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# Distribution and Diversity of *Salmonella* Strains in Shipments of Hatchling Poultry, United States, 2013

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## Summary

Multistate outbreaks of salmonellosis associated with live poultry contact have been occurring with increasing frequency. In 2013, multistate outbreaks of salmonellosis were traced back to exposure to live poultry, some of which were purchased at a national chain of farm stores (Farm store chain Y). This study was conducted at 36 stores of Farm store chain Y and was concurrent with the timing of exposure for the human outbreaks of salmonellosis in 2013. We used environmental swabs of arriving shipment boxes of hatchling poultry and shipment tracking information to examine the distribution, diversity and anti-microbial resistance of non-typhoidal *Salmonella* (NTS) across farm stores and hatcheries. Isolates recovered from shipment boxes underwent serotyping, anti-microbial resistance (AMR) testing and pulsed-field gel electrophoresis (PFGE). Postal service tracking codes from the shipment boxes were used to determine the hatchery of origin. The PFGE patterns were compared with the PFGE patterns of NTS causing outbreaks of salmonellosis in 2013. A total of 219 hatchling boxes from 36 stores in 13 states were swabbed between 15 March 2013 and 18 April 2013. NTS were recovered from 59 (27%) of 219 hatchling boxes. Recovery was not significantly associated with species of hatchlings, number of birds in the shipment box, or the presence of dead, injured or sick birds. Four of the 23 PFGE patterns and 23 of 50 isolates were indistinguishable from strains causing human outbreaks in 2013. For serotypes associated with human illnesses, PFGE patterns most frequently recovered from shipment boxes were also more frequent causes of human illness. Boxes positive for the same PFGE pattern most frequently originated from the same mail-order hatchery. Only one of 59 isolates was resistant to anti-microbials used to treat *Salmonella* infections in people. This study provides critical information to address recurrent human outbreaks of salmonellosis associated with mail-order hatchling poultry.

### Keywords

*Salmonella*; poultry; zoonoses; disease outbreaks; anti-microbial resistance; chickens

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## Introduction

Non-typhoidal *Salmonella enterica* (NTS) is a worldwide cause of foodborne illness in people. NTS is the leading cause of foodborne hospitalisations and death in the United States, and the incidence of illnesses is particularly high in persons with suboptimal immune systems, including children (Scallan 2011). Although most frequently transmitted through food, zoonotic transmission through direct contact with animals accounts for up to 11% of infections with NTS (Hale et al., 2012). Furthermore, the frequency of outbreaks of zoonotic salmonellosis associated with live poultry contact has increased over the previous 20 years. A total of 45 outbreaks of *Salmonella* infections have been linked to contact with live poultry between 1990 and 2012 (Barton Behravesh et al., 2014), and such outbreaks are frequently reported within the literature (Wilkins et al., 2002; Gaffga et al., 2012; Loharikar et al., 2013). The largest reported outbreak occurred in 2013, when a strain of *Salmonella* Typhimurium caused 356 laboratory-confirmed illnesses and was traced back to live poultry purchased at multiple farm stores across the United States supplied by 18 different mail-order hatcheries (CDC, 2013a). In the same year, contact with live poultry was associated with a total of 158 illnesses caused by strains of serovars Infantis, Lille, Newport and Mbandaka (CDC, 2013b). Epidemiologic investigations trace the illnesses back to the store where hatchling poultry were purchased; however, farm stores typically receive hatchlings by mail from multiple mail-order hatcheries, and the complex distribution practices of hatchling poultry makes it difficult to understand the mail-order hatchery sources of outbreak-associated strains (Barton Behravesh et al., 2014). Furthermore, despite being a recurrent source of outbreaks, little is known about the diversity and anti-microbial resistance of NTS in poultry from mailorder hatcheries. This study was designed to characterise the prevalence, distribution of serovars and PFGE patterns, and anti-microbial resistance of NTS across farm stores and mail-order hatchery sources. Concurrent with the timing of sampling, several multistate outbreaks of salmonellosis were linked to exposure to live poultry, some of which were purchased from the same national chain of farm stores where sampling occurred (CDC, 2013a,b). Thus, the results of this study provide additional context to understand the strain diversity of NTS at the time and within the same environment where human exposures resulted in multiple outbreaks of salmonellosis.

## Materials and Methods

### Sample collection

A sample of 40 stores across 13 states was chosen by randomly selecting five stores within each of the eight territories of Farm store chain Y. A total of 1283 stores in 48 states sold hatchlings, and were eligible for inclusion in the study. Each store was provided 10 sampling kits, a short questionnaire and written directions for collecting environmental samples from the bottom of incoming hatchling shipment boxes. Instructions provided to each store directed the employee responsible for handling poultry to wear nitrile gloves and swab the bottom of shipment boxes on the day of arrival with clean gauze sponges soaked with buffered peptone water. Individual swabs were used to sample no more than two shipment boxes per week. Employees returned the swabs, questionnaire and the U.S. postal

service (USPS) label from the shipment box (with tracking number) using overnight shipment to the Ohio State University.

### Laboratory methods

Gauze sponges were added to 36 ml of tetrathionate and incubated at 37°C for 18–24 h. Subsequently, 0.10 ml of Tetrathionate broth was added to 10.0 ml of rapport-vassiliadis broth and incubated at 42°C for 18–24 h, then plated onto XLT-4 agar and incubated at 37°C for 18–24 h. Colonies typical for NTS were confirmed using triple-sugar iron agar and urea agar and were shipped to the National Veterinary Services Laboratories (NVSL) in Ames, Iowa for serovar identification, pulsed-field gel electrophoresis (PFGE) and anti-microbial resistance testing. PFGE was conducted using a CDC-standardised protocol and a single *XbaI* rare-cutting restriction enzyme (Ribot et al., 2006). Patterns were uploaded to PulseNet and named according to CDC protocol (Swaminathan et al., 2001). The minimum inhibitory concentration (MIC) was determined using the broth microdilution technique for a prepared panel of 15 anti-microbials, (NARMS CMV2AGNF, Sensititre, Trek Diagnostics, Cleveland, OH). Breakpoints recommended by the Clinical and Laboratory Standards Institute (CLSI) or used by the National Anti-microbial Resistance Monitoring System (NARMS) were used to classify isolates as having reduced susceptibility (resistant or intermediate) or susceptible. For isolates exhibiting resistance to 3rd generation cephalosporins, PCR was used to test for the presence of the AmpC gene $bla_{CMY}$ using previously described methods (Heider et al., 2009).

### Statistical analysis

The proportion of boxes positive and the frequency of specific PFGE and anti-microbial resistance patterns were calculated. To understand shipment box characteristics associated with the recovery of *Salmonella*, a multivariable logistic regression model (PROC LOGISTIC; SAS v. 9.3, Cary, NC, USA) was used to compare shipments positive and negative for NTS. Variables of interest included species (chicks, ducks or mix), number of hatchlings, and presence of dead, sick or injured hatchlings. The statistical significance of these variables was evaluated in the model while controlling for hatchery source as a fixed effect. Significance was tested using the likelihood ratio test, and $P$ values $<0.05$ were considered statistically significant.

## Results

A total of 219 hatchling boxes from 36 stores of the 40 stores that had agreed to participate were swabbed between 15 March 2013 and 18 April 2013. Shipment boxes were swabbed at stores located in the following 13 states: CO (1), DE (1), FL (4), KS (2), KY (5), MD (1), MO (1), NC (1), NJ (1), NY (5), OH (5), TN (4) and TX (5). Mail-order hatcheries often request other hatcheries to ‘drop-ship’ on their behalf with identical labelling. Therefore, USPS tracking codes from shipment boxes of hatchling poultry were used to determine the actual zip code of origin. Tracking codes were available on 153/219 (70%) of the shipments, and indicated that 132/153 (86%) were shipped from directly from one of three primary hatcheries as indicated on the label, and the remaining 21 ‘drop-shipped’ boxes originated (based on USPS tracking codes) from a source other than what was indicated on the label

Seventeen of the 21 drop-shipments were from hatcheries in NY (1), PA (10), MD (1), OH (2) or IA (3), and four of the drop-shipped from Hatchery B, on behalf of Hatchery A.

## Prevalence of NTS in hatchling shipment boxes

Non-typhoidal *Salmonella enterica* were recovered from 59 (27%) of 219 hatchling boxes (Table 1). Twenty-eight per cent (37 of 132) of boxes direct-shipped from one of three primary hatcheries were positive for NTS. Hatchery source was not statistically significant in the model; however, hatchery A had a higher odds of being positive for NTS (OR = 7.72, 95% CI = 0.98–61.21) (Table 2). For drop-shipped boxes, NTS was recovered from only one of 21 (5%) boxes. Controlling for the hatchery source of the shipment box, NTS recovery was not associated with species (chicks, ducks or a mix), number of birds (25, 50, 75 or ≥100), or the presence of dead, injured or sick birds (Tables 1 and 2).

## Serovars and PFGE Patterns

The 59 isolates recovered from shipment boxes represented 10 different serovars and 23 distinguishable PFGE patterns (Table 3). Four of the 10 serovars, including Typhimurium, Mbandaka, Infantis and Lille, were also associated with outbreaks in 2013. These outbreaks were traced back to contact with live poultry, some of which were purchased at the same national farm store chain. A total of four PFGE patterns representing serovars Typhimurium (1), Mbandaka (2) and Infantis (1) recovered from shipment boxes were indistinguishable from outbreak-associated patterns (Table 3). Three patterns of serovar Lille were different than the PFGE pattern of Lille associated with the human outbreak. In total, 39% (23/59) of isolates recovered from hatchling shipment boxes were PFGE patterns that were also associated with multistate human outbreaks the same year, and 56% (33/59) were serovars that were associated with human outbreaks. For the 10 PFGE patterns of the four serovars associated with outbreaks in 2013, the patterns most frequently recovered from shipping boxes also caused the largest number of human illnesses (Table 3). PFGE patterns of serovars Cerro and Senftenberg, although frequently recovered from shipment boxes, were not associated with multistate outbreaks.

## Distribution of NTS PFGE patterns across hatcheries

Shipment boxes positive for the same PFGE patterns were recovered from boxes shipped from the same mail-order hatchery in all but one instance. For instance, outbreak-associated PFGE patterns of Mbandaka and Infantis were specific to boxes shipped from Hatchery A (Table 3). The outbreak-associated PFGE pattern of *Salmonella* Typhimurium, associated with 356 laboratory-confirmed illnesses (CDC, 2013a), was recovered from 13 shipment boxes at 10 different farm stores. Three of the four boxes with available shipment information originated from Hatchery B, while one box positive for the strain of *Salmonella* Typhimurium was drop-shipped from another source.

## Anti-microbial resistance of NTS recovered from shipment boxes

The majority of isolates (48/59) were either pansusceptible or resistant to only tetracycline or streptomycin (Table 4). Eleven of the isolates were multidrug resistant, including serovars Cerro and Senftenberg. A single isolate of *Salmonella* Kentucky was resistant to ceftriaxone,

and PCR confirmed the presence of *bla*$_{CMY-2}$. Of 13 serovar Typhimurium isolates, six were pansusceptible, five were resistant to tetracycline only, and one was resistant to streptomycin and tetracycline.

## Discussion

Trends towards higher backyard poultry ownership may have multiple societal benefits, including increased awareness of food production practices, access to affordable food and emotional benefits associated with livestock care and handling. However, increases in the popularity of backyard poultry necessitate more focused efforts to reduce the zoonotic transmission of NTS. Approximately 4% of households in 2010 planned to have chickens within 5 years (USDA, 2012), and fewer than half of backyard poultry owners are aware of the risk of salmonellosis when in contact with poultry (Beam et al., 2013). Additionally, 50% of backyard poultry owners reported that the 'learning experience for kids' was an important reason for having chickens (Beam et al., 2013). Accordingly, children <10 years of age accounted for between 41% and 58% of the infections in the 2013 outbreaks of salmonellosis (CDC, 2013a,b). In this study, the combination of environmental swabs of hatchling shipment boxes, molecular genotyping and shipment tracking information was useful to describe the distribution of NTS genotypes across farm stores and hatcheries. Understanding the prevalence and distribution of NTS strains can lead to increased awareness and more focused efforts for interrupting the zoonotic transmission of salmonellosis.

Contamination of hatchling boxes arriving at farm stores with NTS was common, with the proportion of boxes positive for NTS not <17% for the three primary hatcheries. Hatchlings are highly susceptible to NTS infections within the first few days of life, and substantial stressors associated with shipment over long distances may result in increased NTS shedding (Rostagno, 2009). Although the characteristics of shipment boxes (presence of sick or dead chicks on arrival, number of birds in the box and species in the box) were not associated with the NTS status, the qualitative nature of environmental swabs may not accurately reflect the level of contamination within the box. Additional research is necessary to clarify if variations in shipment practices and decreases in stress could reduce the prevalence of NTS in hatchlings.

Comparisons of the identified PFGE patterns to the PulseNet system enabled the description of hatchery sources of outbreak-associated strains of NTS. Rather than distributed across multiple hatcheries, shipment boxes positive for the same outbreak strain most frequently originated from the same hatchery. Hatchery-specific strains of NTS may be due to either colonisation of the hatchery or an upstream supplier of eggs or chicks. Mail-order hatcheries sometimes receive eggs or day-old chicks from multiple sources (Loharikar et al., 2013). Recovery of the *Salmonella* Typhimurium strain (JPXX01.0286) from boxes originating in two different locations may indicate more widespread dissemination across multiple poultry populations. Epidemiologic investigations by the CDC, however, indicate a substantial link between Hatchery B and the JPXX01.0286 Typhimurium strain (CDC, 2013a).

The timing of sampling in this study was concurrent with the timing of exposures for salmonellosis outbreaks that were traced back to poultry, some of which were purchased from the same population of feed stores. Thus, this study is useful to better understand the diversity of NTS at the time and place of human exposures. Many of the strains frequently recovered from shipment boxes were not causes of human outbreaks, including serovars Cerro and Senftenberg. These serovars have been previously shown to be widely distributed but uncommon causes of human illness, suggesting a lower pathogenicity or virulence for strains of these serovars (CDC, 2012; Tewari et al., 2012). For the serovars that were associated with human outbreaks in 2013 (Typhimurium, Mbandaka, Infantis and Lille), the largest number of human illnesses was caused by the strains with the highest frequency of contamination of shipment boxes, suggesting that reductions in contamination of shipment boxes through interventions at the hatchery may lead to concurrent reductions in human illnesses. This finding, however, is based on a limited number of strains, and studies with a larger scope would be necessary to confirm the observation.

Resistance to anti-microbials was uncommon in the population of NTS recovered from hatchling boxes. Only 19% of the isolates (11/59) were resistant to more than one class of anti-microbials. None of the outbreak strains were resistant to anti-microbials commonly used for the treatment of human infections in adults (fluoroquinolones), and only one of the isolates was resistant to anti-microbial used to treat NTS infections in children (3rd generation cephalosporins). A single MDR isolate of *Salmonella* Kentucky harbouring the $bla_{CMY}$ gene was resistant to ceftriaxone, an anti-microbial important for treating infections in children. Anti-microbial use within hatcheries has previously been associated with increases in ceftiofur-resistant NTS infections in people (Dutil et al., 2010). The lack of resistance to 3rd generation cephalosporins is an important finding, and continued monitoring for the emergence of resistant strains in NTS colonising mail-order hatchling poultry is warranted.

## Conclusions

This study provides essential information to understand the distribution and diversity of NTS strains in shipments of mail-order hatchlings. In total, four of the 23 PFGE patterns and 23 of the 59 isolates recovered were associated with concurrent multistate salmonellosis outbreaks traced back to multiple farm stores, including the same farm store chain where sampling occurred. Shipment boxes positive for the same NTS PFGE pattern most frequently originated from the same mail-order hatchery. The population of NTS infecting mail-order hatchling chicks is generally susceptible to anti-microbials. Only one of 59 isolates was resistant to anti-microbials used to treat NTS infections in people. This study focused a description of *Salmonella* strains colonising mail-order hatchling poultry. Additional research is necessary to design effective interventions to further limit zoonotic transmission of *Salmonella.*

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### Impacts

- Non-typhoidal *Salmonella enterica* (NTS) was recovered from 27% (59/219) of hatchling poultry shipment boxes arriving at 36 different stores from a single farm store chain.
- NTS genotypes associated with multistate outbreaks of salmonellosis in 2013 were recovered from 23 of 219 hatchling shipment boxes. Shipment boxes containing indistinguishable genotypes originated from the same mail-order hatchery in all but one instance.
- Nineteen per cent (11/59) of isolates were resistant to more than one class of anti-microbial, but only one of 59 isolates was resistant to anti-microbials used to treat NTS infections in people.

**Table 1**

Proportion of hatchling shipment boxes that contained chicks, ducklings or a mixture of species that were positive for NTS

| | No. positive/total no. (%) | | | |
|---|---|---|---|---|
| | **Total** | **Chicks** | **Ducklings** | **Mix** |
| Hatchery A | 28/86 (32.6) | 23/67 (34.3) | 3/12 (25.0) | 2/7 (28.6) |
| Hatchery B | 6/33 (18.2) | 4/18 (22.2) | 0/4 (0) | 2/11 (18.2) |
| Hatchery C | 3/17 (17.6) | 2/13 (15) | 1/3 (33) | 0/1 (0) |
| Other source | 1/17 (5.9) | 0/15 (0) | 1/2 (50) | 0/0 (0) |
| No shipping information | 21/66 (31.8) | 15/49 (30.6) | 4/9 (44.4) | 1/5 (20.0) |
| Total | 59/219 (26.9) | 44/162 (27.1) | 9/30 (30.0) | 5/24 (20.8) |

## Table 2

Results of a multivariable logistic regression model testing the odds of a NTS positive result from environmental swabs of hatchling boxes arriving at a farm store chain. The significance of box characteristics was evaluated while controlling for 'Source' as a fixed effect

| Variable | Category | *Salmonella* result pos/total (%) | Odds ratio (95% CI) |
|---|---|---|---|
| Source | Hatchery A | 28/86 (33%) | 7.72 (0.98–61.21) |
| | Hatchery B | 6/33 (18%) | 3.56 (0.39–32.26) |
| | Hatchery C | 3/17 (18%) | 3.43 (0.32–36.83) |
| | Other | 1/17 (6%) | Ref |
| Number of hatchlings in box | ≥100 | 21/67 (31%) | 1.83 (0.6–5.59) |
| | 75 | 8/46 (17%) | 1.01 (0.31–3.31) |
| | 50 | 16/42 (38%) | 2.84 (0.92–8.74) |
| | 25 | 13/57 (23%) | Ref |
| At least one dead hatchling in the box | Yes | 4/13 (31%) | 0.95 (0.34–2.65) |
| | No | 55/206 (27%) | Ref |
| At least one sick/injured hatchling in the box | Yes | 12/46 (26%) | 0.4 (0.05–3.48) |
| | No | 47/173 (27%) | Ref |

**Table 3**

Frequency and hatchery sources of PFGE patterns of 59 NTS isolates recovered from hatchling shipping boxes. Pulsed-field gel electrophoresis patterns are listed in descending frequency of recovery

| | | | | No. isolates from each hatchery source* | | | | |
|---|---|---|---|---|---|---|---|---|
| **PFGE pattern** | **Serovar** | **No. shipment boxes positive** | **No. stores where strain was recovered** | **A** | **B** | **C** | **Drop shipper** | **Human outbreak size (2013)†** |
| JPXX01.0286 | Typhimurium | 13 | 10 | | 3 | | 1 | 356 |
| JCGX01.0001 | Cerro | 7 | 5 | 6 | | | | |
| JFXX01.0531 | Infantis | 5 | 4 | 3 | | | | 131 |
| LPPX01.0007 | Lille | 5 | 3 | 3 | | | | |
| JMPX01.0020 | Senftenberg | 4 | 1 | 1 | | | | |
| TDRX01.0261 | Mbandaka | 3 | 1 | 3 | | | | 4 |
| TDRX01.0071 | Mbandaka | 2 | 2 | 2 | | | | 19 |
| JGPX01.0004 | Kentucky | 2 | 2 | | | 2 | | |
| JGPX01.0027 | Kentucky | 2 | 2 | | | | | |
| JMPX01.0079 | Senftenberg | 2 | 1 | | | | | |
| JMPX01.0386 | Senftenberg | 2 | 2 | 1 | | | | |
| TDRX01.0411 | Mbandaka | 1 | 1 | 1 | | | | |
| TDRX01.0414 | Mbandaka | 1 | 1 | 1 | | | | |
| TDRX01.0415 | Mbandaka | 1 | 1 | 1 | | | | |
| LPPX01.0013 | Lille | 1 | 1 | 1 | | | | |
| LPPX01.0014 | Lille | 1 | 1 | 1 | | | | |
| JCGX01.0061 | Cerro | 1 | 1 | 1 | | | | |
| JEGX01.0004 | Enteritidis | 1 | 1 | | | 1 | | |
| JEGX01.0005 | Enteritidis | 1 | 1 | | | | | |
| JIXX01.0049 | Montevideo | 1 | 1 | | 1 | | | |
| JMPX01.0264 | Senftenberg | 1 | 1 | 1 | | | | |
| JMPX01.0388 | Senftenberg | 1 | 1 | 1 | | | | |
| JNXX01.0016 | Tennessee | 1 | 1 | | | 1 | | |

*Based on postal service tracking codes attached to each shipment box.

†Number of laboratory-confirmed human illnesses in published multistate outbreaks of salmonellosis.

## Table 4

Frequency of anti-microbial resistance phenotypes among 59 NTS isolates recovered from hatchling shipping boxes

| **Anti-microbial resistance phenotype** | **No. (%) of isolates (*n* = 59)** |
|---|---|
| Pansusceptible | 26 (44.1) |
| Tet | 21 (35.6) |
| StrTet | 4 (6.8) |
| GenSulStr | 2 (3.4) |
| KanTet | 2 (3.4) |
| AmcAmpAxoFoxTioStrTet | 1 (1.7) |
| GenSul | 1 (1.7) |
| KanSulTet | 1 (1.7) |
| Str | 1 (1.7) |

Isolates were tested for reduced susceptibility to the following anti-microbials: AUG, amoxicillin/clavulanic acid; AMP, Ampicillin; AZI, azithromycin; FOX, cefoxitin; TIO, ceftiofur; AXO, ceftriaxone; CHL, chloramphenicol; CIP, ciprofloxacin; GEN, gentamicin; KAN, kanamycin; NAL, nalidixic acid; STR, streptomycin; SUL, sulfisoxazole; TET, tetracycline; TMP/SMX, trimethoprim/sulphamethoxazole.