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## The Effectiveness of Antibiotics in the Prevention of *Salmonella* Typhimurium in Growing Chickens

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### Authors' contributions

This work was carried out in collaboration between all authors. Authors SC and HA designed the study. Author SC did the laboratory work. Authors SC and AAJV 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.

Research Article

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### ABSTRACT

**Aims:** The aim of this study was to investigate the effectiveness of medication in the prevention of *Salmonella* in growing chickens.

**Material 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. Chickens were given a single dose of a wild-type multiresistant strain of *Salmonella* Typhimurium (resistant to many antibiotics including cloramphenicol and bactrim) by oral route ( $3 \times 10^9$  cfu/bird) as a challenge.

**Results:** The effectiveness of some medications in the reduction of *Salmonella* infections in growing chicken revealed that none of the groups including the control group showed

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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.

**Conclusions:** Antibiotics including Trisulvitrim, Menorox and Neochlore reduced significantly *Salmonella* Typhimurium infection in growing chickens. There was a very little information in the literature about the use of antibiotics to treat *Salmonella* infections in chickens that is one of the most prevalence zoonosis.

*Keywords: Salmonella; chicken; antibiotics; Jamaica.*

## 1. 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 Typhimurium. That is among serovars the 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]. Multi-drug resistant *Salmonella* strains have been consistently reported in The United States, where major outbreaks have happened [6-10].

In the prevention of salmonellosis in Poultry antibiotics are used, for example Norfloxacin is reported as an ingredient of Menorox in various countries including South Africa and is intended for the treatment of bacterial infections in veterinary medicine. Norfloxacin is a synthetic chemotherapeutic antibacterial agent. It is a first generation synthetic fluoroquinolone (quinolone) [11]. Trisulvitrim is a trade mark of a Canadian company (Vétoquinol N.A. Inc.) and is used in the prevention of bacterial infections. The company does not reveal the active component of this drug when we inquired due to patent protection [12]. Neochlore or Neomycin is an antibiotic that interferes with bacterial protein synthesis by binding primarily to the 30S subunit of bacterial ribosomes [13]. The current literature does not record the use of any of these antibiotics in chickens for prevention of *Salmonellae*. The aim of this study was to investigate the effectiveness of these antibiotics in the prevention of *Salmonella* Typhimurium in growing chickens.

## 2. MATERIALS AND METHODS

### 2.1 The Effectiveness of Medication in the Prevention of *Salmonella* Infection in Growing Chickens

Four groups of 60, day-old chicks, healthy and weighting 25 grams average in a parallel study design were kept in a small poultry house made available for the experiment by an ex-poultry farmer in Jamaica during the period of November-December 2011. Group 1 received Trisulvitrim, group 2 Menorox, group 3 Neochlore and group 4 did not receive medication. It 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 at week six coinciding with a single dose of a wild-type multiresistant strain of *Salmonella* Typhimurium (resistant to some antibiotics including chloramphenicol and trimethoprim/sulfamethoxazole, ampicillin and streptomycin by oral route ( $3 \times 10^9$  cfu/bird). Chickens 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.

## 2.2 Isolation of *Salmonella* from Specimens

The isolation of *Salmonella* was carried out using previously described procedures [14]. 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 2.5g of each type of specimen was dissolved in 250ml pre-enrichment broth (buffered peptone water 1%). 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 cysteine broth, and Tetrathionate broth) and further incubated at 37°C 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 37°C for 24-48 hours.

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 [15].

## 2.3 Identification by Slide Agglutination

Presumptive *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 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. Statistically, a P value <0.05 was considered significant in the comparison of each case with the control.

## 2.4 Antibiotic Susceptibility Test

All *Salmonella* isolates 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), ceftiofur (30 µg), nalidixic acid (30 µg), ciprofloxacin (5 µg), norfloxacin (10 µg), tetracycline (30 µg) and imipenem (10 µg).

## 2.5 Statistical Analysis

Statistical data were analyzed using the SPSS version 18. A P value <0.05 was considered significant.

## 3. RESULTS AND DISCUSSION

### 3.1 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 and crops 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.

**Table 1. Effectiveness of antibiotics in the prevention of *Salmonella* infection in growing chickens**

Antibiotic	Positive/n	Prevalence rates (%)	P value
Trisulvitrim	0/60	0	p<0.05
Menorox	1/60	1.6	p<0.05
Neochlore	1/60	1.6	p<0.05
Control	14/60	23.3	

*When comparing with the control group the three antibiotics used in chicks to treat the artificial infection with S. Typhimurium there with a highly statistically significant difference in all cases, p < 0.05. This proved that the three antibiotics used were effective to combat S. Typhimurium infection.*

*Salmonella* Enterica is recognized as one of the most common bacterial causes of food-borne diarrheal illness worldwide. *Salmonella* spp widely distributed in nature and food animals are the main reservoir for human infections. This manuscript describes the efficacy of drug's treatment to prevent chicken *Salmonella* Typhimurium infections. The authors compared the effectiveness of three agents (Trisulvitrim, Menorox and Neochlore) and found that the all were effective, reducing the prevalence of *Salmonella* infection in more than 90%.

Table 2 represents the organs where *Salmonella* Typhimurium were isolated from sick birds. There were 16 cases of salmonellosis out of 240 birds tested as shown in Table 1, which represents an overall prevalence of 6.67%. The prevalence rates of infection in caeca and crops were statistically significant (p<0.05) among sick birds (15 out of 16 caeca tested positive for *Salmonella* infection, in addition to 12 out of 16 crops). On the other hand the prevalence rates of infection in hearts, gizzards and livers were no statistically significant

(NS).The total specimens assessed were 80, 16 of each organ since there were 16 cases of salmonellosis and five organs per each bird were assessed.

**Table 2. Prevalence of *Salmonellae* in specimens from post-mortem chickens**

<b>Organs infected</b>	<b>n</b>	<b>Prevalence rates (%)</b>	<b>p value</b>
Caeca	15	93.5	$p < 0.001$
Hearts	1	6.3	NS
Crops	12	75.0	$p < 0.05$
Gizzards	1	6.3	NS
Livers	3	18.8	NS

*The prevalence rates of infection in caeca and crops were statistically significant ( $p < 0.05$ ). On the other hand the prevalence rates of hearts, gizzards and livers were no significant (NS).The total specimens assessed were 16 of each organ since there were 16 cases of salmonellosis.*

This study 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 [16].

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 infections 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 the most 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].

Concerns have been raised because many strains of *Salmonella* have become resistant to several antibiotics that have traditionally been used to treat the infection in both animals and humans [8-9]. 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 [15]. There has been enough information as to whether food sourced from China, European Union countries or elsewhere is also becoming increasingly contaminated with *Salmonella* strains that are resistant to antibiotics [16-22].

#### 4. CONCLUSION

Antibiotics including Trisulvitrim, Menorox and Neochlore reduced significantly *Salmonella* Typhimurium infection in growing chickens. There was a very little information in the literature about the use of antibiotics to treat *Salmonella* infections in chickens that is one of the most prevalence zoonosis.

#### 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 West Indies, Mona campus, Jamaica.

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#### COMPETING INTERESTS

The authors declared that no competing interests exist.

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