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Research Paper

Occurrence, Risk Factors, Serotypes, and Antimicrobial Resistance of *Salmonella* Strains Isolated from Imported Fertile Hatching Eggs, Hatcheries, and Broiler Farms in Trinidad and Tobago

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ABSTRACT

This cross-sectional study was conducted to determine the occurrence, risk factors, and characteristics of *Salmonella* isolates recovered from imported fertile broiler hatching eggs, hatcheries, and broiler farms in Trinidad and Tobago. Standard methods were used to isolate and characterize *Salmonella* isolates from two broiler hatcheries and 27 broiler farms in the country. The frequency of isolation of *Salmonella* was 0.0% for imported fertile hatching eggs (0 of 45 pools of 10 eggs each, i.e., 450 eggs), 7.6% for hatcheries (12 of 158 samples), and 2.8% for broiler farms (24 of 866 samples) ($P = 0.006$). Stillborn chicks at hatcheries had the highest prevalence of *Salmonella* (7 of 28 samples, 28.0%), whereas on broiler farms the cloacal swabs had the highest prevalence of *Salmonella* (15 of 675 samples, 2.2%). None of the 15 farm management and production practices investigated were significantly associated ($P > 0.05$) with the isolation of *Salmonella*. The predominant *Salmonella* serotypes were Kentucky (83.3%) and Infantis (62.5%) among hatchery and farm isolates, respectively. The disk diffusion method revealed frequencies of antimicrobial resistance (i.e., resistance to one or more agents) of 44.0% (11 of 25 isolates) and 87.5% (35 of 40 isolates) at hatcheries and broiler farms, respectively ($P = 0.0002$). Antimicrobial resistance among hatchery isolates was highest (28.0%) to doxycycline and kanamycin and was very high (>65%) among farm isolates to sulfamethoxazole-trimethoprim, gentamicin, ceftriaxone, kanamycin, and doxycycline. Multidrug resistance (MDR; i.e., resistance to antimicrobial agents from three or more classes) was exhibited by 4.0 and 85.7% of *Salmonella* isolates recovered from several environmental and animal sources at the hatcheries and farms, respectively ($P < 0.0001$). The high level of antimicrobial resistance and the presence of MDR among *Salmonella* isolates from broiler farms highlight the therapeutic implications and the potential for MDR strains to enter the food chain.

HIGHLIGHTS

- *Salmonella* was evaluated in imported fertile hatching eggs, at hatcheries, and at poultry farms.
- Prevalences were 0.0, 7.6, and 2.8% for eggs, hatcheries, and broiler farms, respectively.
- Predominant serotypes were *Salmonella* Kentucky (83.3%) in hatcheries and Infantis (62.5%) on farms.
- Prevalence of antimicrobial resistance was 44.0 and 87.5% for hatchery and farm isolates, respectively.
- Resistant *Salmonella* strains in the broiler production chain have implications for food safety.

Key words: Antimicrobial resistance; Broiler farms; Hatcheries; Imported fertile hatching eggs; *Salmonella*; Trinidad and Tobago

Salmonella is one of the foremost pathogens in the poultry industry *(20)* and a major cause of bacterial foodborne gastroenteritis in humans. Poultry has been considered an important source of *Salmonella* infections in consumers of improperly cooked contaminated chicken and chicken products *(53)*. Salmonellosis in broiler flocks results in severe economic loss due to a reduction in chicken production from extremely high and variable morbidity and mortality *(47)*.

Imported fertile hatching eggs can be a source of *Salmonella* introduction and transmission through the poultry production chain. Vertical (transovarial) transmission occurs when *Salmonella*-infected breeder hens with

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contaminated reproductive tracts lay fertile eggs that harbor *Salmonella (17)*. Eggs may be contaminated at the breeder farm *(65),* and chicks may be infected with *Salmonella* by vertical transmission via the infected hen or horizontal transmission when *Salmonella*-positive eggs contaminate *Salmonella*-free eggs at the breeder farm. *Salmonella* associated with poultry can persist in the hatchery environment, contaminating subsequent batches of eggs and birds *(10)*. Adesiyun et al. *(6)* reported that 91% of the 27 Caribbean countries (including Trinidad and Tobago) imported egg and egg products from foreign sources. The increasing international trade poses an increased risk of transfer of microbes from one country to another *(48)*.

Hatcheries are considered major sources of *Salmonella* infection in young chicks because *Salmonella*-negative chicks can be exposed to *Salmonella*-positive chicks in a confined environment *(22)*. Broiler chicks are most susceptible to pathogen colonization during the first few days after hatching because their immune system and intestinal flora are immature *(58)*. Vertical transmission can occur from infected breeders, and horizontal transmission can occur during handling and transportation *(13)*. Several risk factors have been associated with contamination of hatcheries by *Salmonella (40)*.

The presence of *Salmonella* on broiler farms depends on-farm management practices and on the *Salmonella* status of day-old chicks and feed. On-farm surveillance programs can effectively control and eradicate foodborne pathogens *(56)* and significantly reduce the pressure on the food safety management systems implemented at subsequent steps in the poultry chain, such as at the processing plants *(49)*. A vertical integration model established for producing chicken meat has been recommended as one of the main tools to reduce the presence of *Salmonella* in the final product *(31)* because the control measures and interventions established at each integration sector contribute to the reduction in *Salmonella*. Water quality (microbial, physical, and chemical), presence of pests (e.g., rodents and wild birds) and insects or their larvae, inappropriate disposal of dead *Salmonella*-positive birds, poor feed and drinker management, soiled feathers, and poor litter condition have all been associated with *Salmonella* isolation at broiler farms *(56)*.

The broiler industry in Trinidad and Tobago is operated primarily by vertically integrated companies that have contractual agreements with farmers who are paid to produce grow-out chicks to processing age. Operating costs, such as for repairs and maintenance of the broiler houses, are the farmers' responsibility. However, the integrated companies take responsibility for the chicks, the supply and distribution of feed, the provision of veterinary and management services, the transportation of birds to processing plants for processing, and subsequent marketing. The integrated broiler companies also are responsible for hatching of imported fertile hatching eggs at their hatcheries. This approach results in holistic control of the introduction, propagation, and dissemination of *Salmonella* from the hatchery to the country's retail outlets. The management and veterinary services provided by the integrated poultry companies to the contracted farmers and hatcheries are independent of government agencies, and interventions take place only when notifiable diseases are suspected. All fertile hatching broiler eggs are imported into the country. Broiler production in Trinidad and Tobago is estimated to be 42 million chickens per year where approximately 1 million chickens are consumed weekly, 80% of which are produced locally *(63)*. Broiler production is solely for the local market because chickens produced locally are not exported.

The objectives of this cross-sectional study were (i) to determine the occurrence of *Salmonella* in imported fertile hatching broiler eggs, hatcheries, and broiler farms to identify the risk factors and management practices associated with *Salmonella* contamination and (ii) to determine the resistance of these isolates to antimicrobial agents commonly used in the poultry industry in Trinidad and Tobago.

MATERIALS AND METHODS

Sampling sites and sources of samples. The study was conducted in Trinidad and Tobago, the twin-island Caribbean country located in the southern Caribbean. The poultry industry in Trinidad is vertically integrated. Each company imports fertile hatching eggs for their own hatchery. The companies also provide day-old chicks to their contracted farmers to produce a regular supply of broilers for slaughter at their processing plants. Fertile hatching eggs are imported weekly into the country from two foreign companies based outside the Caribbean region. The breeds of chickens imported are Cobb × Cobb, Ross × Ross, or Hubbard × Cobb. Four commercial broiler hatcheries and approximately 400 contracted broiler farms operate in Trinidad, where three integrated poultry companies own most farms. In this study, the hatcheries and farms owned and operated by the two largest integrated poultry companies, representing 11.2% (27) of the 242 farms owned by both integrators, were sampled during the following periods: February to July 2019 for farms, August to September 2019 for hatcheries, and July to August 2019 for imported fertile hatching eggs. The 27 farms sampled were dispersed across the country (Fig. 1).

Determination of sample size for the study. The sample size for this study estimated for an infinite population using $n_o = Z_u^2 P_{ex}(1 - P_{ex})/d^2$ *(55),* where n_o is the estimated sample size, Z_u is the degree of confidence (1.96), P_{ex} is the expected prevalence (50%), and d is the desired absolute precision.

Because the imported fertile hatching eggs were pooled for processing, a convenience sampling approach was used. The study design stipulated random collection of a crate consisting of 30 eggs during each of 15 sampling visits to the port of entry at the airport. From each crate of 30 eggs, 10 eggs were pooled to constitute a composite sample for processing.

For the hatcheries, the d value was 8%. Thus, $n_o = [1.96^2 \times 0.5(1 - 0.5)]/0.08^2 = 150$. For this study, a total 158 samples were collected from the two hatcheries.

For the broiler farms, the d value was 3.5%. Thus, $n_o = [1.96^2 \times 0.5(1 - 0.5)]/0.035^2 = 784$. For this study, 866 samples were collected from the 27 broiler farms.

Fertile hatching eggs imported by two of the integrated poultry companies in Trinidad and Tobago destined for their respective hatcheries were randomly collected on 15 sampling visits on the day they arrived at the airport. During each visit, one crate (30 eggs) was randomly collected from the shipment by a

FIGURE 1. *Map of Trinidad showing farm and hatchery locations sampled.*

person wearing sterile gloves, transported in a cooler with ice packs to the laboratory, and processed within 3 h of collection. Ten eggs were pooled to constitute a composite sample from which eggshell and egg content samples were separately processed as previously described *(4, 8)*. Overall, 45 eggshell and egg content samples, each consisting of 10 eggs (i.e., a total of 450 eggshell and 450 egg contents samples), were processed.

Samples were collected from the destination broiler hatcheries (A and B) during five sampling visits (three to hatchery A and two to hatchery B). The samples collected were representative of each step of the hatching process. Table 1 shows the types and number of samples collected at both hatcheries. All samples were collected using the methods previously reported *(8)* with slight modifications; in the present study, a pool of 10 instead of 6 eggs was used. At 27 of the broiler farms (Fig. 1) that were owned by the two integrated poultry companies and that received broilers from the two hatcheries, samples of randomly selected individual broilers and the environment (drag swab of litter, boot swabs, and samples of feed and water) to determine the presence of *Salmonella*. Farms were selected proportionally based on their geographical locations, the total number of contract farms owned by the respective company, the broiler throughput, and the number of pens. Of these 27 farms, 4 were supplied by hatchery A and 23 were supplied by hatchery B. Table 2 shows the type and number of samples collected from the broiler farms. Sample collection was conducted using the method previously reported *(8)*.

Administration of questionnaires at hatcheries and broiler farms. Questionnaires were administered at each hatchery and broiler farm from which samples were collected to obtain demographic data, operational information, and information on risk factors for *Salmonella* contamination. Some of the questions included the number of workers employed, the types of pests encountered, the average mortality rates at the farms, the sources of hatching eggs, the types of sanitizers used, and the vaccines administered at hatcheries. The questionnaires are provided as supplemental material (Appendix 1, Questionnaire administered at the hatcheries; Appendix 2, Questionnaire administered at the broiler farms).

Processing of samples collected. Published standard procedures were used to process the samples collected from imported fertile hatching eggs, the hatcheries, and the broiler farms. To process hatching eggs, the procedures earlier described for eggs *(4, 5)* were used. The only modification made was the pooling of 10 eggs to constitute a composite sample. For the 10 pooled eggs, the egg contents and eggshells were processed separately for detection of *Salmonella*. The 45 pools each of eggshells (450 eggs) and egg contents (the same 450 eggs) were processed.

TABLE 1. *Type and number of samples collected at two broiler hatcheries*

Type of sample	No. of samples		
	Hatchery A	Hatchery B	Total
Swabs of eggs in incubator[a]	7	5	12
Swabs of eggs in hatcher[a]	8	4	12
Swabs of incubator interior[b]	7	5	12
Swabs of hatcher interior[b]	8	4	12
Stillborn chicks	15	10	25
Broken eggshells[c]	15	11	26
Hatcher fluff[c]	15	10	25
Incubator air unit	7	4	11
Hatcher exhaust fans	2	4	6
Meconium samples[c]	0	10	10
Egg transfer machine	1	0	1
Chick conveyor belt	2	0	2
Drain	2	2	4
Total	89	69	158

[a] One sample comprised pooled swabs of 10 eggs in one crate: adjacent eggs at each of the four corners (2 eggs per corner) and the center (2 eggs) of each crate or tray.

[b] One sample comprised four surface area (12.7 by 12.7 cm) swabs of each side of the incubator or hatcher, i.e., a total 100-cm^2 area was sampled.

[c] Each sample collected as a composite sample representative of each hatcher.

TABLE 2. *Type and number of samples collected at 27 broiler farms in Trinidad and Tobago*

Type of sample	No. of samples	
	Per farm	Total
Cloacal swab	25	675[a]
Feed[b]	1 or 2[c]	53
Water	2	54
Boot swab	1–3[c]	27
Drag swab	1–3[c]	57
Total		866

[a] Collected from 675 individual broilers.
[b] Two feed samples were collected from feeding containers and newly opened bags, but only one sample was collected from one farm because of limited available containers and bags.
[c] Samples collected differed because of differences in the number of active pens and the frequency of the use of boots.

To process hatchery samples of eggs, chicks, and the environment, the procedures earlier described were used *(8)*. To process broiler farm samples (water, feeds, cloacal swabs, boot swabs, and drag swabs of litter), the procedure used to sample layer farms in three Caribbean countries was used *(8)*.

Isolation and identification of *Salmonella*. For the isolation of *Salmonella,* the procedures earlier described were used *(8, 37)* with slight modifications. All samples collected from the three sources (the airport, hatcheries, and farms) were initially preenriched (1:10) in buffered peptone water (Oxoid, Basingstoke, UK) incubated at 37°C for 18 to 24 h and then selectively enriched in tetrathionate (TT) broth (Oxoid) and Rappaport-Vassiliadis soya (RVS) broth (Oxoid) and incubated for 18 to 24 h at 37 and 42°C, respectively.

Samples enriched in the selective broths were subcultured onto xylose lysine Tergitol 4 (XLT4; Oxoid) and brilliant green agar (BGA; Oxoid) and incubated at 37°C for 18 to 24 h. Suspected *Salmonella* colonies, based on their phenotypic appearance on the respective agars, were subjected to a panel of biochemical tests (triple sugar iron agar, lysine iron agar, urea, citrate, methyl red, sulfide indole motility medium, and *o*-nitrophenyl-β-D-galactopyranoside) (Oxoid) for preliminary identification of *Salmonella* using standard methods. Isolates biochemically confirmed as *Salmonella* were then subjected to a slide agglutination test using *Salmonella* polyvalent antisera (A-I and Vi, Difco, BD, Detroit, MI). Initial complete confirmation and serotyping of *Salmonella* isolates, representative of those recovered in RVS and TT broths or BGA and XLT4, were performed using the phase reversal technique, and the results were interpreted according to the Kauffman-White scheme *(29)* at the Public Health Laboratory (Ministry of Health, St. Michael, Barbados).

Determination of antimicrobial resistance. The antimicrobial resistance of 65 *Salmonella* isolates recovered from the hatcheries and farms was determined using the disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines *(19)*. Eight antimicrobial agents commonly available and used in the local market and frequently used the country's poultry industry were included in the panel. Chloramphenicol was included primarily for comparison and for research purposes. The antimicrobial agents (Difco, BD) and concentrations used were amoxicillin–clavulanic acid (AMC, 30 μg), doxycycline (DO, 30 μg), ceftriaxone (CRO, 30 μg), gentamicin (CN, 10 μg), kanamycin (K, 30 μg), chloramphenicol (C, 30 μg), sulfamethoxazole-trimethoprim (SXT, 23.75 and 1.25 μg), and ciprofloxacin (CIP, 5 μg). The tests were performed on Mueller-Hinton agar (Difco, BD), followed by aerobic incubation at 37°C for 24 h. The zones of inhibition were interpreted as recommended by the disk manufacturer and CLSI *(19)*. For this investigation, isolates that exhibited intermediate zones of inhibition were classified as resistant.

Statistical analyses. Chi-square analyses were conducted using the Statistical Package for Social Sciences (SPSS version 27, IBM, Armonk, NY) to determine the significance of the association between the isolation frequency of *Salmonella* and (i) the risk factors associated with *Salmonella* contamination on hatcheries and broiler farms, (ii) the types of samples collected, (iii) the antimicrobial resistance by type of sample, and (iv) the antimicrobial resistance by serotype. Fisher's exact test was used for 2×2 tables with expected frequencies of <5. Significance was defined as an alpha level of 0.05.

Approval by the research and ethics committee. The study was approved and conducted under terms approved by the University of the West Indies, St. Augustine Campus Research Committee (research grant 2660-457522). The St. Augustine Campus Ethics Committee granted the project an exemption from ethical review after assessing the research proposal.

RESULTS

Overview of demographic and risk factors. Of a total of 37 demographic and risk factors investigated at both hatcheries (Supplemental Appendix 1), none were significantly associated ($P > 0.05$) with the frequency of isolation of *Salmonella*.

Of the 15 factors (demographic data and management practices) investigated for their association with the isolation of *Salmonella* on the broiler farms, none were significant ($P > 0.05$) (Table 3).

Frequency of *Salmonella* isolation by source and serotype. All 45 composite samples of imported fertile hatching eggs (each consisting of a pool of 10 eggs, i.e., a total of 450 eggs processed separately as eggshells and egg contents) were negative for *Salmonella*.

The frequency of isolation of *Salmonella* from 13 types of samples collected from the two hatcheries was 7.6% (12 of 158 samples) (Table 4). The frequency of isolation ranged from 0.0% (seven sample types: swabs of the incubator interior, chick take-off area, drain, hatcher exhaust fans, incubator air unit, egg transfer machine, and samples of meconium) to 28.0% (stillborn chicks). The frequency of isolation of *Salmonella* from stillborn chicks (7 of 25 chicks, 28.0%) was significantly higher ($P < 0.001$) than that found for all other hatchery samples (5 of 133 samples, 3.8%). *Salmonella* Westhampton was isolated from 3.8% (1 sample) of the 26 broken eggshell samples. *Salmonella* Kentucky was the predominant serotype and was identified in 10 (83.3%) of the 12 isolates recovered from *Salmonella*-positive samples. The two isolates recovered from stillborn chicks and one isolate recovered from

TABLE 3. *Demographic data and risk factors associated with* Salmonella *isolation at broiler farms*

Factor	No. of farms sampled	No. (%) of farms positive for *Salmonella*[a]	*P* value
Farm management; no. of workers			0.25
0–5	24	7 (29.2)	
>5	3	2 (66.7)	
Management system			1
Automatic or semiautomatic	12	4 (33.3)	
Manual	15	5 (33.3)	
Pen type			1
Open sided	25	8 (32.0)	
Tunnel ventilated	2	1 (50.0)	
Pen capacity			0.406
1,000–15,000 birds	17	7 (41.2)	
>15,000 birds	10	2 (20.0)	
No. of pens/farm			0.582
1–5	23	7 (30.4)	
>5	4	2 (50.0)	
Vaccines given on farm			0.582
Yes	23	7 (30.4)	
No	4	2 (50.0)	
Goal wt of birds[b]			0.683
3.8–4.5 lb (1.7–2.0 kg)	15	6 (40.0)	
>4.5 lb	12	3 (25.0)	
Water supply			0.377
Municipal only	14	5 (35.7)	
Municipal or pond	7	1 (14.3)	
Pond and river	3	2 (66.7)	
Other[c]	3	1 (33.3)	
Type of feed used			1
Marsh	2	1 (50.0)	
Pelleted	25	8 (32.0)	
Use of artificial lighting			0.676
Yes	8	2 (25.0)	
No	19	7 (36.8)	
Pests on farm			0.103
Yes	14	7 (50.0)	
No	13	2 (15.4)	
Type of pests			0.115
Rats	5	3 (60.0)	
Mongooses	7	1 (14.3)	
Birds	8	6 (75.0)	
Other[d]	5	2 (40.0)	
Type of pest control			0.54
Poison	6	3 (50.0)	
Bait	5	3 (60.0)	
Other[e]	12	5 (41.7)	
Biosecurity measures			0.992
All-in, all-out	27	9 (33.3)	
Restricted access	23	8 (34.8)	
Foot dip	5	1 (20.0)	
One-way traffic	5	2 (40.0)	
Vehicle dips, wheel baths	4	2 (50.0)	
Closed system, bird proofed	2	1 (50.0)	
Fenced property	2	1 (50.0)	
Protective clothing for workers	2	1 (50.0)	
None	3	1 (33.3)	
Mortality rate (%)			1
0–5.0	20	7 (35.0)	
>5.0	7	2 (28.6)	

[a] Farms were deemed positive when *Salmonella* was detected in any sample.
[b] Each farm aimed to achieve a specific weight per bird at the end of the grow-out period.
[c] Municipal water, ponds, and rain (1), municipal water and river (1), and a spring (1).
[d] Other pests were cats and mattes (lizards).
[e] Other types of pest control methods used included loud music, dogs, insecticides, and herbicides around the pens.

hatcher fluff at hatchery A were all *Salmonella* Kentucky. In hatchery B, *Salmonella* Kentucky was isolated from five samples obtained from stillborn chicks and one sample each from eggs in the incubator and the interior of the hatchers.

At the hatcheries, the overall frequency of isolation of *Salmonella* was 3.4% (3 of 89 samples) at hatchery A and 13.0% (9 of 69 samples) at hatchery B. The difference between the two hatcheries was significant ($P = 0.023$). For the 13 types of samples tested from hatchery A, 15.4% (2 of 13 types) yielded *Salmonella*. The frequency of recovery of *Salmonella* was 6.7% (1 of 15 samples) for the hatcher fluff and 13.3% (2 of 15 samples) for stillborn chicks ($P = 1$) from hatchery A. In Hatchery B, 30.8% (4 of 13) of sample types yielded *Salmonella*. The frequency of isolation of *Salmonella* in the samples tested ranged from 9.1% (1 of 11 samples of broken eggshells) to 50.0% (5 of 10 stillborn chicks), but the differences between the types of samples were not significant ($P = 0.064$).

From a total of 27 farms sampled, the overall frequency of isolation of *Salmonella* among five types of samples was 2.8% (24 of 866 samples) (Table 4). The farm prevalence of *Salmonella* was 33.3% (9 of 27 farms). The frequency of isolation of *Salmonella* was 2.2% (15 of 675 samples) for cloacal swabs and 4.7% (9 of 191 samples) for environmental samples (feed, water, workers' boots, and drag swabs of litter). For the environmental samples, the frequency of isolation of *Salmonella* ranged from 1.9% (1 of 53 samples) in feed to 11.1% (3 of 27 samples) in boot swabs. The differences in the frequency of isolation of *Salmonella* among the several types of samples were significant ($P = 0.049$). *Salmonella* Infantis was the predominant serotype isolated, recovered from four (80.0%) of the five types of samples and accounting for 15 (62.5%) of the 24 isolates recovered from *Salmonella*-positive samples. Four (16.7%), one (4.2%), and four

TABLE 4. *Frequency of isolation of* Salmonella *serotypes from imported fertile hatching eggs, hatcheries, and broiler farms in Trinidad and Tobago*

Source of samples	Type of sample	No. of samples collected	No. (%) of samples positive for *Salmonella*	Serotype (no. of isolates; % of isolates)
Imported fertile hatching eggs	Eggshells and albumen	45[a]	0	
Hatcheries	Stillborn chicks	25	7 (28.0)	Kentucky (7; 100.0)
	Broken eggshells	26	1 (3.8)	Westhampton (1; 100.0)
	Swabs of incubator interior	12	0	NA
	Swabs of hatcher interior	12	1 (8.3)	Kentucky (1; 100.0)
	Swabs of eggs in incubator	12	1 (8.3)	Kentucky (1; 100.0)
	Swabs of eggs in hatcher	12	1 (8.3)	Group D (1; 100.0)
	Chick takeoff	2	0	
	Hatcher fluff	25	1 (4.0)	Kentucky (1, 100.0)
	Drain	4	0	
	Hatcher exhaust fans	6	0	
	Incubator air unit	11	0	
	Egg transfer machine	1	0	
	Meconium samples	10	0	
P value			0.075[b]	
Subtotal		158	12 (7.6)	
Farms	Cloacal swab	675	15 (2.2)	Infantis (11; 73.3) Albany (4; 26.7)
	Feed	53	1 (1.9)	Infantis (1; 100.0)
	Water	54	3 (5.6)	ND[c] (3; 100.0)
	Boot swabs	27	3 (11.1)	Infantis (2; 66.7) ND (1; 33.3)
	Drag swabs	57	2 (3.5)	Othmarschen (1; 50.0) Infantis (1; 50.0)
P value			0.049	
Subtotal		866	24 (2.8)	
P value			0.0025	
Total		1024	36 (3.5)	

[a] The 45 composite samples (eggshells and egg contents) were composed of 45 pools each containing 10 eggs, i.e., a total of 450 eggs.
[b] Frequency of isolation of *Salmonella* from stillborn chicks (28.0%, 7 of 25 samples) was significantly higher ($P < 0.001$) than that for all other hatchery samples (3.8%, 5 of 133 samples).
[c] ND, not determined.

(16.7%) isolates were identified as *Salmonella* Albany, *Salmonella* Othmarschen, and an undetermined *Salmonella* serotype, respectively.

Regarding the hatchery sources of chicks supplied to farms, for the farms provided by hatchery A, all seven isolates (100%) from four cloacal swabs, one drag swab, one feed sample, and one boot swab were *Salmonella* Infantis. However, for the farms supplied by hatchery B, *Salmonella* serotypes Albany (four cloacal swabs), Infantis (seven cloacal swabs and one boot swab), and Othmarschen (one drag swab), and an undetermined *Salmonella* serotype (three water samples and one boot swab) were isolated from several types of samples.

Overall, of the 1,024 samples collected from two hatcheries and 27 broiler farms, 36 samples (3.5%) were positive for *Salmonella,* and the frequency of isolation was significantly higher ($P = 0.0025$) for the samples from the hatcheries (12 of 158 samples, 7.6%) than for the samples from the broiler farm (24 of 866 samples, 2.8%).

Frequency of antimicrobial resistance among *Salmonella* isolates. The resistance to eight antimicrobial agents for *Salmonella* isolates recovered from the hatcheries and broiler farms is shown in Table 5.

Among the 25 *Salmonella* isolates from six types of samples collected from the hatcheries, 11 isolates (44.0%) were resistant to one or more antimicrobial agents tested. The differences in the frequency of resistance in *Salmonella* isolates across the type of samples were not significant ($P = 0.10$). Some *Salmonella* isolates were resistant to four of the eight antimicrobial agents: amoxicillin–clavulanic acid (4.0% of isolates), gentamicin (4.0% of isolates), doxycycline (28.0% of isolates), and kanamycin (28.0% of isolates). For the six types of samples assessed, the frequency of resistance among the *Salmonella* isolates was significant only for kanamycin ($P = 0.043$).

For the 40 *Salmonella* isolates recovered from five types of samples collected from the farms, the overall frequency of resistance to antimicrobial agents was 87.5% (35 of 40 isolates) with a range of 40.0% (water) to 100.0% (feed, litter drag swabs, and boot swabs). The differences were significant ($P = 0.02$). Some *Salmonella* isolates were resistant to six of the eight antimicrobial agents with a range in frequency from 2.5% (1 of 40 isolates) for ciprofloxacin

TABLE 5. *Frequency of antimicrobial resistance among* Salmonella *isolates at hatcheries and broiler farms based on type of samples*

Source of sample	Type of sample	No. of isolates tested	No. (%) of resistant isolates[a]	No. (%) isolates resistant to[b]:							
				AMC	DO	CRO	CN	K	C	SXT	CIP
Hatcheries	Stillborn chicks	17	6 (35.3)	1 (5.9)	6 (35.3)	0	1 (5.9)	3 (17.6)	0	0	0
	Broken eggshells[c]	2	2 (100.0)	0	0	0	0	2 (100.0)	0	0	0
	Swab of eggs in hatcher[c]	1	1 (100.0)	0	0	0	0	1 (100.0)	0	0	0
	Swab of eggs in incubator[c]	1	0	0	0	0	0	0	0	0	0
	Swab of hatcher walls[c]	2	0	0	0	0	0	0	0	0	0
	Hatcher fluff[c]	2	2 (100.0)	0	0	0	0	0	0	0	0
P value			0.1	0.993	0.591		0.993	0.043			
Subtotal		25	11 (44.0)	1 (4.0)	7 (28.0)	0	1 (4.0)	7 (28.0)	0	0	0
Farms	Cloacal swab	27	25 (92.6)	0	25 (92.6)	24 (88.9)	23 (85.2)	24 (88.9)	0	21 (77.8)	1 (3.7)
	Feed	1	1 (100.0)	0	1 (100.0)	1 (100.0)	1 (100.0)	0	0	1 (100.0)	0
	Water	5	2 (40.0)	0	2 (40.0)	0	0	2 (40.0)	0	0	0
	Drag swab	2	2 (100.0)	0	2 (100.0)	1 (50.0)	2 (100.0)	2 (100.0)	0	1 (50.0)	0
	Boot swabs	5	5 (100.0)	0	4 (80.0)	4 (80.0)	3 (60.0)	5 (100.0)	0	3 (60.0)	0
P value			0.02		0.044	0.001	0.002	0.01		0.017	0.974
Subtotal		40	35 (87.5)	0	34 (85.0)	30 (75.0)	29 (72.5)	33 (82.5)	0	26 (65.0)	1 (2.5)
P value			<0.001	0.203	<0.001	<0.001	<0.001	<0.001		<0.001	0.426
Total		65	46 (70.8)	1 (1.5)	41 (63.1)	30 (46.2)	30 (46.2)	40 (61.5)	0	26 (40.0)	1 (1.5)

[a] Resistance to one or more agents tested.
[b] AMC, amoxicillin–clavulanic acid; DO, doxycycline; CRO, ceftriaxone; CN, gentamicin; K, kanamycin; C, chloramphenicol; SXT, sulfamethoxazole-trimethoprim; CIP, ciprofloxacin.
[c] Pooled composite sample representative of individual hatcher, incubator, or pen.

to 85.0% (34 of 40 isolates) for doxycycline ($P < 0.001$). The frequency of resistance of *Salmonella* isolates to antimicrobial agents based on the type of sample varied significantly for doxycycline ($P = 0.044$), ceftriaxone ($P = 0.001$), gentamicin ($P = 0.002$), kanamycin ($P = 0.01$), and sulfamethoxazole-trimethoprim ($P = 0.017$).

Resistance of *Salmonella* isolates based on serotype. From the study conducted at the hatcheries, the frequencies of antimicrobial resistance among *Salmonella* Kentucky and *Salmonella* Westhampton isolates were 36.4 and 100%, respectively (Table 6). For the predominant *Salmonella* Kentucky, the overall frequency of resistance to antimicrobial agents was 36.4% (8 of 22 isolates), and the highest frequency of resistance was to doxycycline (36.4%).

For the *Salmonella* isolates from broiler farms, the overall frequencies of resistance to antimicrobial agents were 100.0, 50.0, and 100.0% for *Salmonella* serotypes Infantis, Albany, and Othmarschen, respectively. All (100.0%) isolates of *Salmonella* Infantis were resistant to doxycycline.

Resistance patterns of *Salmonella* isolates from hatcheries and broiler farms. Only 4.0% of the *Salmonella* isolates (1 of 25 isolates) from the hatcheries were multidrug resistant (MDR; i.e., resistant to antimicrobial agents from three or more classes). Among the 11 isolates from hatcheries, four antimicrobial resistance patterns were observed: DO alone (36.4% of isolates), DO-K (27.3%), K (27.3%), and AMC-DO-CN-K (9.1%). Among the 35 resistant isolates from broiler farms, 30 (85.7%) were MDR, and seven resistance patterns were found. Among the 35 resistant isolates, DO-CRO-CN-K-SXT was the predominant pattern (24 isolates, 68.6%), and the other patterns were DO-K (3 isolates, 8.6%), DO-CRO-CN-SXT (2 isolates, 5.7%), DO-CRO-K (2 isolates, 5.7%), DO-CRO-CN-K (2 isolates, 5.7%), DO-CN-K (1 isolate, 2.9%), and K (1 isolate, 2.9%).

DISCUSSION

Our study is part of a group of cross-sectional studies conducted at three levels of the broiler processing industry in Trinidad and Tobago that use the "farm to fork" approach. We previously reported on the prevalence and characteristics of *Salmonella* isolated from samples from retail outlets (supermarkets and cottage poultry processors "pluck shops") *(36, 37)* and processing plants *(38)* in this country. The present study was conducted to evaluate the occurrence of *Salmonella* and the risk factors and characteristics of *Salmonella* isolates recovered from imported fertile hatching eggs, hatcheries, and poultry farms in Trinidad and Tobago. All 45 eggshell and 45 egg content composite samples (comprising 450 pooled fertile hatching broiler eggs) that were collected at the airport were negative for *Salmonella*. This finding is important because imported eggs could introduce *Salmonella* serotypes into the country *(44)*. However, our data indicate that this risk is minimal or nonexistent. Our failure to isolate *Salmonella* from imported fertile hatching eggs agrees with findings for imported table eggs and hatching layer eggs in a previous

TABLE 6. *Frequency of antimicrobial resistance of* Salmonella *isolates based on serotype*

Source of samples	*Salmonella* serotype	No. of isolates tested	No. (%) of isolates resistant[a]	No. (%) isolates resistant to[b]:							
				AMC	DO	CRO	CN	K	C	SXT	CIP
Hatcheries	Kentucky	22	8 (36.4)	1 (4.5)	8 (36.4)	0	1 (4.5)	4 (18.2)	0	0	0
	Westhampton	2	2 (100.0)	0	0	0	0	2 (100.0)	0	0	0
P value			0.163	1	0.536		1	0.054			
Subtotal		24	10 (41.7)	1 (4.2)	8 (33.3)	0	1 (4.2)	6 (25.0)	0	0	0
Farms	Infantis	16	16 (100.0)	0	16 (100.0)	16 (100.0)	16 (100.0)	15 (93.8)	0	16 (100.0)	0
	Albany	4	2 (50.0)	0	2 (50.0)	1 (25.0)	0	2 (50.0)	0	0	1 (25.0)
	Othmarschen	1	1 (100.0)	0	1 (100.0)	0	1 (100.0)	1 (100.0)	0	0	0
P value			0.01		0.009	<0.001	<0.001	0.075		<0.001	0.107
Subtotal		21	19 (90.5)	0	19 (90.5)	17 (90.0)	17 (90.0)	18 (85.7)	0	16 (76.2)	1 (4.8)

[a] Resistance to one or more agents tested.
[b] AMC, amoxicillin–clavulanic acid; DO, doxycycline; CRO, ceftriaxone; CN, gentamicin; K, kanamycin; C, chloramphenicol; SXT, sulfamethoxazole-trimethoprim; CIP, ciprofloxacin.

study in Trinidad, Grenada, and St. Lucia *(8)*. However, to fully understand the risk posed by imported fertile hatching eggs as a vehicle to introduce *Salmonella* into the country, a longitudinal study with a larger sample size is needed. Fertile hatching broiler eggs from *Salmonella*-infected breeder flocks can serve as a source of the pathogen for hatched day-old chicks, the hatchery environment, and broiler flocks on farms *(39)*. *Salmonella* can move from the hatcheries to the farms and then to the processing plants, thus possibly entering the food chain directly through the consumption of broiler meat contaminated with *Salmonella* during broiler production, processing, and retailing *(45)*.

The finding that 6 (46.2%) of 13 sample types collected from the hatcheries were positive for *Salmonella,* even though all the imported fertile hatching eggs were negative for the pathogen, is of interest. This finding may be explained in part by factors such as the prior horizontal contamination of the hatchery environment *(28),* the low numbers of fertile hatching eggs sampled, and the fact that the hatching eggs sampled at the airport may not directly represent the eggs already in the incubators and hatchers sampled in the study. Another explanation for cross-contamination is that both hatcheries did not sanitize the eggs before incubation, which is a frequent practice used to reduce contamination *(60)*.

In our study, the hatchery practices and the findings at both integrated companies were similar, except for the mortality rates (0.01 versus 0.05%), production system (semiautomated versus manual), vaccination method (in ovo versus subcutaneous injection), and the frequency of isolation of *Salmonella* (3.4 versus 13.0%). Some of the practices common to both hatcheries included the lack of restriction of workers' movements, candle egg prehatcher placement, and removal of chicks via a machine, practices that have been implicated in *Salmonella* contamination *(60)*. Variable frequencies of isolation of *Salmonella* from hatcheries have been reported and attributed to the differences in the enforcement of regulations by the responsible agencies. In several studies, the isolation rate of *Salmonella* from hatcheries was 0.3% in The Netherlands *(57),* 4.3% in China *(52),* and 34.0% in Korea *(31)*.

The frequency of isolation of *Salmonella* was significantly higher in stillborn chicks (28.0%) than in the other 12 types of samples (3.8%) collected from the hatcheries. These findings indicate that vertical transmission of *Salmonella* to the fertile hatching eggs may have occurred based on the low overall frequency of horizontal contamination at the hatcheries. These findings suggest that *Salmonella* may be the cause of these stillbirths. A considerably higher frequency of isolation of *Salmonella* (34%) was reported for hatcheries in Korea *(31)*. Although in our study a low frequency of environmental *Salmonella* contamination was found at the two hatcheries, considerably higher frequencies of isolation of *Salmonella* from hatchery environments have been reported by others. *Salmonella* was isolated from eggshell fragments (71%), in the swabs of conveyor belts (80%), and in chick liners (74%) in the United States *(21)* and from hatcher interiors (75%), chick sorting and dispatch areas (100%), meconium (50%), ventilation outlets (50%), and waste areas (75%) in Korea *(39)*. Unlike in our study in which all of the meconium samples were negative for *Salmonella,* Byrd et al. *(15)* found that 12% of chick tray liner (meconium) samples were positive for *Salmonella*.

The potential etiologic role of *Salmonella* Kentucky in stillbirth chicks was evident; all seven (100.0%) *Salmonella*-positive stillbirth chicks yielded only this serotype, which also accounted for 83.3% of the isolates recovered from both hatcheries. *Salmonella* serotypes, including serotype Kentucky, have been responsible for stillbirth chicks in hatcheries, and *Salmonella* Kentucky has emerged as the most important serovar in poultry processing plants *(12)*. In our study, *Salmonella* Kentucky also was recovered from other environmental samples (swabs of the hatcher interior, swabs of eggs in the incubator, and hatcher fluff), albeit at lower frequencies, which is not surprising serotype Kentucky is one of the serotypes commonly associated with hatcheries and broilers *(15)*. Recent studies conducted at broiler processing plants *(38)* and retail outlets *(37)* in Trinidad and Tobago also detected *Salmonella* Kentucky, confirming the local circulation of this serotype. However, various predominant serotypes of *Salmonella* have been isolated from hatcheries in other countries, such as

serotypes Pullorum, Enteritidis, Indiana, and Thompson in China *(64)* and serotypes Enteritidis, Heidelberg, and Senftenberg in Korea *(39)*. The differences in the *Salmonella* serotypes isolated from hatcheries in various countries were expected because serotype dominance may be multifactorial, depending on the prevalent serotypes infecting breeder flocks and contaminating hatchery environments, management systems, and sanitary practices in the respective countries *(40)*. In our study, the farm prevalence of *Salmonella* was 33.3% (9 of 27 farms) compared with 36.7% in Algeria *(26),* 47.9% in Nigeria *(33),* and 27.3% in Ireland *(30)*. For all the samples from the 27 broiler farms assessed in our study, only 2.8% (24 of 866 samples) yielded *Salmonella,* in agreement with similar low frequencies reported on broiler farms in Spain (1.0%) *(42),* Algeria (1.7%) *(26),* Sweden (2.3%) *(62),* and Colombia (2.8%) *(18)*. However, considerably higher overall prevalences of *Salmonella* have been reported elsewhere, such as 15.9% *(33)* and 59.1% *(25)* in Nigeria, 19.8% in Ireland *(30),* and 26.6% in Bangladesh *(9)*.

In the present study, the prevalence of *Salmonella* in the cloacal swabs of broilers reared on conventional broiler farms was considered low at 2.2% (15 of 675 samples), but the possibility of cross-contamination of carcasses with this pathogen during processing cannot be ignored. In another cross-sectional study conducted in four processing plants where broilers from the 27 farms are slaughtered, the overall frequency of *Salmonella* in the cloacal swabs (preslaughter) was 2.2% (2 of 90 samples), and 44.4% of chilled whole carcasses (20 of 45 carcasses) were contaminated with *Salmonella (38)*. The 2.2% *Salmonella* prevalence in cloacal swabs in our study is lower than that reported for conventional farms by researchers of 5.9% in The Netherlands *(57),* 14.8% in Ethiopia *(1),* 38.8% in the United States *(11),* and 55.6% in Korea *(39)*.

Salmonella Infantis was the predominant serotype circulating on the 27 broiler farms sampled across Trinidad and Tobago; 75.0% of the 20 isolates recovered from *Salmonella*-positive samples that were serotypeable were identified as *Salmonella* Infantis, and this serotype was isolated from 80.0% of the five types of samples tested. These findings indicate that *Salmonella* Infantis may be important in the epidemiology of *Salmonella* infections on broiler farms in this country; it has also been isolated from table eggs *(4),* human samples *(16),* and chickens processed at broiler processing plants *(38)*. As in our study, *Salmonella* Infantis was the predominant *Salmonella* serotype isolated from broiler farms in South America *(50)* and Japan *(24)*. However, unlike our findings, other serotypes of *Salmonella* have been predominant on broiler farms in other countries: Enteritidis in Brazil *(27),* Indiana in Zimbabwe *(46),* and Typhimurium in Bangladesh *(9)*.

In this study, among the environmental samples with the potential to be vehicles for the transmission of *Salmonella* and other pathogens on the farm, the boots of workers (11.1%) pose the highest risk, followed by water in drinkers (5.6%), the litter (based on drag swab samples) (3.5%), and feed (1.9%). A slightly lower frequency of isolation (5.9%) was reported for plastic boot covers and fresh droppings in Dutch broiler flocks *(57)*. The need to use disposable boot covers with regular changes between pens cannot be overemphasized. Regarding the contamination of broiler drinking water (storage tanks or drinkers in pens), a considerably lower frequency of *Salmonella* contamination was reported by Bailey et al. *(14),* who found that contamination was affected by several factors such as the type of drinker (trough, plastic bell, or nipple), the method for disposal of dead birds, and the infestation of farms by pests such as rats and wild birds *(34, 51)*.

The frequency of isolation of *Salmonella* (3.5%) in litter drag swab samples in the present study is lower than the 26.7% reported for these samples on layer farms in Trinidad and Tobago *(8)*. In contrast to our findings on broiler farms, in Korea *(39)* 30% of drag swab samples were positive for *Salmonella*. Multiuse litter, which is used on broiler farms in Trinidad and Tobago, where dirty or "caked" litter is removed and fresh litter is added over older litter and treated before placement of chicks, can increase pathogen contamination of the litter *(59)*.

In our study, only 1.9% of the feed samples collected from the 27 farms were contaminated with *Salmonella,* a finding consistent with the low frequency (2.8%) reported for feed samples collected from 23 layer farms in Trinidad and Tobago *(8)*. However, possible contamination of the feed by pests cannot be ignored; in the present study, 14 (51.9%) of the 27 farms sampled reported pest problems, including infestation by rats (60%), mongooses (14.3%), and wild birds (75%), which are potential carriers or shedders of *Salmonella*. These pests can play a role in the transmission of *Salmonella* on poultry farms *(56)*.

Overall frequency of resistance to antimicrobial agents among hatchery *Salmonella* isolates was moderate at 44.0% of isolates, and resistance to only four of the eight agents was found, ranging from 4.0 to 28.0% of isolates. These findings may reflect the exposure of *Salmonella* isolates to these antimicrobial agents, particularly to kanamycin and doxycycline, on the breeder farms from where the hatching eggs originated. The possibility of development of resistance by *Salmonella* isolates recovered from environmental hatchery sources resulting from the overuse of antimicrobial agents in the local poultry industry cannot be ignored. Some reports on the frequency of resistance to antimicrobial agents are not in accordance with our findings *(32, 52)*. A low frequency of antimicrobial resistance (<10%) to ceftriaxone, gentamicin, kanamycin, chloramphenicol, ciprofloxacin, and sulfamethoxazole-trimethoprim was found among *Salmonella* isolates recovered from hatcheries in China *(52)* in agreement with the 0 to 28.0% prevalence of resistance among the *Salmonella* isolates in the present study. In our study all *Salmonella* isolates were susceptible to ciprofloxacin. Shang et al. *(54)* also reported no resistance to ciprofloxacin, gentamicin, and amoxicillin–clavulanic acid among *Salmonella* isolates from hatcheries in Korea. In contrast, these authors detected a high frequency (52.8%) of MDR isolates compared with only 4.0% in our study. These differences in antimicrobial resistance and in the prevalence of MDR isolates may reflect the types and spectra of antimicrobial agents used in the poultry industry in various countries.

The frequency of resistance to antimicrobial agents among *Salmonella* isolates from farms (87.5%) was significantly higher than that among hatchery isolates (44.0%), as was the prevalence of MDR isolates (85.7 and 4.0%, respectively). These findings can be explained in part by the fact that broilers on the farms are also exposed to antimicrobial agents used for prophylaxis, therapy, and growth promotion, as revealed by the responses to the questionnaire administered. In Trinidad and Tobago, regulations on the use of antimicrobial agents in the livestock industry exist but are not enforced routinely.

The frequency of resistance to antimicrobial agents among *Salmonella* isolates from broiler farms (87.5%) is similar to the 100% resistance detected in China *(66),* 100% in Ethiopia *(1),* and 100% in Colombia *(23),* but a lower frequency of resistance (73.1%) was documented on broiler farms in Algeria *(26)*. The high frequencies of resistance (77.8 to 92.6%) to five antimicrobial agents (doxycycline, ceftriaxone, gentamicin, kanamycin, and sulfamethoxazole-trimethoprim) exhibited by *Salmonella* isolates recovered from the cloacal swabs of individual broilers could therefore have therapeutic implications because tetracycline and trimethoprim-sulfa are readily available and used locally in the poultry industry. The need for prudent use and regulation of antimicrobial agents on broiler farms cannot be overemphasized *(2)*. Resistance to these six antimicrobial agents also has been reported among *Salmonella* isolates recovered from chickens at retail outlets *(36),* from layer farms *(7),* and from table eggs *(7)* in Trinidad and Tobago.

Among *Salmonella* Kentucky isolates, the predominant serotype recovered from the hatchery samples, resistance to antimicrobial agents in our study was moderate, particularly to doxycycline (36.4%) and kanamycin (36.4%). However, antimicrobial resistant isolates of this serotype may be transferred to broiler farms. Although the prevalence of MDR *Salmonella* Kentucky isolates was low (4.0%) in our study, this serotype has been identified as a potential emerging MDR human pathogen in Europe, North Africa, and Asia based on the increase in the number of cases over the last decade for which poultry was identified as the source of infection *(43, 61)*.

All isolates (100.0%) of *Salmonella* Infantis were resistant to doxycycline, ceftriaxone, gentamicin, and sulfamethoxazole-trimethoprim, which has potential therapeutic significance may compromise therapeutic interventions. Resistant *Salmonella* serotypes have been recovered from poultry products *(3, 41),* and the broad antimicrobial resistance patterns for some strains of *Salmonella* Infantis are cause for concern *(35)*.

A limitation of this study is that only two of the four functional hatcheries in the country agreed to participate, thereby reducing the number of samples available for the study.

In conclusion, *Salmonella* Kentucky was isolated at a much higher frequency from stillborn chicks than from environmental samples, indicative of vertical transmission of *Salmonella* at the breeder farms from where the hatched eggs originated. The failure to isolate *Salmonella* from the imported fertile hatching eggs may be partly attributed to the small sample size and the fact that the study was cross-sectional rather than longitudinal, which may limit the inferences that can be drawn from our findings. Future investigations should include larger sample sizes and a longitudinal design. The low frequency of isolation of *Salmonella* from the cloacal swabs of broilers does not reduce the risk to food safety from slaughtered birds because *Salmonella*-positive broilers may cross-contaminate multiple other carcasses during processing. The different *Salmonella* serotypes predominant at the hatcheries (Kentucky) and at the broiler farms (Infantis) supplied with chicks from the hatcheries is an indication that other sources of contamination of farm environments may be important; for example, 51.9% of the farms had pest problems (rats and wild birds). To reduce or prevent the on-farm spread of *Salmonella* within and across pens, interventions such as mandatory use of disposable boots by workers may be effective. The high prevalence of resistance (85.7%) to commonly used antimicrobial agents (sulfamethoxazole-trimethoprim, gentamicin, ceftriaxone, kanamycin, and doxycycline) detected among the *Salmonella* isolates recovered from the farm samples may pose therapeutic and food safety risks. Therefore, existing regulations regarding access to and use of antimicrobial agents in Trinidad and Tobago must be enforced through institution of a surveillance system.

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SUPPLEMENTAL MATERIAL

Supplemental material associated with this article can be found online at: https://doi.org/10.4315/JFP-21-236.s1; https://doi.org/10.4315/JFP-21-236.s2

REFERENCES

1. Abdi, R. D., F. Mengstie, A. F. Beyi, T. Beyene, H. Waktole, B. Mammo, D. Ayana, and F. Abunna. 2017. Determination of the sources and antimicrobial resistance patterns of *Salmonella* isolated from the poultry industry in southern Ethiopia. *BMC Infect. Dis.* 17:352. https://doi.org/10.1186/s12879-017-2437-2
2. Adam, C. J. M., N. Fortane, C. Ducrot, and M. C. Paul. 2020. Transition pathways toward the prudent use of antimicrobials: the case of free-range broiler farmers in France. *Front. Vet. Sci.* 7. https://doi.org/ https://doi.org/10.3389/fvets.2020.548483
3. Adesiyun, A., N. Offiah, N. Seepersadsingh, S. Rodrigo, V. Lashley, and L. Musai. 2007. Antimicrobial resistance of *Salmonella* spp. and *Escherichia coli* isolated from table eggs. *Food Control* 18:306–311. https://doi.org/10.1016/j.foodcont.2005.10.013
4. Adesiyun, A., N. Offiah, N. Seepersadsingh, S. Rodrigo, V. Lashley, L. Musai, and K. Georges. 2005. Microbial health risk posed by table eggs in Trinidad. *Epidemiol. Infect*. 133:1049–1056. https://doi.org/10.1017/S0950268805004565
5. Adesiyun, A. A., C. Nkuna, M. Mokgoatlheng-Mamogobo, K. Malepe, and L. Simanda. 2020. Food safety risk posed to consumers of table eggs from layer farms in Gauteng Province, South Africa: prevalence of *Salmonella* species and *Escherichia coli,* antimicrobial

residues, and antimicrobial resistant bacteria. *J. Food Saf.* 40:e12783. https://doi.org/10.1111/jfs.12783
6. Adesiyun, A. A., A. Stewart-Jo, and S. Rodrigo. 2019. Questionnaire survey on salmonellosis and the egg industry in the English-speaking Caribbean. *Int. J. Poult. Sci*. 18:334–339. https://doi.org/10.3923/ijps.2019.334.339
7. Adesiyun, A. A., L. Webb, L. Musai, B. Louison, G. Joseph, A. Stewart-Johnson, S. Samlal, and S. Rodrigo. 2014. Resistance to antimicrobial agents among *Salmonella* isolates recovered from layer farms and eggs in the Caribbean region. *J. Food Prot*. 77:2153–2160. https://doi.org/10.4315/0362-028X.JFP-14-162
8. Adesiyun, A. A., L. Webb, L. Musai, B. Louison, G. Joseph, A. Stewart-Johnson, S. Samlal, and S. Rodrigo. 2014. Survey of *Salmonella* contamination in chicken layer farms in three Caribbean countries. *J. Food Prot*. 77:1471–1480. https://doi.org/10.4315/0362-028X.JFP-14-021
9. Akond, M. A., M. Shirin, S. Alam, S. Hassan, M. M. Rahman, and M. Hoq. 2012. Frequency of drug resistant *Salmonella* spp. isolated from poultry samples in Bangladesh. *Stamford J. Microbiol*. 2:15–19. https://doi.org/10.3329/sjm.v2i1.15207
10. Alali, W. Q., and C. L. Hofacre. 2016. Preharvest food safety in broiler chicken production. *Microbiol. Spectr.* 4. https://doi.org/10.1128/microbiolspec.PFS-0002-2014
11. Alali, W. Q., S. Thakur, R. D. Berghaus, M. P. Martin, and W. A. Gebreyes. 2010. Prevalence and distribution of *Salmonella* in organic and conventional broiler poultry farms. *Foodborne Pathog. Dis*. 7:1363–1371. https://doi.org/10.1089/fpd.2010.0566
12. Ammar, A. M., E. E. Abdeen, U. H. Abo-Shama, E. Fekry, and E. Kotb Elmahallawy. 2019. Molecular characterization of virulence and antibiotic resistance genes among *Salmonella* serovars isolated from broilers in Egypt. *Lett. Appl. Microbiol*. 68:188–195. https://doi.org/10.1111/lam.13106
13. Bailey, J. S., N. A. Cox, S. E. Craven, and D. E. Cosby. 2002. Serotype tracking of *Salmonella* through integrated broiler chicken operations. *J. Food Prot*. 65:742–745. https://doi.org/10.4315/0362-028x-65.5.742
14. Bailey, J. S., N. J. Stern, P. Fedorka-Cray, S. E. Craven, N. A. Cox, D. E. Cosby, S. Ladely, and M. T. Musgrove. 2001. Sources and movement of *Salmonella* through integrated poultry operations: a multistate epidemiological investigation. *J. Food Prot*. 64:1690–1697. https://doi.org/10.4315/0362-028x-64.11.1690
15. Byrd, J. A., J. R. DeLoach, D. E. Corrier, D. J. Nisbet, and L. H. Stanker. 1999. Evaluation of *Salmonella* serotype distributions from commercial broiler hatcheries and grower houses. *Avian Dis*. 43:39–47.
16. Caribbean Public Health Agency. 2013. State of public health in the Caribbean region, inaugural report. Caribbean Public Health Agency, Port of Spain, Trinidad and Tobago.
17. Cason, J. A., N. A. Cox, and J. S. Bailey. 1994. Transmission of *Salmonella* Typhimurium during hatching of broiler chicks. *Avian Dis*. 38:583–588.
18. Castro-Vargas, R., L. Fandiño-de-Rubio, A. Vega, and I. Rondón-Barragán. 2019. Phenotypic and genotypic resistance of *Salmonella* Heidelberg isolated from one of the largest poultry production region from Colombia. *Int. J. Poult. Sci*. 18:610–617.
19. Clinical and Laboratory Standards Institute. 2017. Performance standards for antimicrobial susceptibility testing. Clinical and Laboratory Standards Institute, Wayne, PA.
20. Cosby, D. E., N. A. Cox, M. A. Harrison, J. L. Wilson, R. J. Buhr, and P. J. Fedorka-Cray. 2015. *Salmonella* and antimicrobial resistance in broilers: a review. *J. Appl. Poult. Res*. 24:408–426. https://doi.org/10.3382/japr/pfv038
21. Cox, N. A., J. S. Bailey, J. M. Mauldin, and L. C. Blankenship. 1990. Research note: presence and impact of *Salmonella* contamination in commercial broiler hatcheries. *Poult. Sci. J*. 69:1606–1609. https://doi.org/10.3382/ps.0691606
22. Cox, N. A., J. S. Bailey, J. M. Mauldin, L. C. Blankenship, and J. L. Wilson. 1991. Research note: extent of salmonellae contamination in breeder hatcheries. *Poult. Sci. J*. 70:416–418. https://doi.org/10.3382/ps.0700416
23. Donado-Godoy, P., I. Gardner, B. A. Byrne, M. Leon, E. Perez-Gutierrez, M. V. Ovalle, M. A. Tafur, and W. Miller. 2012. Prevalence, risk factors, and antimicrobial resistance profiles of *Salmonella* from commercial broiler farms in two important poultry-producing regions of Colombia. *J. Food Prot*. 75:874–883. https://doi.org/10.4315/0362-028X.JFP-11-458
24. Duc, V. M., Y. Nakamoto, A. Fujiwara, H. Toyofuku, T. Obi, and T. Chuma. 2019. Prevalence of *Salmonella* in broiler chickens in Kagoshima, Japan in 2009 to 2012 and the relationship between serovars changing and antimicrobial resistance. *BMC Vet. Res.* 15:108. https://doi.org/10.1186/s12917-019-1836-6
25. Ejeh, F. E., F. A. Lawan, H. Abdulsalam, P. H. Mamman, and C. N. Kwanashie. 2017. Multiple antimicrobial resistance of *Escherichia coli* and *Salmonella* species isolated from broilers and local chickens retailed along the roadside in Zaria, Nigeria. *Sokoto J. Vet. Sci.* 15:45. https://doi.org/10.4314/sokjvs.v15i3.7
26. Elgroud, R., F. Zerdoumi, M. Benazzouz, C. Bouzitouna-Bentchouala, S. A. Granier, S. Frémy, A. Brisabois, B. Dufour, and Y. Millemann. 2009. Characteristics of *Salmonella* contamination of broilers and slaughterhouses in the region of Constantine (Algeria). *Zoonoses Public Health* 56:84–93. https://doi.org/10.1111/j.1863-2378.2008.01164.x
27. Fitch, F. M., M. S. Carmo-Rodrigues, V. G. Oliveira, M. V. Gaspari, A. Dos Santos, J. B. de Freitas, and A. C. Pignatari. 2016. β-Lactam resistance genes: characterization, epidemiology, and first detection of $bla_{\text{CTX-M-1}}$ and $bla_{\text{CTX-M-14}}$ in *Salmonella* spp. isolated from poultry in Brazil—Brazil Ministry of Agriculture's pathogen reduction program. *Microb. Drug Resist*. 22:164–171. https://doi.org/10.1089/mdr.2015.0143
28. Girh, Z. M. S., N. S. Rabie, and M. S. Zaki. 2020. Effect of *Salmonella* on hatchability and fertility. *Stem Cell* 11:17–22. https://doi.org/10.7537/marsscj110320.03
29. Grimont, P. A. D., and F.-X. Weill. 2007. Antigenic formulae of the *Salmonella* serovars. World Health Organization and Institut Pasteur, Paris.
30. Gutierrez, M., J. Fanning, A. Murphy, G. Murray, M. Griffin, A. Flack, N. Leonard, and J. Egan. 2009. *Salmonella* in broiler flocks in the republic of Ireland. *Foodborne Pathog. Dis*. 6:111–120. https://doi.org/10.1089/fpd.2008.0163
31. Ha, J. S., K. W. Seo, Y. B. Kim, M. S. Kang, C. S. Song, and Y. J. Lee. 2018. Prevalence and characterization of *Salmonella* in two integrated broiler operations in Korea. *Irish Vet. J.* 71:3. https://doi.org/10.1186/s13620-018-0114-4
32. Habing, G. G., S. E. Kessler, D. F. Mollenkopf, T. E. Wittum, T. C. Anderson, C. Barton Behravesh, L. A. Joseph, and M. M. Erdman. 2015. Distribution and diversity of *Salmonella* strains in shipments of hatchling poultry, United States, 2013. *Zoonoses Public Health* 62:375–380. https://doi.org/10.1111/zph.12157
33. Jibril, A. H., I. N. Okeke, A. Dalsgaard, E. Kudirkiene, O. C. Akinlabi, M. B. Bello, and J. E. Olsen. 2020. Prevalence and risk factors of *Salmonella* in commercial poultry farms in Nigeria. *PLoS One* 15:e0238190. https://doi.org/10.1371/journal.pone.0238190
34. Juricova, H., P. Videnska, M. Lukac, M. Faldynova, V. Babak, H. Havlickova, F. Sisak, and I. Rychlik. 2013. Influence of *Salmonella enterica* serovar Enteritidis infection on the development of the cecum microbiota in newly hatched chicks. *Appl. Environ. Microbiol*. 79:745–747. https://doi.org/10.1128/AEM.02628-12
35. Kehrenberg, C., S. Friederichs, A. de Jong, G. B. Michael, and S. Schwarz. 2006. Identification of the plasmid-borne quinolone resistance gene *qnrS* in *Salmonella enterica* serovar Infantis. *J. Antimicrob. Chemother*. 58:18–22. https://doi.org/10.1093/jac/dkl213
36. Khan, A. S., K. Georges, S. Rahaman, W. Abdela, and A. A. Adesiyun. 2018. Antimicrobial resistance of *Salmonella* isolates recovered from chickens sold at retail outlets in Trinidad. *J. Food Prot*. 81:1880–1889. https://doi.org/10.4315/0362-028X.JFP-18-223
37. Khan, A. S., K. Georges, S. Rahaman, W. Abdela, and A. A. Adesiyun. 2018. Prevalence and serotypes of *Salmonella* spp. on chickens sold at retail outlets in Trinidad. *PLoS One* 13:e0202108. https://doi.org/10.1371/journal.pone.0202108

38. Khan, A. S., K. Georges, S. Rahaman, W. Abebe, and A. A. Adesiyun. 2021. Characterization of *Salmonella* isolates recovered from stages of the processing lines at four broiler processing plants in Trinidad and Tobago. *Microorganisms* 9:1048. https://doi.org/10.3390/microorganisms9051048
39. Kim, A., Y. J. Lee, M. S. Kang, S. I. Kwag, and J. K. Cho. 2007. Dissemination and tracking of *Salmonella* spp. in integrated broiler operation. *J. Vet. Sci*. 8:155–161. https://doi.org/10.4142/jvs.2007.8.2.155
40. Kim, J. H., and K. S. Kim. 2010. Hatchery hygiene evaluation by microbiological examination of hatchery samples. *Poult. Sci*. 89:1389–1398. https://doi.org/10.3382/ps.2010-00661
41. Kumar, N., K. Mohan, K. Georges, F. Dziva, and A. A. Adesiyun. 2019. Prevalence, serovars, and antimicrobial resistance of *Salmonella* in cecal samples of chickens slaughtered in pluck shops in Trinidad. *J. Food Prot*. 82:1560–1567. https://doi.org/10.4315/0362-028X.JFP-18-553
42. Lamas, A., I. C. Fernandez-No, J. M. Miranda, B. Vazquez, A. Cepeda, and C. M. Franco. 2016. Prevalence, molecular characterization and antimicrobial resistance of *Salmonella* serovars isolated from northwestern Spanish broiler flocks (2011–2015). *Poult. Sci. J*. 95:2097–2105. https://doi.org/10.3382/ps/pew150
43. Le Hello, S., A. Bekhit, S. A. Granier, H. Barua, J. Beutlich, M. Zajac, S. Munch, V. Sintchenko, B. Bouchrif, K. Fashae, J. L. Pinsard, L. Sontag, L. Fabre, M. Garnier, V. Guibert, P. Howard, R. S. Hendriksen, J. P. Christensen, P. K. Biswas, A. Cloeckaert, W. Rabsch, D. Wasyl, B. Doublet, and F. X. Weill. 2013. The global establishment of a highly-fluoroquinolone resistant *Salmonella enterica* serotype Kentucky ST198 strain. *Front. Microbiol.* 4:395. https://doi.org/10.3389/fmicb.2013.00395
44. Li, S., Y. He, D. A. Mann, and X. Deng. 2021. Global spread of *Salmonella* Enteritidis via centralized sourcing and international trade of poultry breeding stocks. *Nat. Commun.* 12:5109. https://doi.org/10.1038/s41467-021-25319-7
45. Li, W. W., L. Bai, X. L. Zhang, X. J. Xu, Z. Tang, Z. W. Bi, and Y. C. Guo. 2018. Prevalence and antimicrobial susceptibility of *Salmonella* isolated from broiler whole production process in four provinces of China. *Zhonghua Yu Fang Yi Xue Za Zhi* 52:352–357. https://doi.org/10.3760/cma.j.issn.0253-9624.2018.04.005
46. Makaya, P. V., G. Matope, and D. M. Pfukenyi. 2012. Distribution of *Salmonella* serovars and antimicrobial susceptibility of *Salmonella* Enteritidis from poultry in Zimbabwe. *Avian Pathol*. 41:221–226. https://doi.org/10.1080/03079457.2012.667558
47. Mshelbwala, F. M., N. D. Ibrahim, S. N. Saidu, A. A. Azeez, P. A. Akinduti, C. N. Kwanashie, A. K. Fakilahyel Kadiri, M. Muhammed, I. O. Fagbamila, and P. D. Luka. 2017. Motile *Salmonella* serotypes causing high mortality in poultry farms in three south-western states of Nigeria. *Vet. Rec. Open* 4:e000247. https://doi.org/10.1136/vetreco-2017-000247
48. Osman, K., M. Elhariri, Z. Amin, and A. Nayerah. 2014. Consequences to international trade of chicken hatchlings: *Salmonella enterica* and its public health implications. *Int. J. Adv. Res*. 2:45–63.
49. Pulido-Landínez, M. 2019. Food safety—*Salmonella* update in broilers. *Anim. Feed Sci. Technol*. 250:53–58. https://doi.org/10.1016/j.anifeedsci.2019.01.008
50. Pulido-Landínez, M., A. Banda, and J. Guard. 2015. *Salmonella* emerging serotypes for poultry industry: *Salmonella* Infantis identified in a broiler vertical integration. Presented at the Southern Conference on Avian Diseases (SCAD), International Production and Processing Expo, Atlanta, GA, 27 to 29 January 2015.
51. Raufu, I., V. Bortolaia, C. A. Svendsen, J. A. Ameh, A. G. Ambali, F. M. Aarestrup, and R. S. Hendriksen. 2013. The first attempt of an active integrated laboratory-based *Salmonella* surveillance programme in the north-eastern region of Nigeria. *J. Appl. Microbiol*. 115:1059–1067. https://doi.org/10.1111/jam.12304
52. Ren, X., M. Li, C. Xu, K. Cui, Z. Feng, Y. Fu, J. Zhang, and M. Liao. 2016. Prevalence and molecular characterization of *Salmonella enterica* isolates throughout an integrated broiler supply chain in China. *Epidemiol. Infect*. 144:2989–2999. https://doi.org/10.1017/S0950268816001515
53. Sanchez, S., C. L. Hofacre, M. D. Lee, J. J. Maurer, and M. P. Doyle. 2002. Animal sources of salmonellosis in humans. *J. Am. Vet. Med. Assoc*. 221:492–497. https://doi.org/10.2460/javma.2002.221.492
54. Shang, K., B. Wei, S.-Y. Cha, J.-F. Zhang, J.-Y. Park, Y.-J. Lee, H.-K. Jang, and M. Kang. 2021. The occurrence of antimicrobial-resistant *Salmonella enterica* in hatcheries and dissemination in an integrated broiler chicken operation in Korea. *Animals* 11:154. https://doi.org/10.3390/ani11010154
55. Thrusfield, M. 2007. Veterinary epidemiology. Blackwell, Oxford.
56. Trampel, D. W., T. G. Holder, and R. K. Gast. 2014. Integrated farm management to prevent *Salmonella* Enteritidis contamination of eggs. *J. Appl. Poult. Res*. 23:353–365. https://doi.org/10.3382/japr.2014-00944
57. Van Der Fels-Klerx, H. J., W. F. Jacobs-Reitsma, R. Van Brakel, H. Van Der Voet, and E. D. Van Asselt. 2008. Prevalence of *Salmonella* in the broiler supply chain in the Netherlands. *J. Food Prot*. 71:1974–1980. https://doi.org/10.4315/0362-028x-71.10.1974
58. Varmuzova, K., T. Kubasova, L. Davidova-Gerzova, F. Sisak, H. Havlickova, A. Sebkova, M. Faldynova, and I. Rychlik. 2016. Composition of gut microbiota influences resistance of newly hatched chickens to *Salmonella* Enteritidis infection. *Front. Microbiol.* 7. https://doi.org/10.3389/fmicb.2016.00957
59. Vicente-Salvador, J., S. E. Higgins, B. M. Hargis, and T. Guillermo. 2007. Effect of poultry guard litter amendment on horizontal transmission of *Salmonella* Enteritidis in broiler chicks. *Int. J. Poult. Sci.* 6. https://doi.org/10.3923/ijps.2007.314.317
60. Wales, A., and R. Davies. 2020. Review of hatchery transmission of bacteria with focus on *Salmonella,* chick pathogens and antimicrobial resistance. *Worlds Poult. Sci. J*. 76:517–536. https://doi.org/10.1080/00439339.2020.1789533
61. Wasyl, D., I. Kern-Zdanowicz, K. Domańska-Blicharz, M. Zając, and A. Hoszowski. 2015. High-level fluoroquinolone resistant *Salmonella enterica* serovar Kentucky ST198 epidemic clone with IncA/C conjugative plasmid carrying $bla_{\text{CTX-M-25}}$ gene. *Vet. Microbiol*. 175:85–91. https://doi.org/10.1016/j.vetmic.2014.10.014
62. Wierup, M., H. Wahlström, E. Lahti, H. Eriksson, D. S. Jansson, Å. Odelros, and L. Ernholm. 2017. Occurrence of *Salmonella* spp.: a comparison between indoor and outdoor housing of broilers and laying hens. *Acta Vet. Scand*. 59:13. https://doi.org/10.1186/s13028-017-0281-4
63. Wright, S. 2019. Poultry production in the Caribbean. Available at: https://www.inciner8.com/blog/animal-incineration/poultry/poultry-production-in-the-caribbean/. Accessed 21 February 2021.
64. Xu, Y., X. Zhou, Z. Jiang, Y. Qi, A. Ed-Dra, and M. Yue. 2020. Epidemiological investigation and antimicrobial resistance profiles of *Salmonella* isolated from breeder chicken hatcheries in Henan, China. *Front. Cell. Infect. Microbiol.* 10:497. https://doi.org/10.3389/fcimb.2020.00497
65. Yang, J., S. Gao, Y. Chang, M. Su, Y. Xie, and S. Sun. 2019. Occurrence and characterization of *Salmonella* isolated from large-scale breeder farms in Shandong Province, China. *Biomed. Res. Int.* 2019:8159567. https://doi.org/10.1155/2019/8159567
66. Zhao, X., M. Hu, Q. Zhang, C. Zhao, Y. Zhang, L. Li, J. Qi, Y. Luo, D. Zhou, and Y. Liu. 2020. Characterization of integrons and antimicrobial resistance in *Salmonella* from broilers in Shandong, China. *Poult. Sci. J*. 99:7046–7054. https://doi.org/10.1016/j.psj.2020.09.071