Serotype Tracking of *Salmonella* through Integrated Broiler Chicken Operations

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**ABSTRACT**

The widespread presence of *Salmonella* in all phases of broiler chicken production and processing is well documented. However, little information is available to indicate the identity and movement of specific serotypes of *Salmonella* through the different phases of an integrated operation. In this study, samples were collected from the breeder farm, from the hatchery, from the previous grow-out flock, from the flock during grow-out, and from carcasses after processing. *Salmonella* were recovered from 6, 98, 24, 60, and 7% of the samples, respectively, in the first trial and from 7, 98, 26, 22, and 36% of the samples, respectively, in the second trial. Seven different serotypes were identified in the first trial, and 12 different serotypes were identified in the second trial. For both trials there was poor correlation between the serotypes found in the breeder farms and those found in the hatchery. This finding and the fact that similar serotypes were found in the hatchery in both trials suggests that there was an endemic population of *Salmonella* in the hatchery. An association between the serotypes found in the hatchery and those found on the final processed carcasses was observed in both trials. This study confirms that a successful intervention program for broiler production operations must be multifaceted, with one component being disinfection in the hatchery.

*Salmonella* control in integrated broiler operations is complicated because there are numerous potential sources of *Salmonella* contamination in an integrated poultry operation, including chicks, feed, rodents, wild birds, insects, transportation, and the processing plant environment. Bailey et al. (4) conducted a multistate survey to track the movement of *Salmonella* from the hatchery to the processed carcass, and they found *Salmonella* in each of the 25 sample types they examined. Designing effective strategies to help control *Salmonella* on the processed carcass requires a better understanding of the relative contribution of each of these sources.

Each of these sources of *Salmonella* are potentially important; however, the hatchery may be the most important source for two reasons. First, the newly hatched chick is more susceptible to colonization than older birds are. Miller and Shaffer (11) first observed that the colonization of chicks was dose dependent and varied with the day of challenge. They found that 1-day-old chicks could be colonized with fewer than five cells of *Salmonella* and that later colonization was irregular and required higher doses of *Salmonella*. Two-week-old chicks have a mature gut microflora (5) and are thus more resistant to intestinal colonization by salmonellae. Bailey et al. (2, 3) demonstrated that a single *Salmonella*-contaminated egg could substantially contaminate other eggs and chicks in a hatching cabinet. In a study involving over 8,000,000 broilers, Goren et al. (8) showed that serotypes associated with the final processed carcass were frequently found in hatchery samples but were not found in feed. Second, hatcheries often serve as reservoirs for salmonellae. Cox et al. (6) showed that >25% of samples of egg fragments, belting material, and paper pads from commercial hatcheries contained *Salmonella*, indicating many opportunities for the contamination of newly hatched chicks. Two studies looked at commercial hatcheries and found 5 to 9% of 1-day-old chicks to test positive for salmonellae (9, 10). The high frequency of recovery of *Salmonella* from hatchery samples and the dramatic increase in the prevalence of *Salmonella*-positive chicks leaving the hatchery compared with the low prevalence rate for eggs entering the hatchery indicates that the majority of *Salmonella*-positive chicks must acquire *Salmonella* by passive exposure to *Salmonella* in the hatchery.

Other investigators have found the role of the hatchery to be less important. Although Lahellec and Colin (10) found a considerable amount of *Salmonella* in the hatchery, when isolates were serotyped, they found those originating from the hatchery to be less important in the final product than those present in the grow-out house before the placement of the young chicks or those introduced by vectors into the grow-out house during rearing. The survey of Bailey et al. (4) identified many sources of *Salmonella* but was not designed to determine the contribution of breeder flocks and the hatchery to the *Salmonella* found on the final processed carcass relative to the contribution of the previous grow-out environment. The objectives of this study were to detail the movement of *Salmonella* through all phases of an individual integrated poultry operation. Samples from the breeder farm, from the hatchery, from previous flocks, from grow-out operations, and from carcasses coming out of the chill tank in the processing plant were examined.

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FIGURE 1. Sample sites for trial 1.

FIGURE 2. Sample sites for trial 2.

MATERIALS AND METHODS

Sample collection: sample locations. In trial 1, samples were obtained from breeder farm A, from the hatchery, from broiler farm A, and from the processing plant (Fig. 1). In trial 2, samples were obtained from breeder farms A and B, from the hatchery, from broiler farm B, and from the processing plant (Fig. 2).

Sample collection: sample numbers. For each trial, 50 fecal samples, 10 fluff samples, 10 eggshell samples, and 50 or 100 carcass rinses were obtained from each location at the appropriate times.

Sample collection: fecal samples. Fecal droppings were collected from the broiler farms in week 5 for the previous flock, in weeks 3 and 5 for the current flock, and, for trial 2 only, in week 6 for the current flock. Fecal droppings were collected from the breeder farms at the approximate time of egg setting for the current broiler flock. Fresh fecal droppings (single droppings of ap-

<table>
<thead>
<tr>
<th>Date</th>
<th>Location</th>
<th>No. of positive samples</th>
<th>Serogroup(s)</th>
<th>Salmonella serotype(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>09/24/1999</td>
<td>Broiler farm A, wk 5, previous flock</td>
<td>12/50</td>
<td>A-I (1)</td>
<td>Untypeable (1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>C1 (23)</td>
<td>Ohio (6)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>C3 (24)</td>
<td>Kentucky (2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>E (17)</td>
<td>Senftenberg (3)</td>
</tr>
<tr>
<td>10/01/1999</td>
<td>Breeder flock A</td>
<td>3/50</td>
<td>C3 (18)</td>
<td>Kentucky (3)</td>
</tr>
<tr>
<td>10/22/1999</td>
<td>Hatchery</td>
<td>79/80</td>
<td>B (44)</td>
<td>Heidelberg (5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>C1 (139)</td>
<td>Mbandaka (5), Ohio (5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>E (118)</td>
<td>Senftenberg (9)</td>
</tr>
<tr>
<td>11/15/1999</td>
<td>Broiler farm A, wk 3</td>
<td>18/50</td>
<td>B (39)</td>
<td>Heidelberg (1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>C1 (39)</td>
<td>Ohio (6)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>E (26)</td>
<td>Senftenberg (3)</td>
</tr>
<tr>
<td>11/29/1999</td>
<td>Broiler farm A, wk 5</td>
<td>30/50</td>
<td>B (32)</td>
<td>Heidelberg (9)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>C1 (52)</td>
<td>Mbandaka (3), Ohio (10)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>E (36)</td>
<td>Senftenberg (8)</td>
</tr>
<tr>
<td>12/03/1999</td>
<td>Processing plant</td>
<td>8/100</td>
<td>B (9)</td>
<td>Schwarzengrund (2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>C1 (21)</td>
<td>Ohio (4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>E (5)</td>
<td>Senftenberg (1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>O (6)</td>
<td>Alachua (1)</td>
</tr>
</tbody>
</table>

\(^a\) Numbers in parentheses are the total numbers of isolates picked from plates.

\(^b\) Numbers in parentheses are the numbers of isolates confirmed by serotyping.
proximately 5 to 10 g) were placed aseptically into disposable 50-
ml centrifuge tubes (VWR Brand, West Chester, Pa.) and trans-
ported to the laboratory on ice.

Sample collection: hatchery samples. Hatchery samples (eggshells and fluff from the hatching cabinets) were collected on the
day of hatch. Samples were aseptically placed into quart-sized
ziplock bags (Reynolds Metal Co., Richmond, Va.) and trans-
ported to the laboratory on ice. Fluff samples (3 to 5 g) were
collected from the floors of the hatching cabinets. Eggshell frag-
ments were collected from the hatching trays (from the top, mid-
dle, and bottom of the stack), with three to four half-shell frag-
ments constituting a sample.

Sample collection: carcass rinses. Carcass rinses were col-
clected from the rehang belt prior to the rehanging of carcasses on
the drip line. Each carcass was aseptically placed into a Cryovac
bag (B340, Cryovac, Inc., Simpsonville, S.C.), and 100 ml of
sterile distilled water was added to the bag (7). The bag was shak-
en for 60 s, the carcass was replaced on the line, and the rinse
water was poured into a sterile specimen cup (VWR Brand). The
rinses were transported to the laboratory on ice.

Sample processing: preparation. All fecal droppings were
weighed and diluted 1:10 with buffered peptone water (BPW; Oxo-
id, Ltd., Basingstoke, Hampshire, UK). BPW (10 ml) was added
to all fluff samples, and the samples were thoroughly mixed by
hand. BPW (50 ml) was added to the crushed eggshell fragments

(41) Numbers in parentheses are the total numbers of isolates picked from plates.
(42) Numbers in parentheses are the numbers of isolates confirmed by serotyping.

Sample processing: enrichment. After overnight preenrich-
ment, 0.5 ml of the sample was transferred into 10 ml of tetra-
thionate brilliant green broth (Hajna, Becton Dickinson, Sparks,
Md.) prepared according to the instructions on the package. The
tetrahionate brilliant green broth was incubated for 24 h at 42°C,
and 0.1 ml was transferred into 10 ml of Rappaport-Vassiliadis
broth (Becton Dickinson) prepared according to the instructions
on the package. The Rappaport-Vassiliadis tubes were incubated
overnight at 35°C.

Sample processing: isolation and detection. The samples were
streaked on brilliant green sulfag broth (Becton Dickinson)
with 15 ppm novobiocin (Sigma Chemical Co., St. Louis, Mo.)
added and on modified lysine iron agar (Oxoid Inc., Ogdensburg,
N.Y.) with 15 ppm novobiocin added. The plates were incubated
overnight at 35°C. Typical colonies were picked to triple sugar
iron (Becton Dickinson) and lysine iron agar (Oxoid) slants,
which were incubated at 35°C overnight. Samples showing typical
reactions were serogrouped with Salmonella O antisera (Becton
Dickinson). Because of the number of isolates involved and be-
cause there were multiple isolates from the same somatic group
picked from the same samples, only a representative group of

### TABLE 2. Salmonella serotypes from trial 2 locations

<table>
<thead>
<tr>
<th>Date</th>
<th>Location</th>
<th>No. of positive samples</th>
<th>Serogroups</th>
<th>Salmonella serotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>02/03/2000</td>
<td>Breeder farm A</td>
<td>4/50</td>
<td>C1 (12)</td>
<td>Thompson (2)</td>
</tr>
<tr>
<td>02/03/2000</td>
<td>Breeder farm A</td>
<td>4/50</td>
<td>C3 (6)</td>
<td>Kentucky (1)</td>
</tr>
<tr>
<td>02/03/2000</td>
<td>Breeder farm B, wk 5, previous flock</td>
<td>13/49</td>
<td>B (12)</td>
<td>Schwarzengrund (3)</td>
</tr>
<tr>
<td>02/18/2000</td>
<td>Breeder farm B</td>
<td>3/50</td>
<td>B (10)</td>
<td>Java (1)</td>
</tr>
<tr>
<td>02/23/2000</td>
<td>Hatchery samples, breeder farm A</td>
<td>20/20</td>
<td>E (66)</td>
<td>Senftenberg (17)</td>
</tr>
<tr>
<td>02/23/2000</td>
<td>Hatchery samples, breeder farm B</td>
<td>19/20</td>
<td>E (73)</td>
<td>Schwarzengrund (19)</td>
</tr>
<tr>
<td>03/14/2000</td>
<td>Broiler farm B, wk 3</td>
<td>9/50</td>
<td>C1 (12)</td>
<td>Ohio (2)</td>
</tr>
<tr>
<td>03/27/2000</td>
<td>Broiler farm B, wk 5</td>
<td>11/50</td>
<td>B (48)</td>
<td>Schwarzengrund (8)</td>
</tr>
<tr>
<td>04/03/2000</td>
<td>Broiler farm B, wk 6</td>
<td>15/50</td>
<td>C1 (43)</td>
<td>Mbondaka (13)</td>
</tr>
<tr>
<td>04/03/2000</td>
<td>Processing plant</td>
<td>17/50</td>
<td>B (16)</td>
<td>Schwarzengrund (4)</td>
</tr>
</tbody>
</table>

Sources: (41) Numbers in parentheses are the total numbers of isolates picked from plates.
(42) Numbers in parentheses are the numbers of isolates confirmed by serotyping.
isolates was selected for serotyping. Those serogrouped cultures that were selected for serotyping were transferred to duplicate Trypticase soy agar slants (Becton Dickinson). The cultures were incubated overnight at 35°C. The tubes were sealed; one was stored in the laboratory, and the other was sent to the U.S. Department of Agriculture, Animal Plant Health Inspection Service, National Veterinary Services Laboratory, Ames, Iowa, for serotyping.

RESULTS AND DISCUSSION

*Salmonella* were recovered from the breeder farm, from the hatchery, from the previous grow-out flock, from the current flock at 5 weeks of grow-out, and from carcasses after processing 6, 98, 24, 60, and 7% of the time, respectively, in the first trial (Table 1) and 7, 98, 26, 22, and 36% of the time, respectively, in the second trial (Table 2).

A total of seven different serotypes were observed in trial 1 (Table 1). The predominant processing plant strain was *Salmonella* Ohio, which, along with *Salmonella* Senftenberg, was found in both the hatchery samples and the previous grow-out flock samples. *Salmonella* Swarzen-grund and *Salmonella* Alachua had not been detected at any stage prior to the processing plant, indicating that they might have been picked up by cross-contamination during transport from the farm or during processing.

A total of 12 different serotypes were observed in trial 2 (Table 2). The predominant processing plant strain, *Salmonella* Senftenberg, was found in both the hatchery samples and the previous grow-out flock samples. *Salmonella* Swarzengrund was found in the previous grow-out flock samples but not in the hatchery samples. *Salmonella* Mbandaka had not been seen until the sixth week of grow-out.

In trial 1, *Salmonella* Kentucky, the only serotype found on the breeder farm, was not found in the hatchery, during grow-out, or after processing. In trial 2, four sero-types, *Salmonella* Thompson, *Salmonella* Kentucky, *Salmonella* Java, and *Salmonella* Tennessee, were found on the breeder farms but not in the hatchery. In both trials 1 and 2, the predominant serotype found in hatchery samples was *Salmonella* Senftenberg. From the same hatchery, Bailey and Cosby (1) previously found a substantial airborne *Salmonella* Senftenberg contamination problem that could have contributed to the prevalence of this serotype in the current study. Moreover, the low prevalence rate (6 to 8%) of *Salmonella*-positive fecal grab samples from the breeder farm combined with the number of samples (50 per house) makes it difficult to be certain that all serotypes present on the breeder farm were accounted for in the samples taken. With these caveats, there was no correlation between the serotypes found on the breeder farms and those found in the hatchery.

A frequent association between the serotypes isolated from the hatchery samples and others isolated from the previous grow-out house samples was found. Additional serotypes that had never been found in pre-processing samples were found on final processed carcasses. Again, sample size could have contributed to a failure to find the serotypes earlier, or the carcasses could have become cross-contaminated during processing with *Salmonella* that had come into the processing plant from other locations. Although the results of this study show that different sources can contribute to the *Salmonella* found on the final processed carcass, the central role that the hatchery can play in cross-contamination and the spread of *Salmonella* is affirmed; therefore, a component of any successful *Salmonella* control plan will need to emphasize disinfection in the hatchery. However, hatchery sanitation is not enough to effectively control *Salmonella* during broiler production; a multifaceted plan that addresses all phases of production must be implemented.

REFERENCES