INTRODUCTION

Human illness caused by *Salmonella enterica* subspecies *enterica* serovar Enteritidis has been principally associated with the consumption of contaminated eggs throughout the world (Greig and Ravel, 2009; Jackson et al., 2013). Contamination of the edible interior contents of eggs with *Salmonella* Enteritidis occurs when the pathogen colonizes reproductive tissues in systemically infected hens (Gast et al., 2004; Gantois et al., 2009). In the United States, very little overall progress was made in controlling the incidence of infections with *Salmonella* Enteritidis or other prominent serovars between 1996 and 2010 (Centers for Disease Control and Prevention, 2011; Chai et al., 2012). Substantial public and private resources have been committed to *Salmonella* Enteritidis testing and risk reduction in egg-laying poultry and the sustained international application of such programs has been followed by reported decreases in the occurrence of both egg contamination (Esaki et al., 2013) and human *Salmonella* Enteritidis infections (Poirier et al., 2008; O’Brien, 2013). However, active disease surveillance and retrospective epidemiologic analyses document a continuing association between the prevalence of *Salmonella* Enteritidis in commercial egg flocks and the incidence of human illness (Havelaar et al., 2013; Arnold et al., 2014). Many important environmental influences on laying flocks are shaped by the diverse production systems used in the commercial egg industry. The consequences of these housing systems for laying hens have been actively debated in recent years, but a definitive consensus about their food safety implications has not yet coalesced from the published scientific literature (Holt et al., 2011). Poultry housing systems incorporate numerous and complex facility characteristics and management practices that potentially affect the persistence and transmission of salmonellae. Direct contact

Horizontal transmission of *Salmonella* Enteritidis in experimentally infected laying hens housed in conventional or enriched cages

Richard K. Gast,*1 Rupa Guraya,* Deana R. Jones,* and Kenneth E. Anderson†

*USDA, Agricultural Research Service, Egg Safety and Quality Research Unit, Athens, GA 30605; and †Department of Poultry Science, North Carolina State University, Raleigh 29765

**ABSTRACT** The majority of human illnesses caused by *Salmonella* Enteritidis are attributed to contaminated eggs, and the prevalence of this pathogen in commercial laying flocks has been identified as a leading epidemiologic risk factor. Flock housing and management systems can affect opportunities for the introduction, transmission, and persistence of foodborne pathogens in poultry. The animal welfare implications of different types of housing for laying hens have been widely discussed in recent years, but the food safety consequences of these production systems remain incompletely understood. The present study assessed the effects of 2 different housing systems (conventional cages and colony cages enriched with perching and nesting areas) on the horizontal transmission of experimentally introduced *Salmonella* Enteritidis infection within groups of laying hens. In each of 2 trials, 136 hens were distributed among cages of both housing systems and approximately one-third of the hens in each cage were orally inoculated with doses of $10^8$ cfu of *Salmonella* Enteritidis (phage type 13a in one trial and phage type 4 in the other). At regular intervals through 23 d postinoculation, cloacal swabs were collected from all hens (inoculated and uninoculated) and cultured for *Salmonella* Enteritidis. Horizontal contact transmission of infection was observed for both *Salmonella* Enteritidis strains, reaching peak prevalence values of 27.1% of uninoculated hens in conventional cages and 22.7% in enriched cages. However, no significant differences ($P > 0.05$) in the overall frequencies of horizontal *Salmonella* Enteritidis transmission were evident between the 2 types of housing. These results suggest that opportunities for *Salmonella* Enteritidis infection to spread horizontally throughout laying flocks may be similar in conventional and enriched cage-based production systems.

Key words: *Salmonella* Enteritidis, chicken, horizontal transmission, conventional cage, enriched cage

2014 Poultry Science 93:3145–3151
http://dx.doi.org/10.3382/ps.2014-04237

© 2014 Poultry Science Association Inc.
Received June 8, 2014.
Accepted September 5, 2014.
1Corresponding author: Richard.Gast@ars.usda.gov
between hens, ingestion of contaminated feed or feces, movement of personnel and equipment, and airborne circulation of contaminated dust and aerosols can all facilitate rapid and extensive horizontal dissemination of Salmonella Enteritidis throughout laying flocks (Gast et al., 1998; Thomas et al., 2009). Stress caused by deprivation of feed or water or by environmental heat can increase both the susceptibility of hens to Salmonella Enteritidis colonization and the horizontal transmission of infection between hens (Humphrey, 2006). Individual studies have variously reported higher incidences of environmental Salmonella contamination and flock infection associated with either cage-based or floor-based housing systems (Holt et al., 2011). Enriched (furnished) colony cages and aviaries are intermediate alternatives to conventional battery cages and cage-free systems. The objective of the present study was to determine the effects of 2 different housing systems (conventional cages and colony cages enriched with perching and nesting areas) on the frequency of horizontal transmission of experimentally introduced Salmonella Enteritidis infection within groups of laying hens.

**MATERIALS AND METHODS**

**Experimental Housing of Laying Hens**

In each of 2 similar trials, 136 laying hens were obtained from the specific-pathogen-free flock of Single Comb White Leghorn chickens (negative for antibodies to Salmonella in periodic routine monitoring) at the Southeast Poultry Research Laboratory in Athens, Georgia. These hens (29 and 36 wk old at the beginning of the first and second trials, respectively) were distributed into 4 separately housed groups in different rooms of a disease-containment facility containing cage systems designed to simulate commercial conditions. Hens in 2 rooms (36 per room) were housed in conventional laying cages. Each of these cages housed 6 hens and provided 648 cm² of floor space per bird. Hens in 2 other rooms (32 per room) were housed in enriched laying cages. Each of these cages housed 16 hens and provided 1,216 cm² of floor space per bird, including access to 2 perches and a single enclosed nesting area. The stocking densities in both housing systems represented two-thirds of the maximum levels recommended by the cage manufacturer. All hens were provided with water (via 2 automatic nipple-type drinkers in each conventional cage and 6 in each enriched colony cage) and feed (a pelleted, antibiotic-free layer-breeder ration) ad libitum. All protocols involving these hens were approved by the Institutional Animal Care and Use Committee of the Southeast Poultry Research Laboratory.

**Experimental Infection of Laying Hens with Salmonella Enteritidis**

In each trial, approximately one-third of the laying hens in each cage (2 of 6 birds in each conventional cage and 5 of 16 birds in each enriched colony cage) were orally inoculated with a measured dose of Salmonella Enteritidis. The remaining unoinoculated birds in each cage were designated as contact–exposed to infection by horizontal transmission. Inoculated and unoinoculated hens in each cage were differentiated by leg bands of different colors. In trial 1, hens were infected with a phage type 13a isolate, originally isolated from a contaminated egg yolk by C. Benson at the University of Pennsylvania (Kennett Square). In trial 2, hens were infected with a phage type 4 isolate, originally isolated from the liver of an infected chicken by D. Munro (Scottish Salmonella Reference Laboratory; Glasgow, UK). Two different Salmonella Enteritidis phage types (both of which are epidemiologically important) were included in this study to minimize the strain specificity of results. The inoculum strains were resuscitated by transfer into tryptic soy broth (Acumedia, Neogen Corp., Lansing, MI) for 2 successive cycles of 24-h incubation at 37°C. After cell numbers in each incubated culture were estimated by determining its optical density at 600 nm, further serial 10-fold dilutions in 0.85% saline produced a desired final cell concentration in each oral dose of approximately 1.0 × 10⁸ cfu (confirmed by subsequent plate counts).

**Cloacal Swab Samples**

Immediately before inoculation and at intervals of 3, 6, 9, 13, 16, 20, and 23 d postinoculation, cloacal swabs were obtained to monitor the establishment and persistence of Salmonella Enteritidis in the intestinal tracts of inoculated and contact-exposed hens. A sterile cotton swab was inserted into the cloaca of each bird and rotated gently to collect a sample. This swab was transferred to a 9-mL tube of Rappaport-Vassiliadis broth (Acumedia) and incubated for 24 h at 37°C. A 10-μL portion from each broth culture was then streaked onto brilliant green agar (Acumedia) supplemented with 0.02 mg/mL of novobiocin (Sigma Chemical Co., St. Louis, MO) and incubated for 24 h at 37°C. The identity of presumptive colonies of Salmonella was confirmed biochemically and serologically (Waltman and Gast, 2008).

**Statistical Analysis**

For each trial (and for both trials combined), significant differences (P < 0.05) between housing systems in the mean frequencies of Salmonella Enteritidis isolation from cloacal swabs were determined by Fisher’s exact test. Similarly, significant differences (P < 0.05) between Salmonella Enteritidis strains in their mean frequencies of recovery from cloacal swabs were determined by Fisher’s exact test for each housing system (and for both systems combined). Because the 2 replicate groups of hens for each housing system did not differ significantly within either trial in the frequency of Salmonella Enteritidis recovery from cloacal swabs,
their results were combined for analysis and presentation. Data were analyzed with Instat biostatistics software (GraphPad Software, San Diego, CA).

RESULTS

In trial 1, Salmonella Enteritidis (phage type 13a) was recovered from as many as 75.0% of orally inoculated hens housed in conventional cages (at 16 d postinoculation) and 65.0% of inoculated hens in enriched colony cages (at 3, 13, and 16 d postinoculation; Table 1). There were no significant differences (P > 0.05) between the 2 housing systems in the isolation frequency of Salmonella Enteritidis phage type 13a from cloacal swabs of inoculated hens at any of the 7 sampling intervals or for all dates combined. The phage type 13a Salmonella Enteritidis strain was recovered at peak levels of 27.1% from uninoculated (contact-exposed) hens in conventional cages (at 13 and 16 d postinoculation) and 22.7% from uninoculated hens in enriched cages (at 16 d postinoculation; Table 1). The frequency of Salmonella Enteritidis phage type 13a recovery from cloacal swabs of contact-exposed hens in enriched cages was significantly (P < 0.022) higher than from hens in conventional cages at 3 d postinoculation, but there were no other significant differences between housing types for the other 6 sampling dates or for all dates combined.

In trial 2, Salmonella Enteritidis (phage type 4) was recovered at peak frequencies of 87.5% from inoculated hens in conventional cages (at 3 d postinoculation) and 80.0% from inoculated hens in enriched cages (at 3 d postinoculation; Table 2). There were no significant differences (P > 0.05) between the 2 cage systems in the isolation frequency of Salmonella Enteritidis phage type 4 from cloacal swabs of inoculated hens for any of the 7 sampling dates or for all dates combined. Recovery of the phage type 4 Salmonella Enteritidis strain reached maximum levels of 27.1% from contact-exposed hens in conventional cages (at 9 d postinoculation) and 22.7% from contact-exposed hens in enriched cages (at 9 d postinoculation; Table 2). There were no significant differences between housing systems in the isolation frequency of Salmonella Enteritidis phage type 4 from cloacal swabs of uninoculated hens (P > 0.05) for any of the 7 sampling dates or for all dates combined.

Table 1. Recovery of Salmonella Enteritidis phage type 13a from cloacal swabs of experimentally infected laying hens in different housing systems

<table>
<thead>
<tr>
<th>Days postinoculation</th>
<th>Conventional cages</th>
<th>Enriched colony cages</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Inoculated</td>
<td>Contact-exposed</td>
</tr>
<tr>
<td>3</td>
<td>15/24 (62.5)a</td>
<td>0/48 (0)b</td>
</tr>
<tr>
<td>6</td>
<td>14/24 (58.3)a</td>
<td>1/48 (2.1)b</td>
</tr>
<tr>
<td>9</td>
<td>15/24 (62.5)a</td>
<td>9/48 (18.8)b</td>
</tr>
<tr>
<td>13</td>
<td>17/24 (70.8)a</td>
<td>13/48 (27.1)b</td>
</tr>
<tr>
<td>16</td>
<td>18/24 (75.0)a</td>
<td>13/48 (27.1)b</td>
</tr>
<tr>
<td>20</td>
<td>11/24 (45.8)a</td>
<td>9/48 (18.8)b</td>
</tr>
<tr>
<td>23</td>
<td>8/24 (33.3)a</td>
<td>5/48 (10.4)b</td>
</tr>
<tr>
<td>All</td>
<td>98/168 (58.3)a</td>
<td>50/336 (14.9)bc</td>
</tr>
</tbody>
</table>

a,bValues in rows that share no common superscripts are significantly (P < 0.05) different.

1Two of 6 hens in each conventional cage and 5 of 16 hens in each enriched cage were orally inoculated with approximately 10⁸ cfu of Salmonella Enteritidis. The remaining hens in each cage were contact-exposed to infection.

Table 2. Recovery of Salmonella Enteritidis phage type 4 from cloacal swabs of experimentally infected laying hens in different housing systems

<table>
<thead>
<tr>
<th>Days postinoculation</th>
<th>Conventional cages</th>
<th>Enriched colony cages</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Inoculated</td>
<td>Contact-exposed</td>
</tr>
<tr>
<td>3</td>
<td>21/24 (87.5)a</td>
<td>3/48 (6.3)b</td>
</tr>
<tr>
<td>6</td>
<td>19/24 (79.2)a</td>
<td>5/48 (10.4)b</td>
</tr>
<tr>
<td>9</td>
<td>17/24 (70.8)a</td>
<td>13/48 (27.1)b</td>
</tr>
<tr>
<td>13</td>
<td>12/24 (50.0)a</td>
<td>9/48 (18.8)b</td>
</tr>
<tr>
<td>16</td>
<td>7/24 (29.2)a</td>
<td>5/48 (10.4)ab</td>
</tr>
<tr>
<td>20</td>
<td>3/24 (12.5)a</td>
<td>2/48 (4.2)a</td>
</tr>
<tr>
<td>23</td>
<td>2/24 (8.3)a</td>
<td>0/48 (0)a</td>
</tr>
<tr>
<td>All</td>
<td>81/168 (48.2)a</td>
<td>37/336 (11.0)bc</td>
</tr>
</tbody>
</table>

a,bValues in rows that share no common superscripts are significantly (P < 0.05) different.

1Two of 6 hens in each conventional cage and 5 of 16 hens in each enriched cage were orally inoculated with approximately 10⁸ cfu of Salmonella Enteritidis. The remaining hens in each cage were contact-exposed to infection.
There were also no significant differences ($P > 0.05$) between the 2 *Salmonella Enteritidis* strains in their associated frequencies of recovery from cloacal swabs taken from inoculated or contact-exposed hens in either production system (at any individual sampling date or for all dates combined). None of the cloacal samples collected before inoculation in either trial were positive for *Salmonella*.

**DISCUSSION**

Both the course and consequences of *Salmonella* infections in chickens can vary significantly between different bacterial strains and serovars. Experimental oral infection with *Salmonella Enteritidis* has typically yielded a higher incidence of egg contamination than other serovars (Gast et al., 2007, 2011a). Egg-contaminating *Salmonella* Enteritidis strains adhere to reproductive tract mucosa and survive in forming eggs better than other environmental salmonellae, associated with the expression of very long lipopolysaccharide O-antigen (Guard-Boudin et al., 2004; Coward et al., 2013). Deposition inside developing eggs requires the sequential expression of different bacterial phenotypic properties, which become relevant at successive stages of intestinal colonization and systemic dissemination (Gast et al., 2002; Guard et al., 2010). Although the differentiation of *Salmonella* Enteritidis strains by phage typing has been valuable for establishing the sources of disease outbreaks, the various phage types are not characterized by intrinsic propensities to contaminate eggs (Gast and Holt, 2000; Gantois et al., 2009). Moreover, isolates of the same phage type can diverge in their invasiveness to internal organs and eggs via the accumulation of small genetic changes (Guard et al., 2011). In the present study, strains of 2 epidemiologically important phage types of *Salmonella* Enteritidis (13a and 4) colonized the intestinal tracts of orally inoculated hens at similar frequencies and were also horizontally transmitted to uninoculated hens at similar frequencies.

Persistent environmental contamination in poultry houses is often cited as an important source for the introduction of *Salmonella* Enteritidis into successive laying flocks (Henzler et al., 1998; Dewaele et al., 2012a,b; Lapuz et al., 2012). Larger flocks (Namata et al., 2008; Hneau-Salaün et al., 2009), older flocks (Namata et al., 2008; Pitesky et al., 2013), multiple-age flocks (Hneau-Salaün et al., 2009; Snow et al., 2010), and older facilities (Van Hoorebeke et al., 2010a) have all been associated with higher environmental prevalence of *Salmonella*. Salmonellae may persist in different environmental reservoirs within the various individual commercial poultry housing systems (Carrique-Mas et al., 2009). Studies comparing the *Salmonella* prevalence in the environment and hens in different housing systems have yielded a wide range of results. Higher frequencies of both environmental contamination and horizontal transmission of infection between birds have sometimes been found in cage-free housing systems than in cage systems (De Vylder et al., 2011; Hannah et al., 2011; Watanabe et al., 2012), particularly when outdoor areas are vulnerable to *Salmonella* introduction from external environmental sources (Mollenhorst et al., 2005). Lower *Enterobacteriaceae* levels were detected on eggshells laid in conventional cages than on eggs from cage-free systems (Jones and Anderson, 2013). Conversely, other investigators have attributed a greater risk of *Salmonella* infection to cage-based housing, especially when rodent population densities are high (Huneau-Salaün et al., 2009; Snow et al., 2010; Van Hoorebeke et al., 2010b). Moreover, some studies have concluded that no significant differences in the prevalence of either environmental contamination or fecal shedding of *Salmonella* in laying flocks were evident between cage and cage-free systems (Siemon et al., 2007; Jones et al., 2012) or between conventional and enriched cage systems (De Vylder et al., 2009; Nordentoft et al., 2011; Van Hoorebeke et al., 2011). The current study sought to expand the implications of these earlier results by monitoring horizontal transmission of *Salmonella* Enteritidis infection among hens in conventional and enriched colony cage systems.

The horizontal dissemination of *Salmonella* Enteritidis throughout laying flocks also depends on the frequency and duration of infection in individual hens. Cloacal swabs (as collected in the current experiments) are uniquely useful because they provide results about the intestinal colonization status of individual birds within co-housed groups, but this method has sometimes been reported to provide less sensitive detection of *Salmonella* than testing voided feces or other environmental samples (García et al., 2011; Schulz et al., 2011). The observed prevalence of fecal shedding within commercial flocks often varies considerably over time (Wales et al., 2007; Schulz et al., 2011). Because newly hatched chicks are highly susceptible to the establishment of persistent *Salmonella* colonization of the intestinal tract, they can shed the pathogen in their feces for many months (Gast and Holt, 1998; Van Immerseel et al., 2004). Although *Salmonella* colonization usually declines much more rapidly in mature chickens (Gast et al., 2005, 2011b), fecal shedding sometimes continues for an extended interval (Li et al., 2007; Gast et al., 2009). For example, a small fraction of hens in one study continued to shed *Salmonella* Enteritidis in their feces for 8 wk after oral inoculation with $10^6$ cfu (Gast et al., 2013b). Bacterial persistence in even a few hens could prolong the opportunities for transmission to other birds and the consequent production of contaminated eggs. The frequency and duration of fecal shedding, as well as the likelihood of deposition inside eggs, are directly related to the size of the initial oral dose of *Salmonella* Enteritidis (Gast and Holt, 2000; Gast et al., 2011b, 2013a). Exposure by horizontal contact, the probable mechanism for transmission of most naturally occurring infections within flocks, typically...
HOUSING AND SALMONELLA ENTERITIDIS TRANSMISSION

3149

involves low exposure doses and thus leads to correspondingly low overall frequencies of infection and egg contamination (Gast and Holt, 1999; DeWinter et al., 2011; Esaki et al., 2013).

In the present study, cage type (conventional versus enriched colony) did not affect either the frequency and persistence of intestinal colonization of orally inoculated hens by Salmonella Enteritidis or the dynamics of horizontal transmission of infection to un inoculated birds. The size of cohoused groups of chickens was previously observed to have relatively little influence on the rate of bird-to-bird transmission of Salmonella (Thomas et al., 2011). Another recent study reported that different cage systems did not affect the frequency of contamination of the edible contents of eggs following experimental infection with Salmonella Enteritidis (Gast et al., 2014). However, a prior investigation found that Salmonella Enteritidis invaded to reach internal organs (including reproductive tissues) significantly more often among experimentally infected hens housed in conventional than in enriched colony cages (Gast et al., 2013b). This suggested that housing parameters such as stocking density or behavioral restriction (which are 2 of the salient distinctions between conventional and enriched colony cages in these studies and in commercial applications) might influence the susceptibility of birds to systemic dissemination of infection. Stress associated with high-density housing for chickens has been associated with the suppression of both humoral and cellular immunity and with decreased organ invasion by Salmonella Enteritidis (Gomes et al., 2014). Nevertheless, although experimental infection studies such as the present investigation provide an opportunity to evaluate the effects of very precisely defined treatments and responses under controlled conditions, they cannot address the full complexity of environmental and management influences that exist in commercial poultry houses. Accordingly, several significant multiinstitutional field studies are currently underway to assess the microbiological consequences of different housing systems for laying hens under commercial egg production conditions.

ACKNOWLEDGMENTS

We gratefully express appreciation for excellent technical assistance from Robin Woodroof and laboratory support services from Garrett Ward (USDA, Agricultural Research Service, Egg Safety and Quality Research Unit, Athens, GA).

REFERENCES


Gast, R. K., R. Guraya, J. Guard, and P. S. Holt. 2011a. The relationship between the numbers of Salmonella Enteritidis, Salmona-


