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Salmonella spp in Birds from Jamaica

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Authors' contributions

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

Original Research Article

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ABSTRACT

The present work was conducted to study the presence of *Salmonella* and its IgY antibodies in birds from Jamaica. The IgY fraction was isolated from the egg yolks of a variety of birds including chickens, quails, geese and pigeons by the chloroform-polyethylene glycol method. An enzyme-linked immunosorbent assay (ELISA) was used to test for anti-*Salmonella* antibodies. High significant levels of antibodies were detected in three serovars and *Salmonella* species, namely *Salmonella* Typhimurium, *Salmonella* Montevideo and *Salmonella* Yeerongpilly. This preliminary study from Jamaica demonstrates the presence of high levels of anti-*Salmonella* antibodies that warrants further studies with larger number of samples, since there are large amounts of consumption of eggs from these bird species by the general population in the country.

Keywords: Salmonella; chicken; birds; Jamaica; chloroform-polyethylene glycol method; enzyme-linked immunosorbent assay (ELISA).

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1. INTRODUCTION

The Poultry Industry is vulnerable to a wide range of food borne illnesses such as salmonellosis which is responsible for thousands of death globally and billions of loss revenue to the poultry industry. Salmonellae are gram-negative, non-spore-forming facultatively anaerobic bacilli [1] and this genus is a member of the Enterobacteriaceae family [2].

*S. enterica*serovarTyphimurium infects laying hens and may cause human infection when cracked eggs areconsumed. In contrast, *S. enterica*serovarEnteritidis (SE) contaminatesthe contents of intact eggs and is the major egg-associatedhuman pathogen. It is postulated that *S. enterica*serovarEnteritidis colonizes ovaries and oviducts of chickensand subsequently contaminates eggs as they form [3].

Three key interventions aimed at preventing the contamination and growth of SE in eggs have included farm-based programs to prevent SE from being introduced into egg-laying flocks, early and sustained refrigeration of shell eggs, and education of consumers and food workers about the risk of consuming raw or undercooked eggs. Since 1996, the incidence of SE infection in humans has decreased greatly, although many cases and outbreaks due to SE contaminated eggs continue to occur [4].

Control of *Salmonella* is difficult, because there are numerous potential sources of *Salmonella* contamination in an integrated poultry operation, including chicks, feed, rodents, wild birds, insects, transportation, farm environment, and processing plant environments [5]. It is difficult for farmers to identify chickens that have acquired recent infection with *Salmonella* Enteritidis and that are at risk for producing contaminated eggs [6].

The seroprevalence of anti-*Salmonella* antibodies has been carried out in different populations [7-10]. However in Jamaica and in the region is taken place this type of survey for first time. The present work was conducted to study the anti-*Salmonella* antibodies in egg yolk of laying hens, quails, geese, pigeons and backyard chickens, in addition to the presence of *Salmonella* presence in eggs from birds in Jamaica. These eggs are normally consumed by the population and they can be found in local markets or bird aviaries.

2. MATERIALS AND METHODS

2.1 Specimens

A total of 206 specimens including eggs from laying hens, quails, geese and pigeons were investigated for the presence of Salmonellae. The local grocery outlets studied included supermarkets situated in the corporate areas of Kingston and St Andrew, in addition to local markets and a bird aviary where eggs were collected. Samples of eggs purchased were aseptically placed in sterile bags. All specimens were placed in an igloo with ice packs and transported to the laboratory.

2.2 Cases and Controls

The cases were eggs from laying hens, quails, geese and pigeons. Backyard chickens were used as controls, since they are from an environment that commonly lack proper hygiene and are easily predisposed to *Salmonella* species contamination than other animals or birds.

2.3 Immunoglobulin (Ig) Y Isolation

The immunoglobulin IgY fraction was isolated from the egg yolks of a variety of birds including laying hens, quails, geese and pigeons. The IgY fraction was isolated by the chloroform-polyethylene glycol (PEG) (Sigma-Aldrich Co, St. Louis USA) method described by Polson [11]. In brief the eggs were washed with warm water and the egg yolk was separated and a 1:3 solution made in Phosphate buffered saline (PBS) pH 7.4. (Sigma-Aldrich Co, St. Louis USA). An equal volume of chloroform was added mixed thoroughly and centrifuged at (1,000 RPM) for 30 min at (RT). The supernatant was decanted and mixed with PEG 6000 (12%, w/v), stirred, and incubated (RT for 30 min) and again centrifuged. The precipitate containing IgY was dissolved in PBS at pH 7.4. A volume equivalent to 1/6 the volume of egg yolk was dialyzed against 1L PBS at pH 7.4 for 24 h at 4ºC. The IgY was stored at –20ºC until the analysis.

2.4 ELISA for *Salmonella* Antibody in Several Avian Species

The ELISA procedure described by Smith and collaborators was used to detect anti-*Salmonella* antibodies in the different avian species [10]. Ninety-six well polystyrene microplates (U-shaped bottom, Sigma-Aldrich co, St. Louis USA) were incubated with (2 µg/well) of the LPS from *S*. Typhymurium in coating buffer (overnight at 4ºC.) The microplates were washed 4 times, with (PBS-Tween-20) and blocked with 3% non-fat milk in PBS, (25 µl/well) and incubated 1 hour at room temperature (R.T). The microplates were washed 4 times, then a 50 µl aliquot of the previously isolated egg yolk (Ig)Y solutions in concentration of 1.25 mg/ml was added (triplicates). After incubating for one hour at RT the microplates were washed 4 times and 50 µl anti-IgY-HRP conjugate (Sigma-Aldrich Co) diluted to 1:30000 with PBS-Tween-20 added. After a further incubation and washing step, 50µl tetrametylbenzidine (TMB) was added. The microplates were further incubated for 15 minutes in the darkand 50µl 3M HCl was added to stop the reaction. The microplateswere read at 450 nm. Positivity was taken as a mean optical density value (XOD) equal or higer than 0.2.

2.5 *Salmonella* Isolation

At least 0.1 ml of each egg yolk specimen was dissolved in 250ml 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, Bismuth Sulphite and Brilliant green agar, and incubated at 37ºC for 24-48 hour. Egg shells and egg whites were analysed under sterile conditions for the presence of *Salmonella* before discarding. The egg shells or egg whites were swabbed and added to 250 ml of pre-enrichment broth at 37ºC for 24 hours, and followed the same procedure as the *Salmonella* isolation from egg yolk specimens reported above.

Following incubation the cultures were examined and non-lactose fermenting colonies 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].

2.6 Identification by Slide Agglutination

Presumption *Salmonella* isolates were stored on tryptose agar at 18ºC until confirmation as previously described (Kauffman-White Schema, Difco, Laboratory, Detroit, and Michigan U.S.A) [3]. For each isolate 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 all *Salmonella* including *S*. Typhimurium serovar was performed in the *Salmonella* reference laboratory, Department of Microbiology, Faculty of Medical Sciences, and The University of the West Indies.

2.7 Antibiotic Susceptibility Test

All *Salmonella* isolates 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).

2.8 Statistical Analysis

Statistical analyses were conducted using the statistical package for social sciences (SPSS) software (version 18). Differences between cases and controls were tested by the student t-test. A p value<0.05 was considered statistically significant.

3. RESULTS AND DISCUSSION

The presence of S. Typhimurium antibodies in the different birds are shown in Table 1. In this study the chloroform-Polyethylene glycol (PEG) technique was successful in isolating IgY from the egg yolk of different species of birds tested. Presence of S. Typhimurium antibodies were high among all the species, except for “pigeons”. S. Typhimurium antibodies were found in 100% of the chicken antibody samplesas shown in Table 1.

Table 1. Presence of *Salmonella* Typhimurium antibodies in egg yolk from different avian species tested by indirect ELISA

Animal eggs species	Positive (%)	p value*
Laying hen	100	p<0.001
Quail	84	p<0.001
Goose	98	p<0.001
Pigeon	33	P=0.3
Backyard chicken	27	

**Diferencies between cases and control were tested by the student t-test. A p value<0.05 was considered statistically significant.*

The isolates were obtained from 2 eggs from laying hens and were typed as *Salmonella* Typhimurium, and *Salmonella* Montevideo. Furthermore, *Salmonella* Typhimurium was isolated from the shell of a Backyard chicken´s eggs. Quail and goose eggs tested negative for *Salmonella* contamination. In total were isolated 3*Salmonella* strains. Seepersadsingh and Adesiyun [12] 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. They reported also that this serovar may contribute substantially to salmonellosis in dairy cattle in United States of America [12].

Akter et al, 2007[13] reported salmonellosis is a common problem in poultry farms of Bangladesh. The indiscriminate use of antibiotic to control the disease results in drug resistance. In the same study the antibiogram revealed that the isolates were sensitive to ciprofloxacin (80%), nitrofurantoin (100%), sulphamethoxazole/ trimeoprim and amoxycillin (50%), tetracycline (60%), but resistant to penicillin-G and erythromycin. In our study *Salmonella* Montevideo were sensitive to the entire panel of antibiotic tested using the commercial disc test (Difco), and the isolates of *Salmonella* Typhimurium were sensitive to gentamicin, kanamycin, tetracycline, amikacin, cefazolin, cephalothin, cefepime, cefotaxime, streptomycin, ceftazidime, cefoxitin, nalidixic acid, ciprofloxacin, norfloxacin, and imipenem. One isolate of S. Typhimurium was resistant to trimethoprim/sulfamethoxazole and chloramphenicol and the other one to ampicillin.

In Jamaica the prevalence of S. Typhimurium is not known and we wanted to know the exposure of birds to this bacterium that was the reason for studying presence of anti-*Salmonella* antibodies in birds. Infection with *Salmonella* complemented this work because it is the first report on the infection with *Salmonella* in the island in the last 3 decades.

3.1 Limitation of the Study

Despite the very low number of birds species assayed in this study, yet this preliminary report addresses a potential public environmental hygiene health problem that could occur if screening of birds and infection control measures and policies are not implemented in the country.

4. CONCLUSION

This preliminary study from Jamaica demonstrates the presence of high levels of anti-*Salmonella* antibodies that warrants further studies with larger number of samples, since there are large amounts of consumption of eggs from these bird species by the general population in the country.

CONSENT

No applicable.

ETHICAL APPROVAL

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

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COMPETING INTERESTS

The authors declared that no competing interests exist.

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