Rapid Aerosol Transmission of *Salmonella* Among Turkeys in a Simulated Holding-Shed Environment[1]

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ABSTRACT Once turkeys arrive at Midwest processing plants, they are usually held in large open-sided sheds for 1 to 4 h, waiting to be unloaded. In hot, humid weather, large fans are used to cool the birds. The resultant air currents distribute a significant amount of dust to the turkeys. The dust created in this environment could be a factor in the number of *Salmonella*-contaminated turkeys entering slaughter plants. The objective of this study was to determine if rapid transmission of *Salmonella* in turkeys could occur from exposure to *Salmonella*-contaminated dust similar to what may be experienced in holding sheds or in other high-dust environments prior to slaughter. In the first experiment, trials of 3 different concentrations of *Salmonella* (1.2×10^9, 2.6×10^7, and 2.6×10^5 cfu/g) were conducted to determine if transmission of *Salmonella enterica* serovar Typhimurium var. typhimurium$_\chi$4232 to turkeys 2 to 4 h after aerosol exposure to contaminated feces is possible. Results showed that turkeys became infected after 2 h of exposure to airborne-contaminated feces with a concentration level of 2.6×10^5 cfu of *Salmonella* Typhimurium/g. In the second experiment, consisting of 3 trials, 1 bank (5 cages wide and 3 cages high) of turkeys (n = 15 birds per trial) was exposed to another bank of cages of *S.* Typhimurium-inoculated (n = 15) birds for 2 to 4 h using a fan similar to the type in processing-plant cooling sheds. Results from this experiment demonstrated that birds could be contaminated with *S.* Typhimurium after 2 h of exposure. Results of both studies implicate contaminated dust as a route of rapid airborne transmission of *Salmonella* in turkeys. Processes that generate significant dust prior to slaughter should be regarded as critical control points for *Salmonella*.

Key words: *Salmonella* Typhimurium, turkey, cooling shed, respiratory tract, aerosol transmission

INTRODUCTION

Human salmonellosis is a major cause of human foodborne illness in the United States. Consumption of contaminated, undercooked poultry is a major risk factor for human *Salmonella* infections. To illustrate, from 1973 to 1987, approximately 8% of foodborne outbreaks were associated with consumption of turkey meat, and *Salmonella* spp. accounted for 45.6% of these infections (Bean and Griffin, 1990). The Food Safety and Inspection Service young turkey microbial baseline study in 1997 reported *Salmonella* in whole carcass rinses (18.5%) and in ground turkey (49.9%; Food Safety and Inspection Service, 1997).

In 2003, 3.6% of the regulatory samples analyzed by the Food Safety and Inspection Service between January 1 and October 31, 2003, tested positive for *Salmonella* as compared with 4.29% in 2002 and 10.65% in 1998 (Food Safety and Inspection Service, 2004). The decline in human salmonellosis from 1997 to 2003 coincides with the reduction of *Salmonella* isolation from meat and poultry (Centers for Disease Control and Prevention, 2004).

The goal of on-farm pathogen-reduction strategies is to deliver turkeys to the processing plant with low levels of *Salmonella*. Based on cecal sampling, up to 31% of turkeys may harbor *Salmonella* (Cox et al., 2000). *Salmonella*-contaminated or infected live turkeys entering the processing plant are the primary source of processing-plant contamination. Although plant interventions, including chlorinated water in the chill tank, can reduce the numbers of *Salmonella* on carcasses, incoming birds may affect the microbial quality of the end product (McCapes et al., 1991; Clouser et al., 1995; Bailey et al., 2001).

Controlling *Salmonella* contamination of turkeys prior to slaughter may include limiting the turkeys' exposure to bacteria in the last few hours before slaughter during

[1]Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

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on-farm loading, transportation, and holding at the processing plant. These steps have been identified as points of increased *Salmonella* infection in pigs due to rapid (2 to 4 h) infection during the last few hours before slaughter (Hurd et al., 2001, 2002). Recovery rates for *Salmonella* in pigs are reported to be 3 to 10 times higher after slaughter at the processing plant than those from cohorts slaughtered on the farm, suggesting that the pigs become infected sometime during loading, transportation, and holding (Berends et al., 1996; Hurd et al., 2002). In commercial pork production, pigs rest in holding pens for 2 h or more before slaughter, similar to the 2 or more h turkeys rest on trucks in the cooling sheds before slaughter. It may be possible that similar contamination occurs in turkeys prior to slaughter, but limited data are available to document the effects of loading, transportation, and holding on the prevalence of *Salmonella* in commercial poultry, especially turkeys.

There are multiple points in the last few hours before slaughter that may increase contamination of turkeys. The first point of increased exposure, prior to slaughter, may occur when birds are taken off feed for 3 to 6 h before loading. This can increase the flock's contact with *Salmonella* through increased scratching of the litter in the barn in search of food (Ramirez et al., 1997; Corrier et al., 1999). Second, this disturbance of litter increases the amount of dust in the air, which may increase the risk of infection due to inhalation of *Salmonella*-contaminated particles (Corrier et al., 1999; Ellen et al., 2000). Third, a large amount of dust is generated during the load-out process that facilitates inhalation of *Salmonella*-contaminated particles. In addition, turkeys are loaded onto crates that are likely contaminated with *Salmonella* due to improper cleaning and disinfection (Buhr et al., 2000). Finally, once turkeys arrive at the slaughter plant, they are kept in cooling sheds until shackled. Cooling sheds are structures with a roof, no walls, dirt or concrete floors, and accommodate as many trucks as needed for that day's slaughter. The trucks transporting up to 1,152 turkeys in the 144 crates pull into the cooling shed alongside similar vehicles arriving throughout the night. Large fans blow on the turkeys to keep them cool during the summer months. These sheds are also used during the winter to protect the birds from the cold; however; the fans are not used. Turkeys arriving at the cooling sheds may harbor *Salmonella* and may transfer the organism to the environment. From there, it may spread through dust blown by the fans to uninfected birds. These events occur in the last few hours, and all increase exposure to *Salmonella*-laden dust.

Pathogen contamination of the respiratory tract of birds entering the processing plant may contribute to carcass contamination. For example, *Campylobacter* have been detected in the respiratory tract of broilers prior to entering the scald tank. The amount of dust in the grow-out house during catching and transport led Berrang et al. (2003) to hypothesize that the presence of *Campylobacter* in the respiratory tract contaminates the eviscerated carcass without leakage of the gut during processing. In addition,

Salmonella transmission, based on cultures of the intestinal tract and lymph nodes, has been demonstrated to occur solely by the respiratory route in hogs in which the esophagus was transected (Fedorka-Cray et al., 1995). Further, *Salmonella* in dried feces remained viable for up to 13 mo and was infectious to pigs via either the intranasal or oral route (Gray and Fedorka-Cray, 2001).

Very little work has been done to show if rapid airborne infection (less than 4 h) from contaminated fecal dust in turkeys is possible and if it is responsible for increasing the number of *Salmonella*-contaminated turkeys entering commercial turkey slaughter plants. Understanding the kinetics of *Salmonella* transmission to turkeys from the environment during the last few hours before slaughter may identify a critical control point to reduce the number of contaminated turkeys entering the processing plants. If rapid airborne transmission by inhalation occurs immediately prior to slaughter, controlling dust and therefore reducing transmission at this time will be important.

The objective of this study was to determine whether rapid infection (2 to 4 h) with *Salmonella* by inhalation of contaminated dust particles in turkeys was possible. Experiments were designed to determine if *Salmonella* transmission following inhalation of contaminated fecal dust occurred after short-term exposure. Second, experiments were conducted in a simulated holding-shed environment to determine the feasibility of *Salmonella* airborne transmission to turkeys.

MATERIALS AND METHODS

Turkeys

One hundred twenty-two 17-wk-old tom turkeys, which were used in 2 experiments, were purchased from a local commercial farmer in the Midwest. These birds were brought to the National Animal Disease Center (USDA-ARS, Ames, IA), where they were provided feed and water ad libitum. Birds were divided into groups of approximately 30 each and housed in isolation rooms until they were shown, by 3 sequential cultures of cloacal swabs, to be *Salmonella*-negative. Only *Salmonella*-negative birds were used in these trials. Studies were conducted in isolation barns at the National Animal Disease Center in accordance with the Institutional Animal Care and Use Committee's guidelines.

Cloacal swabs were taken from each turkey 14, 7, and 3 d before the experiment to determine if the birds were free from nalidixic acid-resistant *Salmonella enterica* serovar Typhimurium var. typhimurium$_\chi$4232 (Fedorka-Cray et al., 1994; Becton, Dickinson and Co., Sparks, MD). The swabs were cultured for *Salmonella* using the culture method described below. Individual birds that harbored any type of *Salmonella* and their penmates were not used in this experiment.

Challenge Strain

A nalidixic acid-resistant strain of *Salmonella enterica* serovar Typhimurium var. typhimurium$_\chi$4232 was used

(Fedorka-Cray et al., 1994). An aliquot from a pure *Salmonella* stock culture frozen at –80°C was used to inoculate 10 mL of LB broth (Becton, Dickinson and Co.) containing 50 μg/mL of nalidixic acid (Sigma Chemicals, St. Louis, MO). After overnight incubation (37°C), a 1% inoculum of the culture was transferred to prewarmed LB broth containing nalidixic acid (50 μg/mL), and incubated with shaking (200 rpm) for 3.5 h at 37°C. The culture was then centrifuged using a Sorvall Super T-21 with a Sorvall SLC250 rotor (Kendro Labs, New Town, CT; 10,000 rpm for 20 min). The supernatant was removed, and the pellet was resuspended in PBS. The optical density of the resuspended culture was adjusted to an OD_{600} = 1.5 using a Perkin Elmer Lambda EZ 150 spectrophotometer to approximately 3.5×10^9 cfu/mL (Perkin Elmer, Norwalk, CT). The actual concentration was determined by 10-fold serial dilutions and plating onto XLT4 agar (Becton, Dickinson and Co.). The stock bacterial suspension was then diluted to 10^9, 10^7, and 10^5 cfu of *Salmonella*/mL in PBS. The diluted bacterial suspensions were mixed with feces and dried to generate the fecal dust as described below.

Experiment 1: Aerosol Dust Exposure

Challenge Fecal Dust. To form the *Salmonella*-contaminated dust, all fresh fecal droppings for a single day were collected from the turkey rooms in which negative-study birds were housed using sterile wooden sticks and placed into 18-oz Whirl-Pak bags (Nasco, Fort Atkinson, WI). Any litter or feathers attached to the feces were removed using sterile forceps. Approximately 17 g of feces were placed in 16 sterile petri plates and weighed. Four milliliters of the appropriately diluted *Salmonella* culture was mixed into the feces until the feces appeared to be a smooth, consistent mixture. After mixing, the fecal matter was evenly spread over the entire plate using sterile wooden sticks. Plates were dried in a 42°C incubator containing plates of Drierite desiccant (Drierite, Xenia, OH). Pretrials demonstrated that incubating the fecal matter at 42°C and adding plates of Drierite reduced the drying time in the incubators to less than 48 h. By 18 to 36 h, the fecal matter appeared dry. During incubation, dry plates were removed from the incubator and reweighed to determine the amount of moisture lost. Samples that had not lost more than 70% moisture were returned to the incubator to continuing drying. When the fecal matter had dried, the plates were scraped into sterile 18-oz. Whirl-Pak bags using a sterile wooden stick. The fecal matter in the Whirl-Pak bags was macerated into a fine powder using a rubber mallet. The crushed fecal matter was then poured into a large, sterile Whirl-Pak bag and shaken together to create a homogeneous mixture. An average of 25 g of dried fecal material was weighed and used in each cage. A final concentration of *Salmonella* Typhimurium in the dried fecal matter was determined by using 10-fold serial dilution and by plating onto XLT4 agar.

Cage Construction. Fiberglass cages were constructed to be 63.5 cm long, 53 cm wide, and 48 cm high. Inside the cages, a rubberized wire floor was placed 15 cm above the bottom of the cage to protect the birds from the fans. Air filters were placed on the top and along the lower portion of the sides of the cages to allow air to enter and exit the cage without letting *Salmonella* particles escape from the box (Lab Products, Maywood, NJ). Birds were housed in these cages only during the time of the experiment (4 h).

Continuous aerosol suspension of the fecal matter was achieved by constructing 2 fan apparatuses. The first fan apparatus was used to force fecal matter into the box and evenly distributed it throughout the box. This apparatus consisted of a 115-V AC tube axial fan that was 12 cm square × 4 cm deep (Comair Rotron, San Diego, CA). The fan was enclosed in 2 plastic cone-shaped pieces, creating a funnel into which the fecal matter was placed into the first cone and blown into the fan and then distributed from the second cone. The fan circulated the fecal matter throughout the box at a rate of approximately 105 ft^3/min. The second fan apparatus kept the fecal matter suspended inside the box. This apparatus consisted of 8 fans identical to the one used in the first apparatus arranged onto a sloped platform. To assemble this platform, a piece of treated wood was cut to fit the bottom of the cage. This piece of wood was then cut in half lengthwise and reassembled to create a slope in which the sides of the wood were approximately 12 cm from the bottom of the cage. Two fans were spaced evenly on each side of the platform, and 4 fans were evenly spaced at the apex of the slope to create the airflow necessary to suspend the fecal matter.

Experimental Design. This experiment was conducted at 3 *Salmonella* concentration levels (1.2×10^9, 2.6×10^7, and 2.6×10^5 cfu/g) in the dried feces. Four birds each were used at concentrations of 1.2×10^9 and 2.6×10^7 cfu/g. Eight birds were used at the lower concentration level of 2.6×10^5 cfu/g. More turkeys were used at the lower concentration due to the expected higher number of negative samples.

Two trials were conducted as follows. On the day of the study, 5 birds (4 exposed to *Salmonella*-laden fecal dust and 1 negative control) were put into individual cages. The negative control cage was placed in a separate room to prevent the possibility of inhalation of *Salmonella* when the negative control bird was being handled. Approximately 25 g of *Salmonella*-contaminated fecal matter was added to each cage after the fans were started. Due to heat generated by the fans and the birds, dry ice was placed on top of the cages every 30 min to maintain the box temperature at approximately 35°C. This temperature was chosen because it represented a summer temperature that turkeys would experience in the cooling sheds. At this temperature, the dust concentration would most likely be high, and the birds would be panting, thus facilitating transmission. No attempt was made to simulate the humidity of a summer in the Midwest. The temperature was recorded every 15 min using a battery-operated electronic thermohygrometer (VWR Scientific, West Chester, PA). Two hours after the fans were turned on, 2 birds were removed from the boxes and euthanized
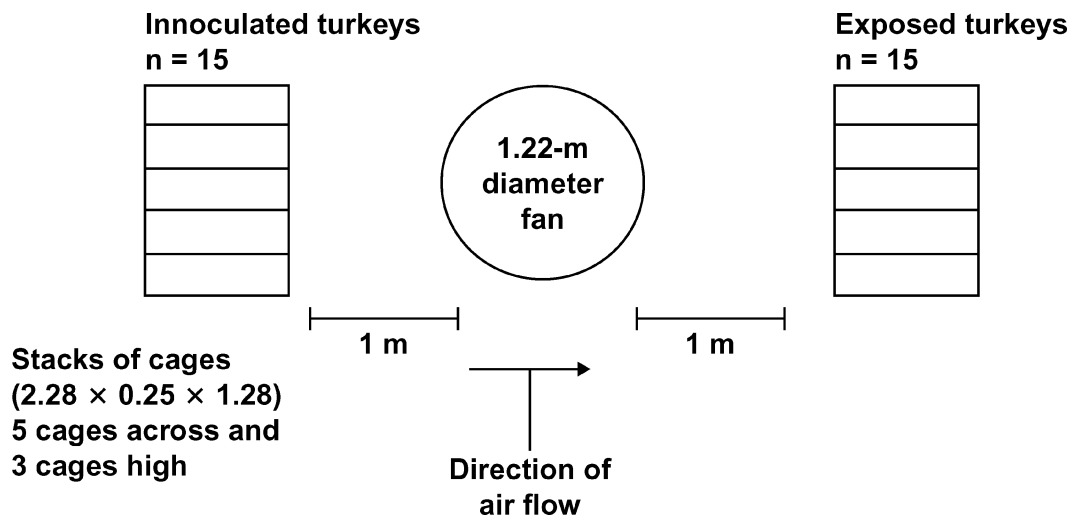
Innoculated turkeys
n = 15

Exposed turkeys
n = 15

1.22-m
diameter
fan

1 m

1 m

Stacks of cages
(2.28 × 0.25 × 1.28)
5 cages across and
3 cages high

Direction of
air flow

Figure 1. Barn scheme for Experiment 2.

by giving a 3-mL injection of Sleepaway (Fort Dodge Laboratories, Fort Dodge, IA), and necropsied. Four hours after the fans started, the remaining birds were removed from the cages and similarly euthanized. At necropsy, nasal-passage swabs, infraorbital sinus swabs, tracheal swabs, air sac swabs, lungs, spleen, a portion of the small intestine, and cecal contents were collected and cultured for *Salmonella* as described below. The cages were cleaned and disinfected between trials using a standard hot water and steam cage-washing system (Basil 4600 cage and rack wash, Steris Corp., Mentor, OH). Ethylene oxide was used to sterilize the electronic equipment between trials. Swabs were taken of the equipment and cultured for *Salmonella*. No *Salmonella* were recovered from any of the swabs taken from the equipment before any of the experiments.

Experiment 2: Simulated Truck Holding Exposure

Experimental Design. Three trials were conducted as follows. Twenty-four hours before the fan was turned on, 15 turkeys received a 1.5-mL dose of LB broth containing 1.0×10^9 cfu/mL of *S.* Typhimurium by oral gavage using a 5-mL sterile syringe. Another 15 turkeys were placed in a separate room in the same barn as the experiment and were not inoculated. The 2 groups were kept in separate rooms until the experiment was initiated. Feed was withdrawn from the turkeys in both groups 12 h before the fan was turned on to simulate fasting before transport (Ramirez et al., 1997; Corrier et al., 1999). Two hours before the fans were turned on, birds in both groups were placed in individual stainless-steel cages (45.72 cm long × 25.4 cm wide × 45.72 cm high) and arranged in 2 rows, 5 cages wide and 3 cages high, as shown in Figure 1. Immediately before the fan was turned on, the bank of inoculated birds was brought into the same room as the bank of unexposed birds (Figure 1). A portable cooler fan (PC4815, Triangle Engineering, Jacksonville, AR), similar in size to that used in the commercial holding shed (ap-

proximately 1.2 m diameter), was placed in the center of the 2 banks of cages. This fan was used to push the air and dust particles from the inoculated group and direct it across the room to the unexposed group at a rate of 19,100 ft³/min. Two hours after the fan was turned on, 7 previously unexposed turkeys (that were now exposed) were randomly selected, euthanized, and necropsied. At the end of 4 h, all remaining exposed turkeys and all birds from the inoculated group were euthanized with a 3-mL wing-vein injection of Sleepaway. All remaining exposed birds (8) and 5 of the 15 inoculated birds were necropsied. Two swabs were taken from nasal passages, infraorbital sinuses, trachea, and air sacs and placed into sterile transport containers. Lungs, spleen, a portion of the small intestine, and cecal contents were placed in sterile Whirl-Pak bags and cultured for *Salmonella*, as described below.

Environmental Sampling. In trials 1 and 2, air-particle sampling was measured in the center of the room directly adjacent to the fan using a tapered element oscillating microbalance (**TEOM**) 1400a sampler (Rupprecht & Patashnick Co. Inc., East Greenbush, NY) and a laser particle counter (model 237, Met One Instruments Inc., Grants Pass, OR). The TEOM and laser particle counter sampled particles approximately 1.5 m above the floor, which was 0.3 m higher than the fan used in this experiment. The instruments operated for approximately 1 h before the beginning of the trial until the end of the experiment, 4 h after exposure to the inoculated birds.

Microbiological Sampling Procedures. All samples were processed within 1 h after collection. One swab of the respiratory tract was placed directly into a vial of 10 mL of tetrathionate broth containing nalidixic acid (50 μg/mL; Becton, Dickinson and Co.), and the second swab was placed in a vial of 10 mL of buffered peptone water (**BPW**) containing nalidixic acid (50 μg/mL; Remel, Lenexa, KS). Lungs and spleens were immersed in 70% ethanol and flamed. These samples were then placed in a separate sterile bag and macerated with a rubber mallet.

Table 1. Number of *Salmonella* challenge strain-positive samples and the number of total samples obtained from turkeys after 2 and 4 h of exposure to various concentrations of *Salmonella*-contaminated fecal dust in Experiment 1

	Sample type																
	Nasal passages		Infraorbital sinuses		Trachea		Lungs		Air sacs		Ceca		Small intestine		Spleen		Any positive samples	
Exposure	2 h	4 h	2 h	4 h	2 h	4 h	2 h	4 h	2 h	4 h	2 h	4 h	2 h	4 h	2 h	4 h	2 h	4 h
Salmonella dose (cfu/g)																		
1.2×10^9	2/4	4/4	2/4	4/4	2/4	2/4	4/4	4/4	2/4	0/4	1/4	3/4	4/4	4/4	2/4	0/4	4/4	4/4
2.6×10^7	1/4	2/4	2/4	1/4	3/4	2/4	3/4	4/4	1/4	0/4	0/4	1/4	3/4	3/4	0/4	0/4	4/4	4/4
2.6×10^5	1/8	0/8	1/8	0/8	0/8	1/8	2/8	3/8	0/8	1/8	1/8	2/8	3/8	4/8	1/8	0/8	5/8	6/8
Nonexposed controls	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1

Twenty-five milliliters of BPW was added to each sample, and each sample was homogenized (220 rpm, 30 s; Seward 80, Seward Medical, London, UK). The homogenized suspension was added to the tetrathionate and BPW enrichment broths containing nalidixic acid (50 μg/mL) and incubated (37°C for 24 h). Ten grams (10% wt/vol) of cecal contents was added to each of the enrichment broths and incubated (37°C for 24 h). Small intestines were incised longitudinally and were directly added (10% wt/vol) to the enrichment broths and incubated (37°C for 24 h). Samples were transferred to a secondary enrichment of Rappaport-Vassiliadis (**RV**) broth (0.1 mL of sample to 10 mL; Becton, Dickinson and Co.), incubated (42°C for 24 h), subcultured to RV broth (0.1 mL of sample to 10 mL), and incubated (42°C for 24 h). After the second enrichment in RV, the samples were plated onto XLT4 agar (37°C for 24 h) containing nalidixic acid (50 μg/mL). Presumptive *Salmonella* colonies were plated onto Rambach agar (DRG International Inc., Mountainside, NJ) and incubated (37°C for 24 h).

RESULTS

Experiment 1: Aerosol Dust Exposure

Results for turkeys necropsied at 2 and 4 h are summarized and shown in Table 1. In the birds exposed to 1.2×10^9 and 2.6×10^7 cfu/g of *S.* Typhimurium, the challenge organism was recovered from all 4 turkeys after 2 and 4 h. All 4 birds at these concentrations had positive lung samples after 4 h but no positive air sacs.

Salmonella Typhimurium was recovered from at least 1 sample in 5 of the 8 turkeys after 2 h of exposure and at least 1 sample from 6 of the 8 turkeys exposed to 2.6×10^5 cfu/g of *S.* Typhimurium in the fecal dust after 4 h of exposure.

All negative control birds were culture negative.

Experiment 2: Simulated Truck Holding Exposure

From the birds exposed by the cooling fan, the challenge strain was recovered from respiratory tract samples of 4 exposed turkeys from trials 1 and 2 (28.6%) at the 2-h time point. Specifically, *Salmonella* were recovered from 2 birds' infraorbital sinuses, 1 bird's lungs, and 1 bird's air sacs. At the 4-h time point, the challenge strain was recovered from only 2 exposed birds in trials 1 and 2 (12.5%). In trial 3, the time the turkeys were necropsied at the 2- and 4-h time points was not recorded, so data are combined as a single time point (Table 2).

The challenge strain of *S.* Typhimurium was recovered from all 15 inoculated birds. *Salmonella* Typhimurium was recovered in all of the birds' nasal passages, cecal contents, and small intestines (Table 2). *Salmonella* were also recovered in the birds' infraorbital sinus swabs (80%), tracheal swabs (93.3%), lungs (86.7%), and spleens (93.3%).

Particle counts and mass-concentration data at sizes 0.05, 1.0, 5, 10, and 25 μm were collected in 2 of the replicates. In all groups, there was a trend of decreasing particles over time with increasing spikes when the door to the room was opened to collect the birds at the 2-h time point. These spikes were large but only lasted a few seconds. Figure 2 shows particles ≤25 μm for the first trial. The instruments recorded a greater number of smaller particles in the air than larger particles. The TEOM recorded an average of 42,000 μm/m^3 of 1.0-μm particles at the time the fan was turned on and a mean of 6,000 μm/m^3 at the end of 4 h. In comparison, there was a mean number of 700 μm/m^3 of 5-μm particles when the fans were turned on and a mean of 100 μm/m^3 at the end of 4 h. Figure 2 shows a decrease in the number of all particles ≤25 μm that were present in the experimental room during trial 1. Only 1 trial is shown because the amount of dust in the air for trial 1 and trial 2 are comparable. By 2 h, the number of particles was reduced by over half.

DISCUSSION

The objective of this study was to determine the feasibility of rapid infection of *Salmonella* by inhalation of contaminated dust particles in turkeys. This study was not designed to determine the minimal infectious dose of *S.* Typhimurium or the minimal time needed for infection. Additional research would be needed to make these determinations. This appears to be a sensitive model for use in exploring these questions because *S.* Typhimurium was recovered from birds in this study.

In Experiment 1, birds were exposed to 10^9 cfu/g of *S.* Typhimurium in a confined space. High concentrations

Table 2. Number of *Salmonella*-positive samples and the number of samples from turkeys exposed to a simulated holding-shed environment inhabited by *Salmonella* inoculated turkeys

| Item	Nasal passages	Infraorbital sinuses	Trachea	Lungs	Air sacs	Ceca	Small intestine	Spleen	Any positive samples
Inoculated birds (all 3 trials)	10/10	14/15	15/15	13/15	10/15	15/15	15/15	14/15	15/15
Trial 1 and 2 (2 h)	0/14	2/14	0/14	1/14	1/14	0/14	0/14	0/14	4/14
Trial 1 and 2 (4 h)	0/16	0/16	0/16	0/16	0/16	1/16	1/16	1/16	1/16
Trial 3 (2 to 4 h combined)	NA[1]	NA	1/15	0/14	1/15	0/15	2/15	0/15	3/15
Total	0/30	2/30	1/45	1/43	2/45	0/43	2/45	0/45	5/45

[1]NA = not tested.

were chosen because of the uncertainty of infection due to inhalation. *Salmonella* were recovered from at least 4 samples from turkeys receiving the 2 highest concentrations of exposure. The number of tissues from which *Salmonella* were recovered declined in birds that received the lowest concentration. *Salmonella* were recovered from 25% fewer birds (regardless of tissue) receiving the lowest concentration of inoculum compared with birds receiving the 2 higher concentrations.

Although this was not an objective of the original study, air-particle analysis was performed in the first trial of Experiment 2 to determine the relationship between the particles in the air (size and number) and the number of infected birds. Over the course of the trial, the mass and number of dust particles in the air of sufficient size to transmit *Salmonella* dramatically declined. The decline in particles over time may be due to the HEPA-filtered ventilation system, which could not be disabled during these studies. The concentration of *S.* Typhimurium in the air during this experiment was not determined. However, based on the air-particle data collected, an approximate number of total respirable particles that could contain *Salmonella* was calculated. At the time the fans were turned on in trial 1, there were approximately 8×10^4 particles in the air. Of the 8×10^4 particles of dust in the air, 7.8×10^4 particles were 1 μm or less in size, and, therefore, are too small to carry *Salmonella*. Thus, approximately 2×10^3 of the remaining particles could carry

Salmonella and be inhaled by the turkeys at the start of trial 1.

In Experiment 2, *Salmonella* were only recovered from respiratory samples from previously unexposed birds during the first 2 h. At 4 h postexposure, *Salmonella* were recovered from intestinal samples only. Therefore, it is possible that no new infections due to inhalation occurred after 2 h. These data, coupled with the dust data, suggest that the direct transmission from infected birds to noninfected birds is possible when the numbers of particles that carry *Salmonella* are high but decreases as the number of particles carrying *Salmonella* declines. We have previously reported that turkeys remain in the holding shed for 5.96 ± 2.17 h (range is 4.38 to 9.75 h; Wesley et al., 2005). During this interval, transmission is undoubtedly facilitated by the humid environment of the commercial holding shed in which transport trailers each hauling 1,154 birds are parked within 1.8 m of each other. This research has shown that turkeys in a confined space can become infected following exposure to high numbers of airborne *Salmonella* associated with fecal dust particles. Furthermore, aerosol transmission to susceptible turkeys may also occur following exposure to air currents carrying dust particles from nearby *Salmonella*-infected turkeys. The latter situation is similar to that experienced by commercial turkeys in cages on transport trucks waiting in holding sheds at the processing plant. Our studies suggest that production steps that generate a large
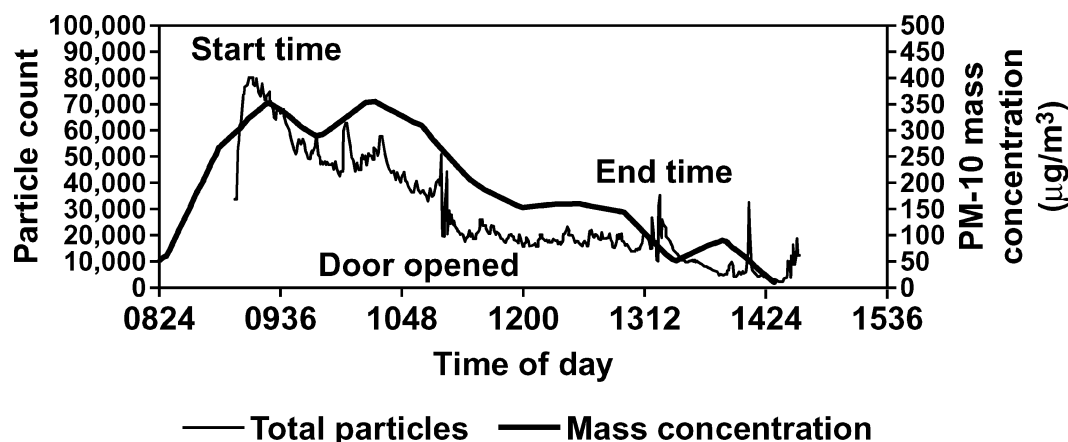
Figure 2. Counts of particles suspended in the air (≤25.0 μg) and mass concentration (μg/m³) were measured with a tapered element oscillating microbalance (Rupprecht & Patashnick Co. Inc., East Greenbush, NY) and recorded in the first trial of Experiment 2 (0900 to 1300 h).

amount of dust, such as feed withdrawal, loading, transportation, and holding are stages that should be reviewed and examined for ways to minimize the amount of dust created and thus reduce the possibility of turkeys becoming exposed to *Salmonella* in the hours just before slaughter.

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