Tracing of Salmonella Contaminations Throughout an Integrated Broiler Production Chain in Dakahlia Governorate, Egypt

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A B S T R A C T
The data available regarding the production chain particularly in poultry flocks in Egypt are yet to be fully elucidated. The study aimed at providing an insight into the cross-contamination with Salmonella spp. at three different stages of an integrated broiler production chain. From 440 collected samples, 152 (34.5%) were positive for Salmonella spp. corresponded to samples collected from broiler farms (n=90, 40.9%), slaughter houses contact surfaces (n=12, 24%) and chicken carcass (n=50, 29.4%) in an integrated broiler supply chain. Isolation and identification of salmonella spp. were followed the standard procedures. Genomic bacterial DNA was extracted and confirmed using specific oligonucleotide primers sequences. Salmonella strains were classified into 11 serotypes. Nine different serotypes were found to contaminate the broiler houses and flocks with the most prevalent serotypes S. Enteritidis (38.8%), S. Kentucky (23.3%) and S. Typhimurium (11.11%). The overall frequency of Salmonella contamination in the live broiler flocks was 40.9% with prevalence of 37.9, 60, 40, 53.3, 60 and 20% from cloaca swabs, litter, feed, water, farms walls and in the hand swabs from workers, respectively. The PCR products of four isolates, (two isolates per S. Typhimurium and S. Enteritidis) were identified by DNA sequencing to determine the potential genetic relationships in these isolates. The findings of the present study suggested that different Salmonella serotypes can persist along the production chain and that contamination could be linked to the contamination of the final product with a potential health hazard to consumers.


INTRODUCTION
Poultry industry has seen a remarkable growth globally and represents approximately 45% of the world commerce (Henchion et al., 2014). In Egypt, this industry provides an integral part of the country’s supply from animal protein and remains trendy among Egyptian consumers across the national income because of its low cost compared to red meat and fish. According to the Organization for Economic Co-operation and Development (OECD) and the Food and Agriculture Organization (FAO) of the United Nations for 2017-2026, ~ 993 thousand tons of chicken are consumed in 2017. Poultry, poultry products and other food of animal’s origin represented the main reservoir for different salmonella (S). enterica serotypes, and human infection with salmonellosis is often associated with poultry borne illness (Berghaus et al., 2013; Ibrahim et al., 2013). The bacterium salmonella, is a facilitative anaerobic gram negative, has ubiquitous nature and can be present in a variety of host and non-host environments (Elmonir et al., 2017). Salmonella infection has long been recognized as one of the most important zoonotic contaminant of food and remains the leading bacterial enteric pathogen responsible for food borne outbreaks in the developed and developing countries (Abd-Elghany et al., 2015).

Food borne illness caused by salmonella infections results in continuous threat to human and animal health and causes serious economic losses in the society (Antunes et al., 2016; Aziz et al., 2018). It has been stated that S. enterica exists in the intestinal tract and reproductive organs of carrier chickens (El-Sharkawy et
al., 2017). Hence, the existence of *salmonella* on live poultry causes its widespread dissemination into the processing chain with a potential contamination of the final product (Antunes et al., 2016; Chao et al., 2007). According to the recent report (WHO, 2015), *S. enterica* causes approximately 180 million illnesses or 9% of the diarrheal illnesses that occur globally each year. Therefore, monitoring the prevalence of *S. enterica* in the whole broiler supply chain is imperative to minimize the hazards of *S. enterica* in poultry. In addition, gaining thorough information about the contribution of risk factors in the integrated broiler production chain is important to implement a successful and cost-effective control program for salmonellosis (EFSA, 2015).

Although there have been extensive documents regarding *salmonella* infections in poultry farms in Egypt, no published reports are found about the occurrence of *salmonella* in an integrated poultry production chain in our country. So, it is of utmost significance to carry out this study to explore the potential occurrence of *salmonella* spp. with special reference to the zoonotic serotypes in broiler poultry farms and to track cross-contamination at broiler farms, slaughter houses and whole chicken carcasses in an integrated production chain and to assess the possible genetic relationships among *Salmonella* isolates by DNA sequencing.

**MATERIALS AND METHODS**

**Sample collection:** During summer 2017, four hundred and forty samples were collected from poultry production system including poultry farms (n=220), slaughter house contact surfaces (n=50) and chicken carcasses (n=170) at the end of the slaughter line. The samples were kept in coolers and transported to the laboratory and processed immediately. The study followed the guidelines for Mansoura University and approved by its Ethical Committee.

**Sampling procedure**

**Broiler poultry farms:** A total of 220 samples (145 cloacae swabs and 15 samples each of feed, litters, water, farm walls and workers hand swabs) were randomly collected from three broiler poultry farms with birds in pre-slaughter age (35 to 40 days) at Dakahlia governorate. A sterile cotton swabs soaked in Buffered Peptone Water (BPW) (Oxoid, Basingstoke, UK) were used for cloacal and hand swabs. Sampling and screening of feed, litter and farm surfaces (walls) were tested according to the methods described by the International Organization for Standardization, Geneva, Switzerland ISO 6579: 2002. All the examined farms have level 1 of biosecurity measures.

**Slaughter houses environmental samples:** Fifty samples (20 knives, 20 tables and 10 abattoir walls) were collected from the contact surfaces in the abattoir according to the methods described by the American Public Health Association (1992). The procedure was done by swabbing four 100-cm² regions of the sample with sterile sponges pre-moistened with 40 mL of BPW. The sponges set were then transferred to sterile bags containing 160 mL of BPW to yield a final volume of 200 mL, corresponding to 400 cm². All samples were thoroughly homogenized before laboratory analyses.

**Samples from chicken carcasses:** One hundred and seventy samples (120 carcass swabs, 25 fillets and 25 livers) were collected from chicken carcasses at the end of slaughter line. Chicken carcass swabs were collected after evisceration using a whole-carcass swab method (McEvoy et al., 2005). Each swab was inoculated in sterilized BPW; while chicken fillets and livers from each individual sample (~25 g of each) were initially incised with a sterile scalpel and then collected into a sterile plastic stomacher bag according to the methods described by ISO 17604: 2003. The samples were transported immediately under aseptic conditions and processed for *salmonella* isolation.

**Salmonella isolation and identification:** Each swab was cultured in 9 ml BPW while chicken fillets and livers sample (~25 g of each) were homogenized in 225 ml of BPW and incubated at 37°C for 24 hrs, then the incubated samples were gently shaken and added to Rappaport-Vassiliadis broth (Oxoid, Basingstoke, UK) at 1:100 and incubated at 42°C for further 24 hrs followed by streaking on Xylose Lysine Desoxycolates at 37°C for 24 hrs. The plates were examined after incubation for the presence of typical and suspected colonies. Up to five colonies from each sample were further identified on the basis of biochemical characterization (Indole test, motility, nitrate reduction, methyl red, Voges proskauer, citrate utilization, and urease) according to the guidelines of ISO 6579: 2002. Biochemically typical *salmonella* isolates were serotyped according to Kauffman–White–Le–Minor techniqueste slide agglutination technique with polyvalent somatic (O) and flagellar (H) antisera (Welcome Diagnostic, UK) which were performed at the Department of Food Hygiene and Control, Faculty of Veterinary Medicine, Benha University Egypt.

**DNA extraction:** Genomic bacterial DNA was extracted and purified from the identified *S. Typhimurium* and *S. Enteritidis* serovar using the QIAamp® DNA Mini Kit Qiagen according to the manufacturer’s guidelines.

**Molecular identification:** Purified DNA was confirmed using specific oligonucleotide primers sequences for the amplification of flic and sefA genes according to Oliveira et al. (2003) and Akbarmehr et al. (2010).

**Amplification, sequencing of flic and sefA Genes, and Data Analyses:** The PCR products of four isolates (including two isolates per *S. Typhimurium* and *S. Enteritidis* that recovered from the farms and two other isolates obtained from chicken carcasses) were identified by DNA sequencing of partially flic and sefA gene. After electrophoresis on a 1.5% agarose gel, the bands were excised and purified using the QIAquick PCR product extraction kits (Qiagen Inc, Valencia, CA) according to the instructions of the manufacturer. Cycle sequencing of the gene fragments was done with the Big Dye ® Terminator v3.1 Cycle Sequencing Kit (Perkin-Elmer, Foster city, CA) according to the manufacturer’s recommendations. Sequencing products were analyzed using applied Biosystems 3130 genetic analyzer (Hitachi, Japan).

RESULTS

Of the total 440 samples screened, *salmonella* was isolated from 152 samples (34.5%) including 90 samples (40.9%) from broiler farms, 12 samples (24%) of slaughter houses contact surfaces and 50 samples (29.4%) collected from the processed chicken. The recovered isolates were assigned to 11 different serovares of *S. enteric* subspecies enteric including *S. Enteritidis* (n=41, 26.9%), *S. Typhimurium* (n=40, 26.3%), *S. Kentucky* (n=29, 19.1%), *S. Bargny* (n=12, 7.9%), *S. Molade* (n=8, 5.3%), *S. Takoradi* (n=6, 3.9%), *S. Labadi* (n=5, 3.3%), *S. Infantis* (n=3, 1.9%), *S. Papuana* (n=3, 1.9%), *S. Tamale* (n=3, 1.9%), and *S. Larochelle* (n=2, 1.3%).

Distribution of *salmonella* spp. in broilers farms: The overall frequency of *salmonella* contamination in the live broiler flocks was 40.9% (90/220) with a prevalence of 37.9% (55/145) from cloacae swabs, and 60% (9/15) from litter, 40% (6/15) from feed, 53.3 % (8/15) from water, 60% (9/15) from farms walls and 20% (3/15), from the worker's hand swabs (Table 1). The isolated serovares from broilers farms were distributed as follows: *S. Enteritidis* 38.8% (35/90), *S. Kentucky* 23.3% (21/90), *S. Typhimurium* 11.1% (10/90), *S. Molade* 7.8% (7/90), *S.Takoradi* 6.7% (6/90), *S. Bargny* 2.2% (2/90) and 3.3% (3/90) for each of *S. Papuana*, *S. Tamale*, *S. Infantis*.

Distribution of different *Salmonella* serotypes in slaughter houses contact surfaces and processed chicken: The data obtained from the processing environment are outlined in Table 2 and 3. In short, 24% (12/50) among slaughterhouses contact surfaces were contaminated with *salmonella* spp. with a higher contamination rate in abattoir walls (60%, 6/10). In general, twelve different *salmonella* isolates were recovered from the screened slaughter house contact surfaces (six isolates of *S. Kentucky* recovered from abattoir walls; two isolates of *S. Enteritidis* recovered from tables and four isolates of *S. Bargny* were recovered from Knives swabs). The occurrence of *salmonella* from the whole chicken carcass was 29.4% (50/170); being higher than that from processing surfaces (24%). In addition, the number of positive samples of *salmonella* spp. that recovered from the whole chicken carcasses swabs, liver and filet were 30/120, 10/25 and 10/25, respectively.

**Table 1**: Occurrence and distribution of different *Salmonella* serotypes in broiler farms

<table>
<thead>
<tr>
<th>Source of samples</th>
<th>No. of samples</th>
<th>No. of positive samples</th>
<th>% of positive</th>
<th>Serotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cloacal swabs</td>
<td>145</td>
<td>55</td>
<td>37.9</td>
<td><em>S. Kentucky</em> (21), <em>S. Molade</em> (7), <em>S. Enteritidis</em> (27)</td>
</tr>
<tr>
<td>Feed</td>
<td>15</td>
<td>6</td>
<td>40</td>
<td><em>S. Takoradi</em></td>
</tr>
<tr>
<td>Litter</td>
<td>15</td>
<td>9</td>
<td>60</td>
<td><em>S. Papuana</em> (3), <em>S. Tamale</em> (3), <em>S. Enteritidis</em> (1), <em>S. Typhimurium</em> (2)</td>
</tr>
<tr>
<td>Water</td>
<td>15</td>
<td>8</td>
<td>50</td>
<td><em>S. Enteritidis</em> (4), <em>S. Bargny</em> (2), <em>S. Typhimurium</em> (2)</td>
</tr>
<tr>
<td>Farm walls</td>
<td>15</td>
<td>9</td>
<td>60</td>
<td><em>S. Enteritidis</em> (3), <em>S. Typhimurium</em> (6)</td>
</tr>
<tr>
<td>Hand swabs from workers</td>
<td>15</td>
<td>3</td>
<td>20</td>
<td><em>S. Infantis</em></td>
</tr>
</tbody>
</table>

**Fig. 1**: Agarose gel electrophoresis of PCR amplification of *fliC* and *sefA* of *S. Typhimurium* and *S. Enteritidis* with expected amplicon size ~ 620 and 310 bp, respectively. Lane5 is DNA ladder; lane 4 and 6 are positive controls, while lane 3 and 7 are negative controls.

**DISCUSSION**

The findings of the present study demonstrated the persistence of different *salmonella* serotypes in an integrated broiler supply that alarms for a potential public health hazard. The frequency reported in the present study was similar to that recently reported in Egypt (El-Sharkawy et al., 2017). In that study, the authors identified *salmonella* infection in 17 out of 41 broiler chicken farms (41%) located in Kafr El-Sheikh Governorate in Northern Egypt during 2014–2015. On the other side, higher isolation rate (90.9 and 57.1%) was reported in the USA (Berghaus et al., 2013) and Egypt (Aziz et al., 2018). In the present study, nine different serotypes were found to contaminate the broiler houses and flocks with the most prevalent serotypes *S. Enteritidis* (38.8%), *S. Kentucky* (23.3%) and *S. Typhimurium* (11.1%). These findings went in parallel with that reported by Abd-Elghany et al. (2015) who stated that *S. Typhimurium* and *S. Enteritidis* were molecularly characterized as *S. Enteritidis* and *S. Typhimurium* isolates: Fig. 1 showed the amplification of *fliC* and *sefA* genes in the selected four *salmonella* isolates. The sequencing of the partially *fliC* and *sefA* genes demonstrated similar DNA profiles for each of *S. Typhimurium* and *S. Enteritidis* that recovered from the farms and the retail products.
considered the most predominant serotypes present in Egyptian poultry farms. On the other side, low frequency of *salmonella* infection was reported in broilers chicken flocks in several studies (Ibrahim et al., 2013; El-Tawab et al., 2015; Ren et al., 2016; Elmonir et al., 2017; Elkenany et al., 2018) corresponded to 16.6, 12.4, 7, 20 and 13.5%, respectively. In an interesting study (Pieskus et al., 2008), the occurrence of *salmonella* infection in conventional broiler stocks located in different areas in Italy, Germany, Lithuania and the Netherlands were 20%, 0, 29 and 11%, respectively. Given that litter, farm walls swabs and water samples provoked a consistently higher number of positive samples than individual chickens or chicken feed, these findings suggest that the farm walls, litter and water might be as a potential reservoir for contamination in the examined broiler farms. Several pathways associated with increase flock colonization by *salmonella* include vertical and horizontal transmission, overcrowding, unhygienic farming activities, lack of adequate biosecurity measures, contamination from previous flocks, exposure to potential sources of the bacterium such as neighboring animals on the farm, insects, rodents, environment, housing system and flock size, season of the year, different hygienic measures, inadequate cleaning and disinfection of broiler rearing houses and contamination of feed, litter and drinking water. These factors could play a vital role for the pathogen to be easily disseminated between the flocks.

The information about the occurrence of *salmonella* contamination at slaughter facilities remains scarce despite being the main source of cross-contamination. In this respect, nearly similar incidences of surface contamination for *salmonella* were reported by several researchers (Adzitey et al., 2012; Wang et al., 2013) in poultry processing environment with a rate of 23.5 and 25%, respectively. On the other side, high positive percentage rate (62.9%, 22/35) was also reported in china slaughter house samples (Ren et al., 2016). However, diverse surveys are still reported from several developing countries. For example, high percentages of positive samples (approximately 35%) were reported from Nigeria (Adeyanju and Ishola 2014) and Egypt (Abd-Elghany et al., 2015). In South America, the detection rates were ranging between ~13 and 39% (Ribeiro et al., 2007; Donado-Godoy et al., 2015), while in Asia, it ranged between 35 and 43% (Ta et al., 2014; Yang et al., 2014).

In China, 54-67% of the 75 retail store raw chicken samples was contaminated with *Salmonella* spp. (Ren et al., 2016). Nevertheless, a comparatively low isolation rate (1.56%) was reported from Morocco in poultry processing plan (Cohen et al., 2007). Different prevalence rates from retail outlets, retail markets and processing plans were reported worldwide. The discrepancy among the previously reported contamination rates of *salmonella* in chicken meat could possibly reflect the diverse hygienic conditions along the production practices, transportation stress and lack of control measures along the slaughter houses environment. The high contamination rate of whole carcass swab would suggest a contamination during de-feathering in the slaughter house and highlight the need for adequate hygienic procedures. It becomes clear that chicken products could be an important vehicle of *S*. enterica, and hence signify its hygienic hazard at this stage.

Although several serotypes of *Salmonella* were recovered separately during the different stages of production, *S*. Enteritidis and *S*. Kentucky were prevailed at all stages of the production chain and *S*. Typhimurium was found in common between the farms and the retail products. *Salmonella* Typhimurium and *S*. Enteritidis demonstrated similar DNA profiles by sequencing and were considered the most prevalent serovars being isolated from the screened broilers on the farms and also recovered from the whole chicken carcasses within the same area. A similar finding was previously given by Hugas and Beloeil (2014) and reported that *S*. Enteritidis and *S*. Typhimurium were commonly associated with poultry and its products and have great public health significance.

The occurrence of *S*. Kentucky at each stage in the production chain was really surprising. The overall prevalence of *S*. Kentucky in the present study was 19.1%. Data reported from a recent study showed that *S*. Kentucky was detected in poultry as a predominant serotype and could be implicated in human *salmonellosis* (Westrell et al., 2014). In that study, 1301 cases of *salmonella* infections were caused by *S*. Kentucky in 12 European countries between 2007 and 2012. The authors added the rationale for increasing the occurrence of *S*. Kentucky could be linked to increase globalisation of food/animal trades. Recently, *S*. Kentucky (particularly the epidemic clone ST198-X1) has been recovered from several livestock reservoirs, particularly from poultry farms in Europe, Africa and Asia (Antunes et al., 2016). In the present study, *S*. Infantis was recovered from the hand swabs of farm workers at the percentage of 20%. According to the European Food Safety Authority (EFSA, 2015), human infection cases due to *S*. Infantis have been increased between 2011 and 2013 and was being considered the fourth most common serotype reported in broilers and broiler meat.

### Table 2: Occurrence and distribution of different Salmonella serotypes from slaughter house environment

<table>
<thead>
<tr>
<th>Source of samples</th>
<th>No. of samples</th>
<th>No. of positive samples</th>
<th>% of positive</th>
<th>Serotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knives swabs</td>
<td>20</td>
<td>5</td>
<td>25</td>
<td>S. Bargny</td>
</tr>
<tr>
<td>Table</td>
<td>20</td>
<td>2</td>
<td>10</td>
<td>S. Enteritidis</td>
</tr>
<tr>
<td>Abattoir wall</td>
<td>10</td>
<td>6</td>
<td>60</td>
<td>S. Kentucky</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>12</td>
<td>24</td>
<td></td>
</tr>
</tbody>
</table>

### Table 3: Occurrence and distribution of different Salmonella serotypes from processed broilers

<table>
<thead>
<tr>
<th>Source of samples</th>
<th>No. of samples</th>
<th>No. of positive samples</th>
<th>% of positive</th>
<th>Serotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carcass</td>
<td>120</td>
<td>30</td>
<td>25.6</td>
<td>S. Typhimurium (21), S. Kentucky (2), S. Molade (1), S. Bargny (6)</td>
</tr>
<tr>
<td>Liver</td>
<td>25</td>
<td>10</td>
<td>40</td>
<td>S. Typhimurium (5), S. Labadi (5)</td>
</tr>
<tr>
<td>Fillet</td>
<td>25</td>
<td>10</td>
<td>40</td>
<td>S. Typhimurium (4), S. Enteritidis (4), S. Larochelle (2)</td>
</tr>
<tr>
<td>Total</td>
<td>170</td>
<td>50</td>
<td>29.4</td>
<td>S. Typhimurium (20), S. Bargny (6), S. Kentucky (2), S. Molade (1), S. Labadi (5), S. Enteritidis (4), S. Larochelle (2)</td>
</tr>
</tbody>
</table>
Taken together, it can be concluded that different Salmonella serotypes can persist along the integrated production chain (on-farm, slaughter operations, equipment, chicken carcasses, handlers and retail meat). Such contamination is significantly linked to the contamination of the final product which highlights a serious public health hazard. Additionally, an integrated surveillance (i.e. collaboration between different authorities), containment strategies and continuous monitoring are urgently needed through a farm-to-table approach at every stage of the production chain in order to minimize contamination and also reduce the emergence of potential Salmonella serotype along the food chain which is of critical importance for public health.

Authors contribution: All authors contributed equally in study design, samples collection and processing and data interpretation. All authors revised the manuscript and approved the final version before publication.

REFERENCES


