Application of Negative Air Ionization for Reducing Experimental Airborne Transmission of \textit{Salmonella enteritidis} to Chicks

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ABSTRACT Electrostatic space chargers were used to impart a negative charge to airborne dust particles and thereby cause them to be attracted to grounded surfaces. To determine whether negative air ionization could affect the airborne transmission of \textit{Salmonella enteritidis}, chicks were housed in four controlled-environment isolation cabinets in which airflow was directed across an unoccupied central area from one ("upstream") group of birds to another ("downstream") group. Negative air ionizers were installed in two of these cabinets. In three replicate trials, groups of chicks were placed in the upstream ends of the transmission cabinets and orally inoculated with \textit{S. enteritidis} at 1 wk of age. On the following day, 1-d-old chicks were placed in the downstream ends of the cabinets. When chicks were sampled at 3 and 8 d postinoculation, \textit{S. enteritidis} was found on the surface of 89.6% of the downstream chicks from cabinets without negative air ionizers, but on only 39.6% of the downstream chicks in the presence of the ionizers. Similarly, \textit{S. enteritidis} was recovered from the ceca of 53.1% of sampled downstream chicks in cabinets without ionizers, but from only 1.0% of the ceca of chicks in cabinets in which ionizers were installed. The presence of the ionizers was also associated with reduced levels of circulating airborne dust particles. Reducing airborne dust levels may thus offer an opportunity to limit the spread of \textit{S. enteritidis} infections throughout poultry flocks.

(Key words: \textit{Salmonella enteritidis}, chicken, dust, negative air ionization)

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INTRODUCTION

The transmission of \textit{Salmonella enteritidis} infection to humans by contaminated eggs has been a significant international public health issue for more than a decade (Centers for Disease Control, 1996; Tauxe, 1997). Controlling the incidence of \textit{S. enteritidis} infections in egg laying flocks has become one of the principal objectives of programs designed to ensure the microbial safety of eggs and egg products (Hogue \textit{et al}., 1998). The success of such programs depends on the accurate identification of the sources and mechanisms of dissemination of \textit{S. enteritidis} in poultry and on the development of effective strategies for preventing the introduction and spread of infection in commercial flocks. Direct contact between birds, contamination of environmental surfaces, biological and mechanical vectors, and air circulation all provide opportunities for pathogens to spread throughout poultry flocks and are thus among the leading prospective targets for control efforts.

Airborne movement of dust and other particles has frequently been implicated as a potential mechanism for transmitting \textit{Salmonella} infection in poultry houses. Davies and Wray (1994) concluded that \textit{Salmonella} contamination in commercial broiler hatcheries could be reduced most effectively by minimizing dust, fluff, and aerosol production. Dust samples from poultry houses have been found to harbor \textit{Salmonella} contamination long after the houses were depopulated (Davies and Wray, 1996a) and even following cleaning and disinfection (Higgins \textit{et al}., 1982). Cason \textit{et al}.
(1994) correlated the isolation of \textit{Salmonella typhimurium} from hatching cabinet air samples with a high incidence of horizontal spread of \textit{Salmonella} infection among newly hatched chicks. Nakamura \textit{et al}.
(1997) observed that the rate at which \textit{S. enteritidis} infection spread horizontally along a row of caged chickens was diminished by airflow in the opposite direction. After Lever and Williams (1996) administered oral doses of \textit{S. enteritidis} to day-old chicks housed in floor pens, air samples taken from these rooms were positive for \textit{S. enteritidis} and un inoculated chicks in nonadjacent pens soon became infected. Holt \textit{et al}.
(1998) similarly reported that the transmission of \textit{S. enteritidis} infection between groups of laying hens housed in separated rows of cages was associated with frequent \textit{S. enteritidis} isolation from air samples.

\begin{itemize}
\item \textbf{Abbreviation Key}: BG = brilliant green; TBG = tetrathionate brilliant green; TS = tryptone soya.
\end{itemize}
Electrostatic charging of particles in enclosed spaces has been found to reduce airborne dust levels by causing the charged dust particles to be attracted to (and collect on) grounded surfaces (Mitchell, 1996; Mitchell et al., 1998b). The generation of a strong negative electrostatic charge by an ionizer was recently used to reduce airborne bacterial levels in poultry hatching cabinets (Mitchell et al., 1998a). Negative air ionizers were previously shown to reduce the frequency of experimental airborne transmission of Newcastle disease virus in environmental isolation cabinets (Mitchell and King, 1994). Gast et al. (1998) recently observed transmission of *S. enteritidis* infection between groups of chicks in nonadjacent areas of isolation cabinets after airborne contamination of environmental surfaces apparently led to subsequent oral ingestion of the pathogen by the chicks. The objective of the present study was to determine whether negative air ionizers, by reducing the levels of dust and other particles in the air of controlled-environment isolation cabinets, could affect the airborne dissemination of *S. enteritidis* and the transmission of *S. enteritidis* infection between groups of chicks in nonadjacent sections of the cabinets.

**MATERIALS AND METHODS**

**Environmental Isolation Cabinets**

All trials were conducted in stainless steel environmental cabinets (Mitchell et al., 1972) in an isolation building operated under conditions in excess of biosafety level 2 (U.S. Department of Health and Human Services, 1993). In each cabinet, two wire-floored areas for housing chicks (0.85 m² each) were separated by an unoccupied central area. The wire partitions (30.5 cm apart) at the boundaries of this central area allowed the passage of air but prevented any direct contact between the two groups of chicks. Separate externally supplied feeders and nipple automatic drinkers were present in each end of the cabinets. Separate pass-through air-locks and glove ports allowed access to each end of the cabinets. The isolation cabinets were maintained at a negative static pressure relative to the surrounding room and this room was likewise negative relative to the exterior of the building. Filtered air was brought into one end of each cabinet and exhausted from the opposite end, so that airflow was directed from one group of “upstream” chicks, across the uninhabited central area, to the other “downstream” group of chicks. Exhausted air from the cabinets was passed through high efficiency filters before leaving the isolation building. A ventilation rate of 0.34 m³/min (with no recirculation) led to 12 complete air exchanges each hour. Internal conditions of 29.4 C and 50% relative humidity were maintained in the isolation cabinets.

**Negative Air Ionizers**

Negative air ionizers were installed in two of the four environmental isolation cabinets used in each trial. Individual ionizer bars were placed in the upstream, central, and downstream portions of these cabinets. Each ionizer bar was 50.8 cm long, with 1.27 × 1.27 cm cross sections and electrodes every 1.27 cm. The bars were situated 7.62 cm below the stainless steel ceilings of the cabinets and were operated at ~20,000 V direct current by a current-limited power supply. The supply current was limited to less than 0.5 mA for safety. The cabinets were grounded to improve the attraction of negatively charged dust particles.

**Experimental Design**

In each of three replicate trials, 25 1-d-old Single Comb White Leghorn chicks from our laboratory’s specific-pathogen-free flock were placed in the upstream end of each of four isolation cabinets (two containing negative air ionizers and two with no ionizers). At 7 d of age, each upstream chick was inoculated orally with 1 mL of an overnight broth culture of a phage type 13a *S. enteritidis* strain, diluted to contain approximately 1.0 × 10⁸ cfu per dose. One day after inoculation of the upstream chicks, 25 uninoculated 1-d-old chicks were placed in the downstream end of each isolation cabinet. All chicks were given *ad libitum* access to water and antibiotic-free feed (23% CP, 3,100 kcal ME/kg, 1.0% Ca, 0.48% P). At 3 d postinoculation, all chicks in two cabinets (one with and one without ionizers) were euthanatized by cervical dislocation and 4 upstream and 16 downstream chicks were sampled for *S. enteritidis*. At 8 d postinoculation, the chicks in the remaining two cabinets were similarly euthanatized and sampled for *S. enteritidis*.

**Determination of Airborne Dust Concentrations and Particle Counts**

On the 4th d after chicks were placed in the upstream ends of the isolators in Trial 3, air samples were collected approximately 30 cm above the center of the floor in the downstream end of each cabinet. To determine the airborne dust concentration, air samples were tested at 1-s intervals for 1 h using a TSI DustTrak² (a laser-based instrument that has a sensitivity of 0.001 mg/m³ and is capable of measuring concentrations up to 200 mg/m³). To determine the concentration of airborne particles of various sizes, air samples were drawn and analyzed over 1-min intervals for a period of 1 h using a CI-500 Climet Particle Counter³ (a laser-based instrument capable of counting particles as small as 0.3 μm in six size ranges). Dust and particle count data from similar cabinets (with or without ionizers) were combined for analysis.

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²TSI Incorporated, Shoreview, MN 55126.
³Climet Instruments, Inc., Redlands, CA 92374.
**Culture Methods to Detect Salmonella enteritidis**

To test for the presence of *S. enteritidis* on the feathers and skin of sampled chicks, each euthanatized bird was placed into a separate sterile plastic bag containing 150 mL of TS broth and massaged gently through the bag for 30 s. After the chicks were removed, the surface rinse samples were incubated for 24 h at 37°C. A 1-mL portion from each incubated TS broth culture was transferred to 9 mL of TBG broth and incubated for 24 h at 37°C. A loopful of each TBG broth culture was then streaked onto plates of BG agar supplemented with 0.02 mg/mL novobiocin and incubated 24 h at 37°C. The identity of prospective *S. enteritidis* colonies was confirmed biochemically and serologically (Mallinson and Snoeyenbos, 1989).

To test for the presence of *S. enteritidis* in the intestinal tract, the chicks were removed from the TS broth rinse bags and samples of each bird’s cecum were aseptically extracted, manually macerated with cotton swabs, and transferred to approximately 10 vol of TBG broth. As described above, each TBG broth sample was incubated and streaked onto BG agar to isolate individual colonies of *S. enteritidis*.

**Statistical Analysis**

Significant differences (*P* < 0.05) in the mean frequency of recovery of *S. enteritidis* from surface rinses and cecal samples between different treatment groups of chicks (housed with or without negative air ionizers) or between groups sampled on different days were determined by applying Fisher’s exact test to data organized into 2 × 2 contingency tables using Instat biostatistics software. Differences between treatments and sampling days were determined both within individual trials and for all trials combined.

**RESULTS AND DISCUSSION**

When air samples were collected and tested on the 4th d after chicks were placed in the upstream ends of each isolation cabinet in Trial 3, the presence of negative air ionizers was associated with a 77.7% reduction in mean airborne dust concentrations in comparison to control cabinets (Figure 1a). Similarly, negative air ionizers were associated with an overall 81.9% reduction in the mean counts of airborne particles in these cabinets; reductions ranging from 68.4 to 91.7% were observed for particles of six different size ranges (Figure 1b).

All upstream chicks sampled throughout the three trials were positive for *S. enteritidis* recovery from both surface rinses and ceca. In each of the three trials, *S. enteritidis* was isolated from significantly (*P* < 0.005) fewer surface rinses of downstream chicks in cabinets with negative air ionizers than from rinses of chicks in control cabinets (Figure 2a). For the three trials combined, *S. enteritidis* was recovered from 89.6% of surface rinses from chicks in control cabinets and from 39.6% of the rinses from cabinets with ionizers. Similarly, the presence of negative air ionizers was also associated with a significant (*P* < 0.001) reduction in the frequency of isolation of *S. enteritidis* from cecal samples taken from downstream chicks in each of the three trials (Figure 2b). For all trials combined, *S. enteritidis* was found in 53.1% of cecal samples from chicks in control cabinets, but from only 1.0% of samples from chicks in cabinets with ionizers.

In a prior study using these same cabinets, Gast et al. (1998) concluded that downstream chicks evidently became infected with *S. enteritidis* by ingesting the

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4Oxoid, Ogdensburg, NY 13669.
5Difco Laboratories, Detroit, MI 48232.
6Sigma Chemical Co., St. Louis, MO 63178-9916.
7GraphPad Software, San Diego, CA 92121.
pathogen following airborne contamination of downstream environmental surfaces. The magnitude of the observed effect of the ionizers on the transmission of intestinal infection in the present study may thus have been accomplished by reducing downstream environmental levels of _S. enteritidis_ sufficiently that chicks were unlikely to ingest an infectious dose. The changes in _S. enteritidis_ isolation frequencies between the 2 sampling d in this study may help illustrate this relationship (Figure 3). The frequency of _S. enteritidis_ isolation from surface rinses of control chicks for the three trials combined increased significantly ($P < 0.005$) between 3 and 8 d postinoculation (from 79.2 to 100%), as did the corresponding frequency of _S. enteritidis_ recovery from cecal samples (from 14.6 to 91.7%). However, in the presence of the ionizers, the incidence of _S. enteritidis_ recovery from surface rinses actually decreased significantly ($P < 0.025$) over this same interval (from 52.1 to 27.1%), so that fewer chicks were likely to be exposed to an infectious dose of _S. enteritidis_. Accordingly, cecal colonization by _S. enteritidis_ was not detected at 8 d postinoculation in any of the chicks in cabinets with ionizers. Chicks can be readily infected with _Salmonella_ via the mucosa of diverse body orifices (Cox _et al._, 1996). Contamination of feed and water, as well as direct contact between birds, can provide opportunities for infection to spread further (Hinton _et al._, 1989; Gast and Beard, 1990; Nakamura _et al._, 1994). The susceptibility of chickens to the transmission of _S. enteritidis_ infection can be heightened by stresses such as feed restriction (Nakamura _et al._, 1994; Holt _et al._, 1998).

In the present study, a reduced incidence of airborne transmission of _S. enteritidis_ infection was observed in conjunction with the presence of electrostatic space chargers that lowered the dust levels in air. This finding indirectly suggests that airborne movement of dust was at least partly responsible for _S. enteritidis_ transmission in the control groups without ionizers installed. Nakamura _et al._ (1997) also recently implicated contaminated airborne dust particles or water droplets as a probable cause of the horizontal spread of experimental _S. enteritidis_ infection in chickens. Previous investigations have demonstrated that reducing circulating dust levels by electrostatic space charging can reduce the levels of total aerobic bacteria and Enterobacteriaceae in poultry hatching cabinets (Mitchell _et al._, 1998a) and decrease the experimental transmission of viral respiratory infection (Mitchell and King, 1994).

The current experiment shows that effective dust control can have a potent impact on the likelihood of airborne transmission of _S. enteritidis_ in an experimental setting. Although the actual relative significance of dust
in the dissemination of bacterial pathogens in commercial flocks has not been established with certainty, the typically high incidence of Salmonella isolation from poultry house dust (Higgins et al., 1982; Davies and Wray, 1996b) suggests that this mode of transmission is likely to occur. Reducing airborne dust levels could accordingly offer a powerful tool for restricting the opportunities for Salmonella infections to spread extensively throughout poultry flocks. Technologies such as electrostatic space charging, if found to be both feasible and efficacious under commercial conditions, could have a significant impact on efforts to control S. enteritidis infections in poultry and egg-borne transmission of S. enteritidis to humans.

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