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A CASE OF ANTIBIOTIC RESISTANT *SALMONELLA* INFECTION IN BROILER CHICKS

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ABSTRACT

A case of Salmonella pullorum infection in broiler chicks suspected to have been contracted through the hatchery/parent stock is reported. The isolates from the liver, heart and intestines of dead chicks at post mortem (PM) were found to be resistant to two commonly used antibiotics (Gentaryl-D®, Pantex Holland) and Doxy-Gen®, Kepro Holland) which were initially administered to the chicks at the doses of 1 g/1.5 litres of drinking water for 3 days, and 100 g/300 litres of drinking water for 5 days respectively. The organisms were moderately sensitive to ciprofloxacin which on administration at 1 ml/200 litres of drinking water for 3 days failed to alleviate the condition as mortality continued to increase. On administration of Oxyfuravit® [Oxytetracycline hydrochloride, Furaltadone hydrochloride, vitamin A (3000 iu), vitamin B_1 (2 mg), vitamin B_2 (4 mg), vitamin B_6 (2mg), vitamin B_{12} (10 ug), vitamin C (20 mg), vitamin D (1500 iu), vitamin E (2 mg), vitamin K (2 mg), d–Ca – pentothenate (10 mg), inositol (1mg) and soluble excipient (1000 mg)], the condition of the chicks improved remarkably with a drastic drop in mortality. The reduction in mortality and the positive response of the chicks to Oxyfuravit® medication was thought to be as a result of the Furaltadone hydrochloride component, since the sensitivity profile indicated that the Salmonella isolates were resistant to tetracycline. This report suggests that Salmonella isolates in our locality may be resistant to commonly used antibiotics and that there is a compelling need for safe and effective alternative antibiotics for use in such cases of antibiotic resistance in poultry. However, there is need for poultry farmers to always apply strict hatchery sanitation and poultry house management practices while observing the recommended withdrawal periods for antibiotics before poultry meat and eggs are made available to the consumers.

***Keywords*:** *Salmonella*, Antibiotic- resistance, Broiler chicks.

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INTRODUCTION

Bacterial diseases are one of the serious problems facing the poultry industry worldwide. They are responsible for major economic losses to farmers in the industry [1,2,3,4]. *Salmonella* and *Escherichia coli* (collibacillosis) infections are the most commonly diagnosed bacterial infections in poultry [5, 6]. *Salmonella* species can be divided into two broad groups on the basis of pathogenesis and infection

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biology. One group consists of a large number of serovars, including *Salmonella typhimurium* and *Salmonella enteritidis*, which can colonize the alimentary tract of food animals or cause gastrointestinal disease in a range of hosts including man [7]. The other group comprises a small number of serovars that cause systemic typhoid-like disease in a restricted range of host species such as *Salmonella typhi* in humans, *Salmonella dublin* in cattle, *Salmonella pullorum* and *Salmonella gallinarum* in poultry. *Salmonella pullorum* is the causative agent of pullorum disease in poultry, while *Salmonella gallinarium* is the causative agent of fowl typhoid [7].

Pullorum disease is an acute systemic disease that results in a high mortality rate in young chicks but rarely causes such severe clinical disease in adult birds, though it can result in loss of weight, decreased egg lay, diarrhea, and lesions and abnormalities of the reproductive tract (7). *Salmonella pullorum* contaminates eggs in the ovary or oviduct following ovulation. It localizes in the reproductive tract of chickens and as a consequence may be transmitted vertically to chicks through transovarian transmission of the bacteria into developing hatching eggs [7]. Transmission can also occur via direct or indirect contact with infected birds (respiratory or fecal) or contaminated feed, water, or litter. Infection transmitted via egg or hatchery contamination usually results in death during the first few days of life up to 2–3 weeks of age. Transmission between farms is due to poor biosecurity. Watanabe *et al.* [8] found high levels of maternal antibody to *Salmonella pullorum* in the eggs from some infected hens. These were more likely to prevent multiplication of the bacteria within the eggs, thus, leading to an increased chance of embryo survival and the hatching of infected chicks. Control measures have virtually eradicated *Salmonella* pullorum from intensively reared commercial flocks in Europe and North America [9]. Both pullorum disease and fowl typhoid are endemic where free-range rearing of poultry has gained increasing popularity and has continued to cause substantial economic losses poultry farmers. In countries such as Asia and South America where the intensification of the poultry industry is in its infancy, high ambient temperatures and open-sided housing restrict the efficacy of hygiene measures because of environmental contamination [7,9]. Wild and game birds may also act as potential reservoir for *Salmonella* infection. Pheasant chicks are also susceptible to pullorum disease [10].

Persistent and uncontrolled use of antibiotics to control and manage bacterial infections has given rise to bacterial resistance to available antibiotics, which is currently a very serious problem of the poultry industry especially in developing countries of the world [11,12,13,14]. This report documents a case of bacterial infection in broiler chicks caused by drug-resistant *Salmonella pullorium* and its management using a drug containing furaltadone hydrochloride.

CASE HISTORY

The infected chicks were the second out of the three batches of birds raised in the Poultry Unit of the Agriculture Farm, University of Nigeria, Nsukka, as a part of the HUJJI-UNN Broiler Project. The birds were maintained on deep litter. The first (3,465 chicks) and third (2,866 chicks) batches performed very satisfactorily with a mortality of only 55 (1.5%) and 38 (1.3%), respectively at the age of 4 weeks. The infected second batch (2,574 chicks) had no vaccination history and was apparently healthy although some of the chicks had soiled vents on arrival. Progressive daily mortality was recorded with increasing number of the chicks showing weakness, droopy feathers and faecal pasting of the vent. They were placed on Gentamycin, Doxycycline and vitamins (Gentary1D®, Pantex Holland) medication at the dose of 1 g/1.5 litres of drinking water for three consecutive days on arrival. Due to increasing mortality, a different brand of Gentamycin-doxycycline combination (Doxy-Gen®, Kepro Holland) was administered at the dose of 100 g/300 litres of drinking water for five days, while awaiting the results of laboratory pathology and bacteriology tests conducted on some of the dead chicks submitted for examination.

LABORATORY INVESTIGATIONS

Some of the dead, moribund and apparently healthy chicks were examined for gross pathological signs and lesions. Samples of the heart, liver, and intestines obtained at postmortem were sent to Microbiology

Laboratory for bacteriological culture and sensitivity test. Bacteriological culture was done using nutrient agar and MacConkey agar (MAC), (OXOID Ltd., Detroit, Michigan, USA). The samples were inoculated (streaked) onto 5% nutrient and MacConkey agar plates and incubated at 37ºC for 24 - 48 hours. All isolates that fermented lactose within 24 - 48 hours on MacConkey agar were further identified morphologically and biochemically according to standard methods (15). The *in vitro* antimicrobial susceptibility was carried out by the agar - disc diffusion test [16] using Oxoid iso-sensitest agar. Single disc diffusion method was used to assess the susceptibility of the *Salmonella* isolates to commonly used antimicrobial agents as described by National Committee for Clinical Laboratory Standards (NCCLS) [16].

A total of 10 antibiotic discs containing antibiotics: Ampicillin 25 µg; Nalidixic acid 30 µg; Augumentin 50 µg; Norfloxacin 10 µg; Tetracycline 10 µg; Cefuroxine 20 µg; Nitrofurantoin 100 µg; Chloramphenicol 30 µg; Ciprofloxacin 5 µg and Gentamycin 10 µg were used. The antibiotics impregnated discs were applied to the surface of the inoculated plates with sterile forceps. Each disc was gently pressed down onto the agar to ensure complete contact with the agar surface. The plates were inverted and incubated at 37°C for 24 hours. The plates were examined, and the diameters of the zones of complete inhibition were measured to the nearest whole millimeter. The zone diameter for individual antimicrobial agents was then translated into susceptible (more than 20 mm), intermediate (20–14 mm) and resistant (less than 13 mm) categories according to interpretation table [16].

RESULTS

Gross Lesions: At necropsy of some dead chicks, the following lesions were observed: enlargement of the liver with diffused necrotic foci and tiny haemorrhagic spots, the spleen was enlarged and mottled, pericarditis was evident, and the mesenteric arteries were heavily congested.

Microbiological findings: The bacteriological culture of the organs (heart, liver and intestines) collected at necropsy yielded heavy growth of *Salmonella pullorum* (5×10^6 cfu/ml). The isolates showed resistance to Ampicillin – 8 mm, Nalidixic acid – 7 mm, Augumentin – 8 mm, Norfloxacin – 10 mm, Tetracycline – 8 mm, Cefuroxime – 7 mm, Gentamycin – 10 mm, and Nitrofurantoin – 8 mm. The organism was slightly susceptible to Chloramphenicol (13 mm) and moderately susceptible to Ciprofloxacin (17 mm).

DIAGNOSIS

Based on the history, clinical signs, gross lesions, and bacteriological findings, the disease was diagnosed as salmonellosis caused by *Salmonella pullorum.*

TREATMENT

Based on the sensitivity test result, coupled with the very poor response to the previously administered drugs, Ciprofloxacin (Ciprovet solution®, The Arab Pesticides and Veterinary Drug Mfg. Co., Jordan) was introduced at the dose of 1 ml/200 litres of drinking water for 3 days. The mortality did not abate despite medication and the mortality at nine days of age was 549 (21%). Oxyfuravit® (Maridav, Ghana Ltd) was then introduced and administered in drinking water at the dose of 100 g/200 litres of drinking water daily for seven days. The chicks showed marked improvement and there was drastic reduction in mortality following this treatment. Oxyfuravite® contains Oxytetracycline hydrochloride, Furaltadone hydrochloride, vitamin A (3000 iu), vitamin B_1 (2 mg), vitamin B_2 (4 mg), vitamin B_6 (2mg), vitamin B_{12} (10 µg), vitamin C (20 mg), vitamin D (1500 iu), vitamin E (2 mg), vitamin K (2 mg), d – Ca – pentothenate (10 mg), inositol (1 mg) and soluble excipient (1000 mg).

DISCUSSION

It would appear that the infected second batch of chicks was not from the same source as batches one and three. The infection must have been acquired from the hatchery or parent stock considering the high early

mortality. The *Salmonella pullorum* isolated from internal organs of the infected chicks were resistant to a number of antimicrobial agents including Gentamycin and Doxycycline which the birds were initially placed on arrival. Similar reports of anti-microbial resistance of *Salmonella*, and other bacterial organisms are common [12,17]. Antimicrobial resistance has grown to become a global problem [18]. Although the isolates were moderately sensitive to ciprofloxacin, it was not effective in treating the infection. However, no analysis was conducted to determine whether the ineffectiveness of the drug was as a result of insufficient drug concentrations in the formulation [13]. Inappropriate antibiotic usage in terms of overuse and misuse in both man and animals is considered the most important factor promoting the emergence, selection and dissemination of antibiotic resistant micro-organisms in both veterinary and human medicine [18,19,20].

Oxyfuravit® (furaltadone and oxytetracycline combination) was used to control the infection, and the success was attributed to the furaltadone component since the *Salmonella* isolates were also resistant to tetracycline, although antibiogram of the organism using furaltadone was not tested. The use of Furaltadone and other Nitrofurans has been prohibited in food-producing animals in several countries because both the WHO and European Union (EU) are unable to assign a maximum residue limit to them because of the potential carcinogenic effects of their residues on human health [21]. Its use in this case was as a treatment of last resort. Furthermore, lack of stringent policy on the control and use of antibiotics in food animals has allowed the continued circulation and use of the drug by Nigeria poultry farmers because it also serves as immune booster and growth promoter due to its vitamins and mineral content. In any case, farmers are advised to observe the withdrawal period for meat (7 days) and eggs (14 days) before these products are made available to consumers.

CONCLUSION

A case of antibiotic resistant *Salmonella pullorum* infection is reported in broiler chicks, and a drug containing oxytetracycline, furaltadone and some vitamins and minerals was able to effectively treat the disease and prevent further mortality after 21 % of the stock has been lost to the disease.

RECOMMENDATION

To prevent bacterial infection in poultry, it is very necessary to apply strict hatchery sanitation and laying house management practices; purchase chicks only from reliable hatcheries, avoid exposure to carriers or contaminated environment, and provide adequate biosecurity. Also, the routine withdrawal periods for these antibiotics for meat and eggs should be observed before these products are made available for consumption.

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