), 34 (81.25%), 39 (81.25%) wk. At 34 and 39 wks, detection of *Listeria* and *Campylobacter* had decreased, which may have given SE the opportunity to grow. In the egg belt dust, recovery ($P < 0.0001$) of SE inoculum was low at 22 (31.25%) and 26 (37.50%) wks, and high at 30 (50.00%), 34 (93.75%), 39 (100.00%) wk. The dry environment of dust is not an ideal medium for the growth of *Listeria* and *Campylobacter*, which explains the low detection of these pathogens and the introduction of SE via a broth to the sample provided SE the best opportunity for growth. Dust is a good vehicle for transporting bacteria via the air (Zhao et al., 2014). The spread of potential pathogenic

Table 4. Overall recovery of inoculated *Salmonella* Enteritidis (1 log CFU) in environmental samples taken from flocks at 22, 26, 30, 34, and 39 wks of age from in a cage-free aviary housing system.

Sample type	Hen age	Detection % (Prevalence)
Concrete dust	22 wk	0.00% (0/16)
	26 wk	0.00% (0/16)
	30 wk	37.50% (6/16)
	34 wk	81.25% (13/16)
	39 wk	68.75% (11/16)
	P-value	<0.0001
Drag swabs	22 wk	6.25% (1/16)
	26 wk	6.25% (1/16)
	30 wk	56.25% (9/16)
	34 wk	81.25% (13/16)
	39 wk	81.25% (13/16)
	P-value	<0.0001
Egg belt dust	22 wk	31.25% (5/16)
	26 wk	37.50% (6/16)
	30 wk	50.00% (8/16)
	34 wk	93.75% (15/16)
	39 wk	100.00% (16/16)
	P-value	<0.0001
Manure belt scraper	22 wk	0.00% (0/16)
	26 wk	12.50% (2/16)
	30 wk	18.75% (3/16)
	34 wk	75.00% (12/16)
	39 wk	62.50% (10/16)
	P-value	<0.0001
Wall dust	22 wk	31.25% (5/16)
	26 wk	56.25% (9/16)
	30 wk	87.50% (14/16)
	34 wk	100.00% (16/16)
	39 wk	100.00% (16/16)
	P-value	<0.0001

microorganisms such as *Salmonella* spp. has been previously reported (Gast et al., 1998; Calvet et al., 2009; Pal et al., 2022). In the manure belt scraper swabs ($P < 0.0001$), the challenge SE was not recovered at 20 (0%) wk, was low at 26 (12.50%) and 30 (18.75%) wks, and high at 34 (75.00%) and 39 (62.50%) wk. Although a consist trend in the detection levels of *Listeria* and *Campylobacter* were not observed, the detection of these pathogens was moderate and could have impacted the recovery of SE inoculum from the manure belt scraper swabs. In the wall dust, recovery ($P < 0.0001$) of SE inoculum was low at 20 (31.25%) wk and high at 26 (56.25%), 30 (87.50%), 34 (100.00%), and 39 (100.00%) wks. The recovery of the SE inoculum followed a similar trend that was observed in the egg belt dust and was likely caused by the same factors.

The detection of naturally occurring *Listeria* and *Campylobacter* spp. in the cage-free aviary production environment was cyclic but also dependent on the type of sample. Drag swabs (*Listeria*) or manure belt scraper swabs (*Campylobacter*) provided the greatest detection overall, except for *Campylobacter* detection in concrete dust swabs, which appeared to be impacted by rodent excreta. The rate of naturally occurring pathogen detection was cyclical or sporadic depending on the type of sample collected over the 17 wks of sampling. Low level inoculation of *Salmonella* Enteritidis into the environmental samples resulted in very low recovery initially, with the greatest recovery amongst the sample types occurring later in the study. The current study ended at 39 wks of flock age, which is just before the required (FDA, 2009) post-peak sampling period of 40 to 45 wks of age. It is important to note that greatest recovery of low-level *Salmonella* Enteritidis from inoculated swabs occurred at this time; validating the FDA Egg Rule sampling period as ideal in conventional and aviary housing systems. Further research is needed to clarify the interaction of *Listeria, Campylobacter*, and *Salmonella* and the role they may play in the detection/recovery of these pathogens if they are all present in the same environment.

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DISCLOSURES

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

REFERENCES

Arnold, M. E., J. J. Carrique-Mas, and R. H. Davies. 2010. Sensitivity of environmental sampling methods for detecting *Salmonella* Enteritidis in commercial laying flocks relative to the within-flock prevalence. Epidemiol. Infect. 138:330–339.

Arnold, M. E., D. Mueller-Doblies, R. J. Gosling, F. Martelli, and R. H. Davies. 2015. Estimation of the sensitivity of various environmental sampling methods for detection of *Salmonella* in duck flocks. Avian Pathol. 44:423–429.

Calvet, S., H. Van den Weghe, R. Kosch, and F. Estellés. 2009. The influence of the lighting program on broiler activity and dust production. Poult. Sci. 88:2504–2511.

CDC. 2016. Multistate outbreak of *Salmonella* Oranienburg infections linked to Good Earth Egg Company shell eggs (final update). Accessed Jul. 2021. https://www.cdc.gov/salmonella/oranienburg-10-16/index.html.

CDC. 2018. Multistate outbreak of *Salmonella* Braenderup infections linked to Rose Acre Farms shell eggs (final update),. Accessed Jul. 2021 https://www.cdc.gov/salmonella/braenderup-04-18/index.html.

CDC. 2020. Preliminary incidence and trends of infections with pathogens transmitted commonly through food − foodborne diseases active surveillance network, 10 U.S. Sites, 2016-2019. MMWR 69:509–514. Accessed Apr. 2021 https://www.cdc.gov/mmwr/volumes/69/wr/mm6917a1.htm?s_cid=mm6917a1_w.

Chemaly, M., M. T. Toquin, Y. Le Nôtre, and P. Fravalo. 2008. Prevalence of *Listeria monocytogenes* in poultry production in France. J. Food Prot. 71:1996–2000.

FDA. 2009. Prevention of *Salmonella* Enteritidis in shell eggs during production, storage, and transportation. Final Rule. 21. CFR 16 and 118. Accessed Apr. 2021 https://www.govinfo.gov/content/pkg/FR-2009-07-09/pdf/E9-16119.pdf.

Gast, R. K., B. W. Mitchell, and P. S. Holt. 1998. Airborne transmission of *Salmonella* enteritidis infection between groups of chicks in controlled-environment isolation cabinets. Avian. Dis. 42:315–320.

Henzler, D. J., D. C. Kradel, and W. M. Sischo. 1998. Management and environmental risk factors for Salmonella enteritidis contamination of eggs. Am. J. Vet. Res. 59:824–829.

Lohmann Tierzucht. 2019. Management-Recommendations for the Rearing of Pullets in Deep Litter, Perchery and Free-range

Systems. Accessed Aug. 2022. https://hylinena.com/wp-content/uploads/2019/10/Lohmann_Alternative_System-1.pdf

Jones, D. R., M. T. Musgrove, A. B. Caudill, and P. A. Curtis. 2006. Frequency of *Salmonella, Campylobacter, Listeria* and *Enterobacteriaceae* detection in commercially cool water-washed shell eggs. J. Food Saf. 26:264–274.

Jones, D. R., K. E. Anderson, and J. Y. Guard. 2012. Prevalence of coliforms, Salmonella, Listeria, and Campylobacter associated with eggs and the environment of conventional cage and free-range egg production. Poult. Sci. 91:1195–1202.

Jones, D. R., N. A. Cox, J. Guard, P. J. Fedorka-Cray, R. J. Buhr, R. K. Gast, Z. Abdo, L. L. Rigsby, J. R. Plumblee, D. M. Karcher, C. I. Robison, R. A. Blatchford, and M. M. Makagon. 2015. Microbiological impact of three commercial laying hen housing system. Poult. Sci. 94:544–551.

Jones, D. R., J. Guard, R. K. Gast, R. J. Buhr, P. J. Fedorka-Cray, Z. Abdo, J. R. Plumblee, D. V. Bourassa, N. A. Cox, L. L. Rigsby, C. I. Robison, P. Regmi, and D. M. Karcher. 2016. Influence of commercial laying hen housing systems on the incidence and identification of *Salmonella* and *Campylobacter*. Poult. Sci. 95:1116–1124.

Kers, J. G., F. C. Velkers, E. A. J. Fisher, G. D. A Hermes, J. A. Stegeman, and H. Smidt. 2018. Host and environmental factors affecting the intestinal microbiota in chickens. Front. Microbiol. 9:235.

Marmion, M., M. T. Ferone, P. Whyte, and A. G. M. Scannell. 2021. The changing microbiome of poultry meat from farm to fridge. Food. Microbiol. 99:103823.

McDowell, S. W. J., F. D. Menzies, S. H. McBride, A. N. Oza, J. P. McKenna, A. W. Gordon, and S. D. Neill. 2008. *Campylobacter* spp. in conventional broiler flocks in Northern Ireland: epidemiology and risk factors. Prev. Vet. Med. 84:261–276.

Meerburg, B. G., and A. Kijlstra. 2007. Role of rodents in transmission of *Salmonella* and *Campylobacter*. J. Sci. Food Agric. 87:2774–2781.

Meerburg, B. G. 2010. Rodents are a risk factor for the spreading of pathogens on farms. Vet. Microbiol. 142:464–465.

Milillo, S. R., J. C. Stout, I. B. Hanning, A. Clement, E. D. Fortes, H. C. den Bakker, M. Wiedmann, and S. C. Ricke. 2012. *Listeria monocytogenes* and hemolytic *Listeria innocua* in poultry. Poult. Sci. 91:2158–2163.

Pal, A., A. P. Jackson, A. Urrutia, K. S. Macklin, S. B. Price, R. J. Buhr, and D. V. Bourassa. 2022. Bacterial composition of settled dust during growout of broiler chickens. Poult. Sci. 101:101602.

Rolfe, D. L., H. P. Riemann, T. B. Farver, and S. Himathongkham. 2000. Drag swab efficiency factors when sampling chicken manure. Avian. Dis. 44:668–675.

Rothrock, M. J. Jr, M. L. Davis, A. Locatelli, A. Bodie, T. G. McIntosh, J. R. Donaldson, and S. C. Ricke. 2017. *Listeria* occurrence in poultry flocks: detection and potential implications. Front. Vet. Sci. 4:125.

Smith, S., J. Meade, J. Gibbons, K. McGill, D. Bolton, and P. Whyte. 2016. The impact of environmental conditions on *Campylobacter jejuni* survival in broiler faeces and litter. Infect. Ecol. Epidemiol. 6:31685.

Stern, N. J., M. R. S. Clavero, J. S. Bailey, N. A. Cox, and M. C. Robach. 1995. *Campylobacter* spp. in broilers on the farms and after transport. Poult. Sci. 74:937–941.

Zhao, Y., A. J. A. Aarnink, M. C. M. De Jong, and P. W. G. Groot Koerkamp. 2014. Airborne microorganisms from livestock production systems and their relation to dust. Crit. Rev. Environ. Sci. Technol. 44:1071–1128.