# Transmission of *Salmonella* to broilers by contaminated larval and adult lesser mealworms, *Alphitobius diaperinus* (Coleoptera: Tenebrionidae)

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**ABSTRACT** The ability of the lesser mealworm, *Alphitobius diaperinus* (Panzer), commonly known as the darkling beetle, to transmit marker *Salmonella* Typhimurium to day-of-hatch broiler chicks was evaluated, as well as the spread to nonchallenged pen mates. In trial 1, day-of-hatch chicks were orally gavaged with 4 larval or 4 adult beetles that had been exposed to marker *Salmonella*-inoculated feed for 72 h. In addition, chicks were gavaged with the marker *Salmonella* in saline solution. These chicks were then placed into pens to serve as challenged broilers. In trial 2, all pens received 2 challenged chicks that were gavaged with larvae or beetles that had been exposed to marker *Salmonella*-inoculated feed for 24 h and then removed from the inoculated feed for a period of 7 d. At 3 wk of age, cecal samples from the marker *Salmonella*-challenged broilers and from 5 pen mates in trial 1, or 10 pen mates in trial 2, were evaluated for the presence of the marker *Salmonella* in their ceca, and at 6 wk of age, all remaining pen mates were sampled. To monitor the presence of the marker *Salmonella* within pens, stepped-on drag swab litter samples were taken weekly. For the *Salmonella*-saline pens, 29 to 33% of the broilers that had been challenged and 10 to 55% of the pen mates were positive at 3 wk of age, and only 2 to 6% had positive ceca at 6 wk. For the pens challenged with adult beetles, 0 to 57% of the challenged broilers and 20 to 40% of the pen mates had positive ceca at 3 wk, and 4 to 7% were positive at 6 wk. The pens challenged with larvae had the greatest percentage of marker *Salmonella*-positive broilers; 25 to 33% of the challenged broilers and 45 to 58% of pen mates were positive at 3 wk, and 11 to 27% were positive at 6 wk. These results demonstrated that ingestion of larval or adult beetles contaminated with a marker *Salmonella* could be a significant vector for transmission to broilers.

**Key words:** *Alphitobius diaperinus*, *Salmonella* Typhimurium, lesser mealworm, darkling beetle, broiler

2009 Poultry Science 88:44–48
doi:10.3382/ps.2008-00235

## INTRODUCTION

The lesser mealworm, *Alphitobius diaperinus* (Panzer), is a common pest of layer and broiler poultry facilities. It was previously known as a cosmopolitan and secondary stored-product pest of grain and cereal products; however, it is gaining notoriety as a reservoir for poultry and human pathogens (Skov et al., 2004). Commercial broiler farms can suffer financial losses associated with a large population density of the darkling beetle because of structural damage, pest control expenses, and decreased feed efficiency. Economic losses may also result from the ability of darkling beetles to transmit pathogens that have a negative impact on human health (McAllister et al., 1994; Steelman, 1996).

Numerous studies have shown that bacteria and viruses are able to colonize or contaminate, or both, beetles. In one study, 26 pathogenic serogroups of *Escherichia coli* were isolated from 151 adult darkling beetles collected from commercial poultry houses (Harein et al., 1970). In addition, avian leukosis and Marek's disease viruses can be transmitted to broilers that ingest beetles contaminated with the virus (Eidson et al., 1966; Lancaster and Simco, 1967). It has also been shown that darkling beetles can transmit immunosuppressive viruses (McAllister et al., 1995; Goodwin and Waltman, 1996) that may contribute to the infection of broilers by other pathogens.

De las Casas et al. (1972) isolated *E. coli*, *Salmonella*, and other human pathogenic bacteria from larval and


Received June 11, 2008.
Accepted August 24, 2008.
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adult beetles collected from turkey brooder houses. Harein et al. (1970) collected 1,000 adult beetles from turkey brooder houses and found a salmonellae contamination rate of 2.2%, with 5 different serogroups isolated. Adult beetles killed and stored in a sterile environment remained positive for *Salmonella* Typhimurium for up to 45 d (De las Casas et al., 1968). Despins and Axtell (1995) discovered that when chicks were given a choice between starter feed and larval beetles, the average consumption of a 2- to 3-d-old chick was 389 larval beetles per day. McAllister et al. (1994) orally gavaged 10 day-of-hatch chicks with 1 larval or adult beetle contaminated by feed inoculated with *Salmonella* Typhimurium, and tested for colonization in the chicks by cloacal swabs 24 and 48 h after gavage. They found that 9 of the 10 chicks orally gavaged with 1 larval beetle, and 7 of the 10 chicks orally gavaged with 1 adult beetle had positive cloacal swabs within 24 h, and that all 10 of the chicks that were orally gavaged with adult beetles were positive after 48 h.

The objective of this study was to evaluate colonization in broiler chicks and the subsequent transmission to nonchallenged pen mates when seeder chicks were gavaged with larval or adult beetles (contaminated with *Salmonella* Typhimurium), or saline containing the marker *Salmonella* Typhimurium.

## MATERIALS AND METHODS

### Trial 1

Nonmedicated broiler starter crumbles (25 g) were spray-inoculated with 5 mL of a suspension containing a nalidixic acid-resistant (**NAL$^r$**) marker strain of *Salmonella* Typhimurium at a level of $\log_{10}$ 9 cfu/mL in 0.85% sterile saline. Two groups of beetles, one group of 50 adults and another group of 50 larvae, were placed into a container with the inoculated feed for 72 h. Each group of beetles was then removed from the feed and placed into a 15-mL polypropylene conical tube. Preliminary enumeration of marker *Salmonella* Typhimurium from inoculated adult and larval beetles indicated that 4 beetles would have an average level of $\log_{10}$ 4 cfu of the marker *Salmonella* Typhimurium when exposed to inoculated feed under these conditions. Day-of-hatch broiler chicks were orally gavaged with 4 adult or 4 larval beetles by using a modified 1-mL syringe to obtain the above-mentioned inoculation level of the marker *Salmonella* Typhimurium per chick. Additional chicks were inoculated with a saline suspension containing $\log_{10}$ 4.7 cfu/mL of the marker *Salmonella* Typhimurium (*Salmonella*-saline). All challenged chicks were banded on both wings, and the nonchallenged pen mates were not wing banded. Four chick-box paper liners from each trial were individually cultured for natural salmonellae presence.

### Trial 2

Nonmedicated broiler starter crumbles (25 g) were again spray-inoculated with 5 mL of a suspension containing NAL$^r$ *Salmonella* Typhimurium at a level of $\log_{10}$ 9 cfu/mL in 0.85% sterile saline. Two groups of beetles, one group of 100 adults and another group of 100 larvae, were placed into containers with the inoculated feed for 24 h and then removed and placed in a sterile container with noninoculated feed for 7 d. The 7-d period was selected to represent a short house cleanout period between successive broiler flocks. Day-of-hatch chicks were challenged with *Salmonella*-saline, *Salmonella*-larval, and *Salmonella*-adult beetles as described for trial 1. The saline suspension in trial 2 contained $\log_{10}$ 4.58 cfu/mL of the marker *Salmonella* Typhimurium.

### Experimental Rooms

All tests were conducted in a single environmentally controlled building in 6 similarly configured rooms, containing 6 pens each. Before the beginning of the trials, the entire room and all the equipment were pressure washed and foam sanitized (quaternary ammonium), and the room remained empty for 4 wk before reassembly. Pens were 1.06 m wide by 2.29 m long, on cement floors covered with new pine shavings. Each pen contained one tube feeder and nipple drinker line hung from the ceiling. Feed and water were provided ad libitum. Broilers were fed standard nonmedicated starter and grower diets and were managed according to the commercial broiler guidelines and subjected to conventional photoperiod restrictions.

In trial 1, six of the 12 pens had 1 challenged chick, whereas the other 6 pens had 2 challenged chicks for each mode of challenge. In trial 2, two challenged chicks were placed into each of the 12 challenge pens. Chicks inoculated with each mode of challenge were placed in the 2 end pens (separated by 4 pens of chicks in which the marker *Salmonella* was not introduced) in 2 of the 6 rooms, for a total of 4 pens for each challenge route. Each pen contained a total of 40 chicks at a density of 650.3 $cm^2$/chick. All procedures and animal protocols were preapproved by the Institutional Animal Care and Use Committee of the University of Georgia.

### Drag Swabs

To detect the presence of the marker *Salmonella* Typhimurium in the litter, the stepped-on drag swab procedure was performed weekly (Buhr et al., 2007). Two drag swabs (DS-001, Solar Biologicals Inc., Ogdensburg, NY) presoaked in skim milk, were unwound and dragged to create a figure-8 design in the pen. The swabs were stepped on 10 times while being dragged twice around the perimeter of the pens to transfer *Sal-*

*monella* from the litter to the swabs. The 2 swabs were then placed into separate sterile plastic bags.

To recover the marker *Salmonella* Typhimurium from the drag swabs, 100 mL of 1% buffered peptone water was added to each drag swab bag. The bags were then shaken and incubated overnight at 37°C. One loopful (3-mm plastic loops) of the enriched broth was plated onto Brilliant Green Sulfa agar (**BGS**; Acumedia, Baltimore, MD) with the addition of NAL (Sigma, St. Louis, MO; BGS+NAL) at 200 mg/kg (Cox et al., 2007). Nalidixic acid added to the agar was used to depress the growth of background bacteria from the samples and enable growth of only the marker *Salmonella* Typhimurium. Using a marker strain of *Salmonella* Typhimurium with induced NAL resistance precluded the possibility of detecting naturally occurring *Salmonella* introduced through the chicks, from the environment, or from the feed in these trials on BGS plates containing 200 mg/kg of NAL. Plates were then incubated overnight at 37°C and the presence of characteristic *Salmonella* colonies was recorded. Typical colonies were randomly selected and confirmed to be the marker *Salmonella* by latex agglutination (Cox et al. 2007). All drag swab samples that were negative on BGS+NAL plates were removed from the cold room (4°C) and restreaked on BGS plates that did not contain NAL to detect the presence of nonmarker salmonellae.

### Cecal Samples

Cecal samples were collected when the broilers were 3 and 6 wk of age. In trial 1, at 3 wk of age, the wing-banded, challenged broilers and 5 nonchallenged pen mates were killed and their ceca were aseptically removed and placed into sterile plastic sampling bags, labeled accordingly, and transported to the laboratory on ice. In trial 2, the ceca of the wing-banded, challenged broilers and 10 nonchallenged pen mates were removed by using the same methods as above. At 6 wk of age, the ceca from all of the remaining nonchallenged pen mates in each pen were collected by using the methods described.

### Recovery of Marker Salmonella from Ceca

Each cecal sample bag was weighed, the contents were macerated with a rubber mallet, and 1% buffered peptone water was added at 3 times the volume to the weight of the sample. The bags were stomached for 30 s and placed in a 37°C incubator for preenrichment overnight. After preenrichment, 0.1 mL of each sample was streaked onto BGS+NAL agar plates and incubated overnight at 37°C, and the above-mentioned procedures were performed for the marker *Salmonella*. Incidence data for *Salmonella* in cecal samples were analyzed with Fisher's exact test, and significance was determined at $P < 0.05$ (SAS Institute, 2000).

## RESULTS AND DISCUSSION

### Drag Swab Samples

In trial 1, litter in all pens containing 1 and 2 chicks challenged with *Salmonella*-larval beetles tested positive for *Salmonella* Typhimurium for the 6-wk duration. Litter in both pens containing 1 chick that was challenged with *Salmonella*-adult beetles was positive for the 6-wk sampling period. Litter in 1 pen containing 2 chicks challenged with *Salmonella*-adult beetles remained positive for 5 wk, whereas the litter in the other pen was positive for wk 4, negative for wk 5, and then positive again on wk 6. Litter in the pens challenged with *Salmonella*-saline demonstrated more variation in the recovery of *Salmonella*. Litter in 1 pen challenged with *Salmonella*-saline remained positive for 4 wk (1 challenged chick), and litter in 1 pen remained positive through wk 6 (2 challenged chicks). Only litter samples taken through wk 1 to 3 were positive in the pen containing 2 chicks challenged with *Salmonella*-saline. Weeks 1 and 6 were the only litter *Salmonella*-positive samples for the pen containing 1 chick challenged with *Salmonella*-saline. The *Salmonella*-negative litter drag swab results between positive weeks may have been due to the concentration of the marker *Salmonella* Typhimurium in the pens being at or near the level for detection by the stepped-on drag swab technique. In trial 1, the chick-box paper liners were culture positive for naturally occurring salmonellae, which were determined to be serogroups B1 and E. These serogroups were occasionally recovered from drag swabs litter samples that were negative for the $NAL^r$ marker *Salmonella* at 5 and 6 wk.

In trial 2, all pens contained 2 challenged chicks, and the litter in 3 of 4 pens containing marker chicks challenged with *Salmonella*-larval beetles remained *Salmonella* positive for the 6-wk period; in the fourth pen, the litter was *Salmonella* positive only through wk 3. Litter in one of the pens challenged with *Salmonella*-adult beetles remained positive for the 6-wk period, 2 pens remained positive for wk 5, and the 1 pen was positive through wk 4. The marker *Salmonella* Typhimurium was detected in the litter of pens challenged with *Salmonella*-saline through wk 6 in 1 pen, wk 5 in 2 pens, and wk 4 in 1 pen.

Marker *Salmonella* Typhimurium recovery from the litter persisted longer in the pens challenged with *Salmonella*-larvae and *Salmonella*-beetles than in some of the pens challenged with *Salmonella*-saline. Drag swab sample results indicated that within the first week of grow-out, *Salmonella* Typhimurium had contaminated the pen litter for all pens. The continued detection of *Salmonella* from the litter for 3 to 6 wk implied that either the *Salmonella* were able to persist in the litter or the broilers continued to shed marker *Salmonella* over the 6-wk period. This demonstrated that both larval and adult beetles could acquire *Salmonella* Typhimu-

**Table 1.** *Salmonella* incidence (% in parentheses) in cecal samples from challenged broilers and pen mates at 3 wk and pen mates at 6 wk, in trial 1

| Challenge route | 3 wk (challenged broilers) | 3 wk (pen mates) | 6 wk (pen mates) |
|---|---|---|---|
| *Salmonella*, saline | 2/6[1] (33)[2,a] | 2/20 (10)[b] | 3/155 (2)[b] |
| *Salmonella*, larval beetles | 2/6 (33)[a] | 9/20 (45)[a] | 15/154 (10)[a] |
| *Salmonella*, adult beetles | 0/6 (0)[a] | 4/20 (20)[ab] | 11/153 (7)[a] |
| *P*-value | NS | 0.0132 | 0.0034 |

[a,b]Percentages with the same letter within a column are not significantly different ($P < 0.05$).

[1]Number positive/number sampled.

[2]Percentage positive.

rium and transmit a colonization dose when consumed by day-of-hatch chicks. Sufficient *Salmonella* Typhimurium was subsequently excreted with the feces and provided exposure to the other chicks in the same pen in which the challenged chicks were placed.

## Cecal Samples at 3 and 6 wk

Thirty-three percent of both the broilers challenged with *Salmonella*-larvae and those challenged with *Salmonella*-saline were positive at wk 3 (Table 1). All the broilers challenged with *Salmonella*-adult beetles in trial 1 had ceca that tested negative for the marker *Salmonella* Typhimurium at 3 wk. The absence of *Salmonella* Typhimurium from all the broilers challenged with adult beetles would suggest that the *Salmonella* persisted in these broilers for fewer than 3 wk postchallenge, during which time the *Salmonella* may have been eliminated from the ceca or may have been outcompeted by natural flora and excreted.

In trial 2, 57% of the adult beetle-challenged broilers and 40% of the pen mates were *Salmonella* positive and continued to excrete *Salmonella* for 3 through 6 wk (Table 2). Twenty-five percent of the broilers challenged with *Salmonella*-larval beetles and 29% of the broilers challenged with *Salmonella*-saline were positive for *Salmonella* Typhimurium 3 wk after inoculation. The *Salmonella*-larvae and *Salmonella*-saline challenges were able to colonize chicks persistently up to 3 wk after gavage. These results agreed with the report by McAllister et al. (1994) in which day-old-chicks that were gavaged with 1 larval or adult beetle contaminated by feed inoculated with *Salmonella* Typhimurium yielded positive cloacal swabs at 24 to 48 h after inoculation. These data demonstrate that darkling beetles (larvae and adults) can be a source of *Salmonella* Typhimurium for colonization of day-old broiler chicks and can subsequently colonize pen mates.

More pen mates of broilers challenged with *Salmonella*-larvae were positive at 6 wk than pen mates of broilers challenged with *Salmonella*-adult beetles or *Salmonella*-saline. The number of positive pen mates of the broilers challenged with *Salmonella*-saline and *Salmonella*-adult beetles was not different. The positive cecal samples of the pen mates at 3 and 6 wk indicate that *Salmonella* colonized the broilers via the larval or adult beetles and was then transmitted to other broilers in the same pen. The pen mates of the broilers challenged with *Salmonella*-larvae beetles were more likely to be positive than the pen mates of broilers challenged with *Salmonella*-saline at 6 wk for trial 2. Despins and Axtell (1995) reported that darkling beetle larvae contained approximately 20% crude lipids and 67% CP, on a dry weight basis. Elevated lipid levels in beef muscle can result in an increased heat and acid resistance of *Salmonella* Typhimurium DT104 (Juneja and Eblen, 2000). Therefore, the high lipid content of the larval beetles could have improved the ability of *Salmonella* Typhimurium to pass through the acidic proventriculus and gizzard of the chick, thereby remaining intact and able to colonize the ceca. The lipid content of adult darkling beetles has not been determined, so the role of lipids in the relative success of the larval vs. adult beetle challenge requires further evaluation.

The presence of *Salmonella* Typhimurium in 6-wk-old broilers resulting from exposure to pen mates inoculated by 4 *Salmonella* Typhimurium-contaminated larval or adult beetles demonstrated the reservoir competence of beetles for *Salmonella* Typhimurium and the potential for subsequent contamination to future flocks.

**Table 2.** *Salmonella* incidence (% in parentheses) in cecal samples from challenged broilers and pen mates at 3 wk and pen mates at 6 wk, in trial 2

| Challenge route | 3 wk (challenged broilers) | 3 wk (pen mates) | 6 wk (pen mates) |
|---|---|---|---|
| *Salmonella*, saline | 2/7[1] (29)[2,a] | 22/40 (55)[a] | 7/114 (6)[b] |
| *Salmonella*, larval beetles | 2/8 (25)[a] | 23/40 (58)[a] | 35/131 (27)[a] |
| *Salmonella*, adult beetles | 4/7 (57)[a] | 16/40 (40)[a] | 6/141 (4)[b] |
| *P*-value | NS | NS | 0.0001 |

[a,b]Percentages with the same letter within a column are not significantly different ($P < 0.05$).

[1]Number positive/number sampled.

[2]Percentage positive.

Only the challenged broilers were exposed to beetles, and neither larval nor adult beetles were available for consumption by pen mates. If pens had included *Salmonella*-contaminated beetles at population densities comparable with those often seen in commercial broiler houses, there might have been greater spread of *Salmonella* from the continued consumption of beetles living in the litter.

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