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Prevalence of *Salmonella* Organisms in Poultry and Poultry Environments in Jamaica

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Authors' contributions

This work was carried out in collaboration between all authors. Authors HA and MPS coordinated the study. All authors designed the study. Authors SC and AAJV did the laboratory work and managed the literature searches, wrote the protocol, and wrote the first draft of the manuscript. All authors managed the analyses of the study, and read and approved the final manuscript.

Research Article

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ABSTRACT

Aim: This study was undertaken to determine the prevalence of *Salmonella* spp contamination in the Jamaican poultry industry and its environments.
Materials and Methods: A total of 45 farms across 6 Jamaican parishes were selected for this study. A total of 6693 specimens from animals and the environment were investigated for the presence of *Salmonella* spp. All specimens were placed in an igloo with ice packs and transported to the laboratory for analysis. Bacteriological media obtained from Difco Laboratories Detroit MI U.S.A were used for the isolation and identification of *Salmonella* spp. *Salmonella* serological typing was performed to determine the *Salmonella* serovar by standard procedures.
Results: This study revealed a low prevalence of *Salmonella* contamination/infection in both small and large entities in the poultry industry in Jamaica. The overall prevalence was 1 % (79 positive out of 6693 specimens). However, a higher prevalence of *Salmonella* was observed in the case of those operations which practiced "organic" poultry farming. It was shown that two *Salmonella* serovars including Augustenborg and Kentucky, identified

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during the study, are newly reported serovars in Jamaica. The sources of *Salmonella* infection varied from poultry itself to other species, such as rodents, pigs and insects. Improper disposal of broken eggs, wet bedding and other fomites contributed to *Salmonella* contamination.

Conclusions: The results of the study indicate possibility of salmonellosis (zoonosis) in Jamaica, although the prevalence of *Salmonella* spp was low, and the need for improved quality of the food industry, animal care and human health to prevent salmonellosis.

Keywords: Salmonella; prevalence; Jamaica; poultry.

1. INTRODUCTION

Salmonella pandemic was first noted in the late 1980's and has been attributed to contaminated eggs. The proportion of *Salmonella* infections associated with this serovar (S. serovar Enteritidis), seems to have increased over time. In 1995, 36% of *Salmonellae* worldwide were S. serovar Enteritidis, compared to 65% in 2002 [1].

Control of *Salmonella* infection is difficult, because there are numerous potential sources of *Salmonella* contamination in integrated poultry operation, including chicks, feed, rodents, wild birds, insects, transportation, farm environment, and processing plant environments [2].

Since the major reservoir for human infection is poultry and livestock, reducing the number of *Salmonellae* harbored in these animals would significantly reduce human exposure [3]. In Denmark, for example, all animal feeds are treated to kill *Salmonellae* before distribution, resulting in a marked reduction in salmonellosis [4].

Early detection of *Salmonellae* is of utmost importance in the recognition and control of outbreaks of salmonellosis. Therefore an understanding of possible points of cross-contamination and how poultry flocks may become infected is paramount in establishing proper control measures and ultimately minimizing the spread of *Salmonella* infection to humans and other animal species.

Globally *Salmonella* spp is of great economic and health concern [5]. There is widespread anxiety from consumers and regulators of the poultry industry for regular screening of *Salmonella* spp. in poultry which will result in early detection and provide data on the distribution of the bacteria. Beal and collaborators [6] reported that control of *Salmonella* infection in chicken is an important public health issue. Virtually no data exist on the prevalence of *Salmonella* in the Jamaican poultry industry. This study was undertaken to determine the prevalence of *Salmonella* spp contamination in the Jamaican poultry industry and its environments. Consequently poultry farms and their environments, food processing plants and supermarkets were targeted as sites for sampling to investigate the most prevalent *Salmonella* spp. in the Jamaican poultry industry.

2. MATERIALS AND METHODS

2.1 Specimens

It was a cross-sectional study. Samples were collected once from the same farm. The abattoirs, supermarkets and farms selected for participation in the study were selected

randomly. There was not any association between them; the samples were statistically representative of the poultry industry and its environments. A total of 45 farms across 6 parishes were selected for this study. Where possible, 4 large and 4 small farms were sampled from each parish. Locally, large poultry farms range in size from 20,000 to 30,000 birds per farm. Small farms range from 5000 to 9,000 birds. Large poultry houses vary from the sophisticated tunnel ventilated houses which include automated fans, and a misting system for air movement and temperature control to the standard traditional mesh and zinc sheet structure. Small local poultry farms have the standard traditional mesh structure in addition to improvised material including lumber and fabric. The layout of farms varies and is influenced by the style of management.

A total of 6693 specimens including 6120 from animals and 573 from the environment were investigated for the presence of *Salmonellae.* Specimens were collected once weekly for eight months. Of the specimens collected 359 were from the poultry house and surrounding environment.

The local grocery outlets studied included supermarkets situated in the corporate areas of Kingston and St Andrew, in addition to local markets. Samples of various chicken parts and whole chicken were purchased and aseptically placed in sterile bags. All specimens were placed in an igloo with ice packs and transported to the laboratory for analysis.

Chicken carcasses and various chicken parts from the two main poultry houses were collected aseptically, once weekly. Specimens used in analysis were cloacal swabbing, chicken faeces, anatomical caeca, anatomical crop, anatomical gizzard, poultry feed, poultry drinking water, poultry carcass rinse, poultry litter (bedding), and eggs.

Environmental specimens used in analysis were: abandon poultry bedding, flies, larvae (maggot), poultry stagnant water, poultry soak away pit, sick birds, feaces specimens from goats, rats, cattles and pigs.

All bacteriological media were obtained from Difco Laboratories Detroit MI U.S.A 48232-7058. The isolation and identification of *Salmonella* was carried out using previously described procedures [7]. *Salmonella s*erological typing was performed to determine the *Salmonella* serovar by standard procedures [8].

2.2 Isolation of *Salmonella* from Specimens

The isolation of *Salmonella* was carried out using previously described procedures [12]. The exterior of the cloaca of the birds was first cleaned with sterilized moistened cotton balls prior to application of the moistened cotton tips of each swab applicator. The swabs and also samples of caeca and crops were immediately placed in sterile screw cap test tube containing 9 ml of pre-enrichment broth (buffered peptone water 1%).

At least 2.5 g of each type of specimen was dissolved in 250 ml pre-enrichment broth (buffered peptone water 1%). The inoculated pre-enrichment broth was incubated at 37ºC for 24 hours. Following this incubation the pre-enrichment broth was thoroughly mixed using a vortex mixer. A 1ml aliquot of buffered peptone water 1% was added to 9 ml of enrichment broth (Selenite broth, Selenite cystein broth, and Tetrathionate broth) and further incubated at 37ºC for 24 hours. After vortexing 0.15 ml and a 3 mm loopful of inoculum was used to inoculate differential plating media such as MacConkey agar, *Salmonella/Shigella* agar

selective media, Bismuth Sulphite and Brilliant green agar and incubated at 37ºC for 24-48 hours.

Following incubation the cultures were examined and non-lactose fermenting colonies (all non-lactose fermenters are not candidates for biochemical test) were selected and used to inoculate Kleiger iron agar and urea agar slants. After a further 24 hours incubation period at 37ºC colonies which gave the typical *Salmonella/Shigella* reaction were inoculated to the routine line of sugars and again incubated. Confirmation was followed by slide agglutination with somatic "O" and flagella "H" antigens of *Salmonella*. Serological typing was performed to determine the *Salmonella* serovar [13].

2.3 Identification by Slide Agglutination

Presumption *Salmonella* isolates were stored on tryptose agar a room temperature until confirmation as previously described (Kauffman-White Schema, Difco, Laboratory, Detroit, and Michigan U.S.A) [3]. For each isolate each of 2 loopfuls of the growth on tryptose agar was emulsified in one drop of normal saline solution (0.9%) on a clean microscope slide. The preparation was examined for autoagglutination.

If the organism was not self agglutinating one drop of either "H" anti-serum or "O" anti-serum was added to each spot. After mixing the slide was agitated by gently rocking back and forth for 2 to 3 minutes. The slide was examined for agglutination. (Kauffman-White Schema, Difco, Laboratory, Detroit, and Michigan U.S.A). Identification of *Salmonella* Typhimurium serovar was performed in the *Salmonella* reference laboratory, Department of Microbiology, Faculty of Medical Sciences, The University of the West Indies.

2.4 Antibiotic Susceptibility Test

All *Salmonella* isolates (*Salmonella* Typhimurium) tested were investigated for their antibiotic resistance with the disc diffusion test using the following discs (Difco): gentamicin (10 µg), kanamycin (30 µg), ampicillin (10 µg), amikacin (30 µg), trimethoprim/sulfamethoxazole (1.25/23.75 µg), chloramphenicol (30 µg),cefazolin (30 µg), cephalothin (30 µg), cefepime (30 µg), cefotaxime (30 µg), streptomycin (10 µg), ceftazidime (30 µg), cefoxitin (30 µg), nalidixic acid (30 µg), ciprofloxacin (5 µg), norfloxacin (10 µg), tetracycline (30 µg) and imipenem (10 µg).

3. RESULTS AND DISCUSSION

The sources and occurrence of *Salmonellae* in the farm environment are as shown in Table 1. *Salmonella* spp. was isolated from environmental specimens including rat faeces (7/86, 8%) and muscoid flies (4/27, 15%). All other specimens tested negative. The prevalence of *Salmonellae* in faecal specimens from other animals on farms in close proximity to poultry farms is shown in Table 2. *Salmonellae* were isolated from 6% (5/79) of faecal specimens from pigs, but none of the samples from goat and cattle were positive. Table 3 shows the prevalence of *Salmonellae* in specimens from large abattoirs: *Salmonellae* were isolated from 9 out of 1200 caeca specimens, which represent a prevalence of 1%. However, they were not isolated from crops, gizzards, livers, and other specimens taken from large abattoirs. In positive specimens from the large poultry abattoirs the serovar isolated was *Salmonella* Montevideo.

Table 1. Sources and occurrence of *Salmonellae* in the farm environment in Jamaica

Source	Positive/n	Prevalence (%)
Soak away pit	0/4	0
Stagnant water	0/68	0
Foot bath	0/38	0
Abandoned chicken bed	0/76	0
Maggots	0/58	0
Muscoid flies	4/27	15
Rat faeces	7/8	86

Mucoid flies and rats in proximity to chicken houses could act as a vector for the transmission the Salmonella organisms. Four large farms and four small farms were tested. N represents # of sources.

Table 2. Prevalence of *Salmonellae* in farm animals in close proximity to poultry farms in Jamaica

Source	Positive/n	Prevalence (%)
Goat	0/96	0
Cattle	0/89	0
Pig	4/79	6

Fourty samples (15 large farm animals and 25 small farm animals) were tested. Approximately 2-3 sources per farm were tested. The 4 isolates of Salmonella in pigs were from the same small farm animal and from 4 different pigs.

Table 3. Prevalence of *Salmonellae* in specimens from large poultry abattoirs in Jamaica

Source	Positive/n	Prevalence (%)
Caeca	9/1200	0.75
Crop	0/1080	0
Gizzard	0/86	0
Liver	0/86	0
Line swab	0/42	0
Floor Swab	0/48	0
Prechilled Vat	0/39	0
Chilled Vat	0/39	0

Six large poultry abattoirs were sampled. The prevalence of caeca was 0.75 and came from the same abattoirs.

The Prevalence of *Salmonellae* from small poultry abattoirs is shown in Table 4. *Salmonellae* were isolated from 15% (13/86) organic caeca specimens, and 5% (6/120) of caeca, crop and gizzard specimens, respectively. The *Salmonella* serovars found in these specimens were *Salmonella* Austenborg and *Salmonella* Kentucky, which were being reported for the first time in the Jamaican poultry industry.

Table 4. Prevalence of *Salmonellae* in specimens from small poultry abattoirs in Jamaica

Source	Positive/n	Prevalence (%)
Organic caeca	13/86	15
Caeca	6/120	5
Crop	6/120	5
Gizzard	6/120	5

The organic caeca tested positive in 4 of 10 small poultry abattoirs, abattoir 1 (3 positive samples), abattoir 4 (1 positive sample), abattoir 5 (8 positive samples) and abattoir 9 (3 positive samples). Non-organic caeca, crop and gizzard tested positive (5%) in abattoir 5, where 6 out of 20 samples each tested positive.

The prevalence of *Salmonella*e in specimens from poultry farms is shown in Table 5. *Salmonella* was isolated from 1% (3/435) egg samples (meaning unbroken eggs were open and tested), 7% (23/328) of broken eggs (meaning broken egg parts) and 2% (2/98) of wet litter specimens. All specimens collected from market outlets including chicken parts (0/79, 0%) and carcass rinses (0/38, 0%) tested negative for *Salmonella.*

Table 5. Prevalence of *Salmonellae* in poultry farms in Jamaica

Source	Positive/n	Prevalence (%)
Water	0/242	0
Feed	0/285	0
Foot bath	0/38	0
Eggs	3/435	1
Broken eggs	23/328	7
Penn beds	0/104	0
Wet litter	2/98	2
Cloacal swab	0/1500	0

Forty-five poultry farms were sampled and all tested for the eight sources. Small farm 11 tested positive for 1 out of 10 broken eggs, and large farm 3 tested positive for 2 out of 10 eggs. Broken eggs sourced 6 farms, 5 small and 1 large: Small poultry farm 4, 5, 16, 21 and 23 tested positive for 3, 5, 1, 4, and 4 eggs respectively; large farm 2 tested positive for 5 broken eggs. Small farm 16 tested positive for two wet litters.

The overall prevalence was 1 % (79 positive out of 6693 specimens). This study revealed that the prevalence of *Salmonellae* in Jamaica's poultry and its environments is low. Most of the environmental specimens were negative for *Salmonellae* except for few specimens including faeces of pigs collected in proximity to the poultry farms. Measures should be put in place to contain possible propagation of *Salmonellae* from such sources to poultry, including an improvement of hygiene in pig farms. The prevalence of *Salmonellae* found in the environmental specimens and farm animals were lower than that reported from studies conducted by Korsak et al, 2004 [9]; that evaluated the performances of four detection methods for recovery of *Salmonella* spp. in naturally contaminated fecal specimens of porcine origin and showed that 47.8%, 34 of 71 specimens tested positive for *Salmonella* serovars. Seepersadsingh and Adesiyun in 2003 [10] studied the prevalence and antimicrobial resistance of *Salmonella* spp. in different animal species in Trinidad and reported the presence of *S*. Montevideo in one of two isolates recovered from reptiles. In a study conducted by Galland and collaborators in 2001 [11] *S.* Montevideo was found to be

the most frequently isolated serotype. They reported also that this serovar may contribute substantially to salmonellosis in dairy cattle in United States of America.

In this study the fact that although the overall prevalence was low across all samples, those samples from small abattoirs and organic birds had a substantially higher prevalence of *Salmonella*. Factors that may be contributing to this difference are poor hygiene at the facility, poor biosecurity and different sources such as broken eggs, wet litters, and proximity to pig farms among others. The public health impact of this difference is that a lot of Jamaican people consume poultry from small abattoirs and birds from small abattoirs that are marketed at the same types of grocery stores sampled. However a salmonellosis outbreak has not been reported in the last two decades.

In this research "organic" caeca (caeca from chicken which received no medication) sourced from a small poultry abattoir, had a 15% prevalence which represents the highest prevalence among abattoir specimens. Augustenborg serovar was isolated from organic caeca from the northern coastal region of the island; this serovar was sensitive to the panel of antibiotics. This is the first report of this serovar in Jamaican poultry. *S*. Augustenborg was isolated in Scotland for the first time in 2003. This strain was believed to have been acquired abroad [12].

The presence of *Salmonella* spp. in the crop and gizzard of slaughtered chickens was reported [13-14]. In this study the isolation of *Salmonella* spp was performed from samples of crops, gizzard and non-organic caeca from small poultry abattoirs. *Salmonella* Kentucky isolated from these samples collected in the Kingston and St. Andrew area is being reported in Jamaica for the first time and was sensitive to the panel of antibiotics. Weill, and collaborators [15] reported that *S*. Kentucky was frequently isolated from humans, other animals, or environmental sources in France, but It was assumed that this isolate must have been acquired abroad. These authors also noted that poultry products may be of particular interest because poultry is the main animal reservoir of *S*. Kentucky. A possible source of this serovar of *Salmonella* in East Africa was pork [15]. In the present study it was observed that the small local poultry abattoir from which *S*. Kentucky was isolated, also engaged in pig rearing.

The incidence of *Salmonella* observed in Jamaican poultry houses was low, ranging from 2% in sick birds and inanimate objects to 7% in broken eggs. As a result of the study poultry farmers were advised to be vigilant and to repair dripping water nipples and conveyer pipes to avoid poultry bed becoming wet. Similarly other studies have reported no association of *Salmonellae* with bedding regardless of the appearance [16]. It is also suggested that eggs should be removed from poultry houses as soon as possible to avoid breakage and minimize *Salmonella* contamination. The prevalence of *Salmonella spp* in specimens of water, feed and chicken's cloacal swabs collected in poultry farms was zero, suggesting that the *Salmonella's* surveillance programme in the Jamaican poultry industry has improved, with the implementation of recommended cleaning, sanitizing and hygienic practices at poultry houses.

In this study the prevalence of *Salmonella spp* from fly's specimens was 15%. The isolation of the pathogen from this type of environmental samples coincided with the presence of contaminated broken eggs. The pathogen was not isolated from fly's specimens collected in places with no broken eggs. It suggests that salmonellosis could be considered under certain conditions of transmissibility an arthropod-borne disease. A *Salmonella*-infected fly can serve as a vector in the food chain. It may carry the pathogen from a contaminated

broken egg to a non-contaminated broken egg. Some Jamaican farmers feed raw broken eggs to domestic and farm animals which could contribute to salmonellosis. This practice was reported by 40% (4 out of 10) of questioned farmers. Studies tracing infected broken eggs to their sources are needed to reduce the prevalence of this infection.

It is important to note that no *Salmonella* was isolated from poultry products obtained from marketing outlets. This provides some reassurance to the Jamaican public as poultry is a major food source in Jamaica. The two major ways of controlling food borne salmonellosis in humans include reducing *Salmonella* infection in the animal population by good animal husbandry and educating the public. The benefits of these achievable goals are evident in this study. Its results should guide the measures required to eliminate sources of infection.

4. CONCLUSION

The results of the study indicate possibility of salmonellosis (zoonosis) in Jamaica, although the prevalence of *Salmonella* spp was low and the need for improved quality of the food industry, animal care and human health to prevent salmonellosis.

CONSENT

No applicable.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee of the University of West Indies, Mona campus, Jamaica.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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