

THE EFFECTIVENESS OF MEDICATION IN THE PREVENTION OF *SALMONELLA* IN GROWING CHICKENS AND THE SEROPREVALENCE OF ANTI-*SALMONELLA* ANTIBODIES IN BIRDS.

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Authors' contributions

This work was carried out in collaboration between all authors. Author SC and AJV designed the study and did the laboratory work. SC and AJV managed the literature searches, wrote the protocol, and wrote the first draft of the manuscript. All authors managed the analyses of the study, performed the statistical analysis and read and approved the final manuscript.

ABSTRACT

AIMS: The aim of this study was to investigate the effectiveness of medication in the prevention of *Salmonella* in growing chickens. Also we intended to study the seroprevalence of anti-*Salmonella* antibody in several avian species.

MATERIALS AND METHODS: 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. An indirect enzyme-linked immunosorbent assay (ELISA) was used to study the seroprevalence of anti-*Salmonella* antibody in a bird population.

RESULTS: The effectiveness of some medications in the reduction of *Salmonella* infections in growing chicken was study. None of the groups including the control group showed any clinical signs of Salmonellosis. However, post-mortem specimens including caeca showed the presence of *Salmonella* in 1.6 % (1/60) each of chickens treated with Menorox®, and Neochlore®, compared to 23% (14/60) among the control group. In addition, the seroprevalence of anti-*Salmonella* antibodies in birds demonstrated probably the present of natural immunity to this bacteria with the ability to fight the wild types.

CONCLUSIONS: Medication and good hygiene play a role in reducing the risk of salmonellosis and other bacterial infections. The seroprevalence of Salmonellosis was higher than that expected in chickens.

Keywords: *Salmonella*, chicken, antibiotic, Jamaica

INTRODUCTION

The genus *Salmonella* is ubiquitous in the environment and is one of the most common causes of food borne infections worldwide [1]. A characteristic feature of this organism is its wide host range, which comprises most animal species including mammals, birds, and cold blooded animals [2]. The most frequently reported *Salmonella* serovar is *Salmonella* Typhimurium. Typhimurium is among the serovars most commonly associated with human Salmonellosis in most European countries and in the United States [3].

The prevalence of *Salmonella* infection in the United States is also relatively high. Estimated 1000 *Salmonella* related deaths and 4 million cases of food poisoning caused by *Salmonella* are reported each year. Salmonellosis accounts for 60% of all bacterial disease in the United States annually [4]. There are about 5000 cases of *Salmonella* infection in Canada annually [5]. In England and Wales there is an estimated 30% contamination of chicken carcasses by *Salmonella* microbe [6].

The aim of this study was to investigate the effectiveness of medication in the prevention of *Salmonella* in growing chickens. Also we intended to study the seroprevalence of anti-*Salmonella* antibody in several avian species by indirect enzyme-linked immunosorbent assay (ELISA).

MATERIALS AND METHODS

THE EFFECTIVENESS OF MEDICATION IN THE PREVENTION OF *SALMONELLA* INFECTION IN GROWING CHICKENS.

Four groups of 60, one-day old baby chickens were kept in a small poultry house made available for the experiment by an ex-poultry farmer.

Group 1 received Trisulvitrim, group 2 Menorox, group 3 Neochlore and group 4 did not receive medication. Medication was administered according to the manufacturer's instructions. Briefly, one tea spoon was added to a gallon of water and administered for 5 days in all three cases. Chicken were fed water from poultry nipple dispenser. Feed was administered to the groups by way of an automatic feeding system. The chickens were observed daily to minimize signs of stress and to ensure that there was no obstruction in the water and feed line. Wet bedding was

removed weekly or as the need arose. At the end of 7 weeks, mature chickens were slaughtered; the caeca and crop were removed and tested for *Salmonella* species.

ISOLATION OF *SALMONELLA* FROM SPECIMENS

The isolation of *Salmonella* was carried out using previously described procedures [7]. 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 were immediately placed in sterile screw cap test tube containing 9 ml of pre-enrichment broth the swab was completely submerged in the broth to ensure optimal recovery of the organism.

At least 25g of each type of specimen was dissolved in 250ml pre-enrichment broth. The inoculated pre-enrichment broth was incubated at 37°C for 24 hours following this incubation the pre-enrichment broth was thoroughly mixed using a vortex mixer. A 1ml aliquot of the Pre-enrichment broth was added to 9 ml of enrichment broth (Selenite broth, Selenite cystein broth, and Tetrathionate broth) and further incubated at 37°C for 24 hours. After vortexing 0.5 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 37°C for 24-48 hour.

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 37°C 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 [9].

IDENTIFICATION BY SLIDE AGGLUTINATION

Presumption *Salmonella* isolates were stored on tryptose agar at 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 on a clean microscope slide. The preparation was examined for autoagglutination.

If the organism was not self agglutinating one drop each of “H” antiserum to *Salmonella* and “O” anti-serum respectively 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).

IMMUNOGLOBULIN (Ig) Y ISOLATION

The immunoglobulin IgY fraction was isolated from the egg yolks of a variety of birds including chicken, quail, geese and pigeon. The IgY fraction was isolated by the chloroform-polyethylene glycol (PEG) (Sigma-Aldrich Co, St. Louis USA) method described by Polson [9]. 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 (1000 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 4°C. The IgY was stored at –20°C. Until required.

ELISA FOR STUDING THE SEROPREVALENCE OF ANTI-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]. Ninetysix 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 4°C.) 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 (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 diluted to 1:2000 with PBS-Tween-20. added. After a further incubation and washing step 50ul tetrametylbzidine (TMB) was added. The microplates were further incubated for 15 minutes in the dark.and 50ul 3M HCl was added to stop the reaction. The microplates were read at 450 nm. Positivity was taken as a mean optical density value (XOD) equal or higer than 0.2.

RESULTS

THE EFFECTIVENESS OF MEDICATION IN THE PREVENTION OF *SALMONELLA* INFECTION IN GROWING CHICKENS

The effectiveness of some medications in the reduction of *Salmonella* infections in growing chicken is shown in Table 1. None of the groups including the control group showed any clinical signs of Salmonellosis. However, post-mortem specimens including caeca showed the presence of *Salmonella* in 1.6 % (1/60) each of chickens treated with Menorox®, and Neochlore®, compared to 23 % (14/60) among the control group.

SEROPREVALENCE OF ANTI-SAMONELLA ANTIBODIES IN SEVERAL BIRDS

The seroprevalence of anti-S. Typhimurium antibodies in the different birds are shown in Table 2. In this study the chloroform-Polyethyleneglycol (PEG) technique was successful in isolating IgY from the egg yolk of different species of birds tested. The seroprevalence of anti-S. Typhimurium antibodies were high among all the species, except for pigeons. It was significant to note that anti-S. Typhimurium antibodies were found in 100% of the chicken antibody samples.

DISCUSSION

The current investigation was carried out to assess the prevalence of *Salmonella* in growing chicken in the Jamaican poultry industry and also, to detect potential sources of contamination in order to provide critical information and advice as a means of controlling the spread of this organism throughout this sector.

This study also represents the first report in Jamaica on the use of medication in the reduction of *Salmonella* infections in the poultry industry. The results of the clinical trial indicate that medications are effective in reducing the incidence of *Salmonella* infection in growing chickens. Although chicken were subjected to three different types of medication a low prevalence of infection was still encountered indicating that medication does not necessarily offer 100% protection. It is documented that the application of antimicrobial additive in the poultry industry minimizes *Salmonella* colonization in the gastrointestinal tract of chickens [11].

The results of this study revealed a low prevalence of *Salmonella* infection in birds treated with medications in small entities in the poultry industry in Jamaica. However, a higher prevalence of *Salmonella* was observed in the case of those operations which practiced “organic” poultry farming and it is important that consumers be made aware of the increase risk of infection with poultry products produced by the organic method.

Contaminated food continues to be a cause of major concern, causing 10 to 15% of acute gastroenteritis in humans. There is increased testing, surveillance and investigation by the United Kingdom and European Countries regulating authorities, food manufacturers and public health laboratories to minimize *Salmonella* food poisoning [4]. Early detection of *Salmonella* 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 [4].

In the early 1990's concerns have been raised because many strains of *Salmonella* have become resistant to several of the antibiotics that have traditionally been used to treat the infection in both animals and humans [12].

Antibiotic resistance has emerged as a result of widespread use of antibiotics and antimicrobials both prophylactically and therapeutically. The result is that the range of effective antibiotics that can be used on farms to control the spread of this pathogen is severely limited [13].

There has been little information as to whether food sourced from European Union countries or elsewhere is also becoming increasingly contaminated with *Salmonella* strains that are resistant to antibiotics [14]. However recently published statistics from the United States have shown a dramatic increase in the proportion of multiresistant isolates of all strains of *Salmonella* serovar Typhimurium from 7% in 1990 to 28% in 1995, with 83% of the latter being *Salmonella* serovar Typhimurium DT104 [15].

CONCLUSION

The results confirmed the efficacy of medication commonly used in the poultry industry and the importance of this practice in food safety. Medication and good hygiene play a role in reducing the risk of salmonellosis and other bacterial infections. The seroprevalence of *Salmonellosis* was higher than that expected in chickens.

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TABLES 1. Effectiveness of medication in the prevention of *Salmonella* infection in growing chicken.

Groups	n	Number tested	
		Positive	Prevalence (%)
Trisulvitrim	60	0	0
Menorox	60	1	1.6
Neochlore	60	1	1.6
Control	60	14	23

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293 Table 2. The seroprevalence of anti-*S. Typhimurium* antibodies, in different avian species, by
294 indirect ELISA.

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Animal species	Number tested	Positive	Sero-prevalence of anti- <i>S. Typhimurium</i> antibodies
Chicken	32	32	100%
Quail	64	54	84%
Goose	25	24	98%
Pigeon	3	1	33%

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297 The ELISA was performed according to a standard protocol for detecting Avian (Ig)Y.

298 Cut-off point = 2.0

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