CASE REPORT

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Outbreak and management of *Salmonella* Enteritidis infection in 2-week-old Lohmann brown pullets

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Abstract

Avian salmonellosis has huge economic and public health impact. In this manuscript, a case of *S*. Enteritidis and its management within Jos Metropolis was reported. Fifty-three carcasses of 2-weeks old pullets were presented at the poultry and fish clinic of the Veterinary Teaching Hospital, University of Jos, Nigeria for investigation. There was persistent mortality despite 5 days medication with 20% Enrofloxacin (Floxinor®, Shijiazhuang Guanghua Pharmaceutical co. Ltd, Hebei, China). Cumulative mortality within 14 days was 203 birds in a flock of 4,000. Necropsy was done and harvested organs were subjected to microbial analysis for bacterial isolation, identification and antibiotic susceptibility test while portions of these organs were preserved in 10% formalin for histopathology. Necropsy findings were empty crops, hepatitis with petechial hemorrhages, nephritis, congested and consolidated lungs, peritonitis, congested spleens and mild enteritis. Histologically, there were vacuolation and necrosis of renal tubular epithelia cells and interstitial infiltration with heterophils. Severe disorganization of hepatic cords, infiltration with inflammatory cells and mild necrosis of hepatocytes were observed, while there was severe congestion and diffuse hemorrhages in the lungs. Cellular infiltration within the lamina propria of small intestine with stunting and blunting of the villi were observed. Organism isolated on MacConkey agar was identified as *Salmonella* Enteritidis. Antibiotic susceptibility test showed the organism to be most susceptible to Streptomycin, which was administered via drinking water at dosage of 40mg/kg with good recovery of the flock. It was concluded that the occurrence of *Salmonella* Enteritidis infection in this flock might be from the hatchery or via ingestion of contaminated feed and water. Day old chicks should be screened for Salmonella infection and strict biosecurity should be instituted on poultry farms.

Keywords: Mortality, Necrosis, Pullets, *Salmonella* Enteritidis, streptomycin

Introduction

Commercial poultry production has over the years remained a major employer of labour and has been a source of affordable animal protein to many households. Annual poultry egg production in Nigeria as announced by Poultry association of Nigeria (PAN) is estimated to be about 10.3 billion table eggs

(Ekwujuru, 2016). Poultry meat production has significantly increased with the strategies put in place by the Central Bank of Nigeria (CBN) in addition to the efforts of some hatcheries that encourage farmers to buy day old broilers to raise to table size then, buy back these birds and process the birds for consumption. Although, the poultry industry has these great potentials, there are numerous challenges that can slow down its growth. These include periodic scarcity and high cost of feed raw materials, high interest rate of bank loans, high price of day-old chicks, egg glut, disease outbreaks and others. The causes of disease outbreaks in poultry farms are multifactorial, ranging from infectious to non-infectious causes. Over time, it has been shown that many outbreaks are associated with bacterial infections (Barde *et al*., 2012).

One of the very important bacterial diseases affecting poultry is Fowl paratyphoid (Salmonellosis), a condition caused by one of the many non-host adapted motile *Salmonella* spp like *Salmonella* Heidelberg*, Salmonella* Enteritidis or *Salmonella* Typhimurium (Chauhan & Roy,2007*).* These bacteria are Gram-negative rods, oxidase negative and motile non-lactose fermenters. Paratyphoid infection usually affects young birds between day 4 and 2 weeks of age (Chauhan & Roy, 2007). Salmonella contamination in fertile eggs can cause embryo mortality or rapid and high mortality in newly hatched chicks. In the first 14 days of life, mortality and morbidity can be high with weight loss and poor growth (Dhillon *et al.,* 2001).

Clinically, signs observed in infected chicks include: somnolence, droopy wings, huddling and shivering near heat source, ruffled feathers, anorexia, emaciation and diarrhea, which is usually caused by enterotoxin produced by the pathogen. Paratyphoid Salmonellas such as *Salmonella* Enteritidis are responsible for poor performance of breeders, decreased egg production by layers, infertility and mortality (Barrow, 2000). The incidence is usually higher in younger birds and mortality could be up to 20% in first 3 weeks of life.

The economic losses due to Salmonellosis of poultry are enormous in addition to its implication on human health and environmental contamination. Hence, prevention and control of Salmonellosis of poultry is a priority and of high importance. This report is an account of the management of an outbreak of *Salmonella* Enteritidis in 2 weeks old pullets.

Case Management

Case history

On 04-03-2020, a client presented 53 carcasses of 2 weeks old Lohmann brown pullets at the poultry and fish clinic of the Veterinary Teaching hospital (VTH), University of Jos, Nigeria. He complained that mortality had been recurrent, despite over 5 days of administration of a brand of 20% Enrofloxacin (Floxinor®, Shijiazhuang Guanghua Pharmaceutical co. Ltd, Hebei, China). The initial population at day old was 4,000 chicks and as at time of presentation, cumulative mortality had reached 203 with higher peaks in the last 3 days and has been increasing from 30, to 43 and 87, respectively. The client also reported that the feed intake had dropped from 6 bags of chicksmash per day to 4 bags as well as water intake from 200 litres to 160 litres/day. It was revealed that the following vaccines: Mareks HVT (day 3), 1st IBD (day 7) and on day 10, Livacox® (Coccidiosis vaccine) had been administered. Newcastle disease La Sota vaccine could not be administered due to rising mortality before the case was reported to the clinic.

Post mortem

The birds were examined externally for skin lesions and ecto-parasites before immersion in disinfectant solution, prior to post mortem examination. Necropsy of the chicks revealed the following: empty crops, hepatitis with slight petechial hemorrhages; nephritis; (Plates I and II) congested and consolidated lungs; mild peritonitis; splenic congestion and mild enteritis.

Affected organs like liver, lungs, spleen, heart, kidneys and small intestine were harvested and separated into two parts. The first part was used for microbial analysis, while the second part of organs was preserved in 10% formalin for histopathologic investigation.

Microbial Isolation and Identification and histopathology

Under sterile condition, inocula from organs (except the intestine) were streaked on 5% blood and MacConkey agars and incubated aerobically at 37°C for 24 hours. The bacterial colonies that grew on the two different culture media were observed and the morphological characteristics recorded.

Identification of cultured bacteria via Gram staining and biochemical tests such as oxidase, catalase, indole and others were done as described by Olutiola *et al*. (1991). With a sterile inoculating loop, a few colonies of the non-lactose fermenting organism

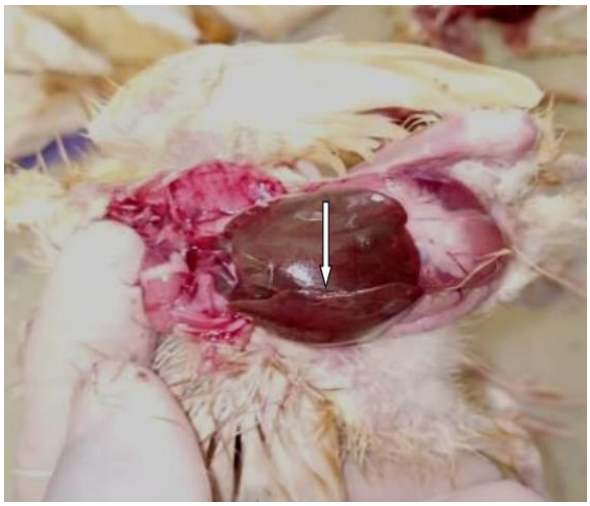

Plate I: Hepatitis with slight petechial hemorrhage (arrow)

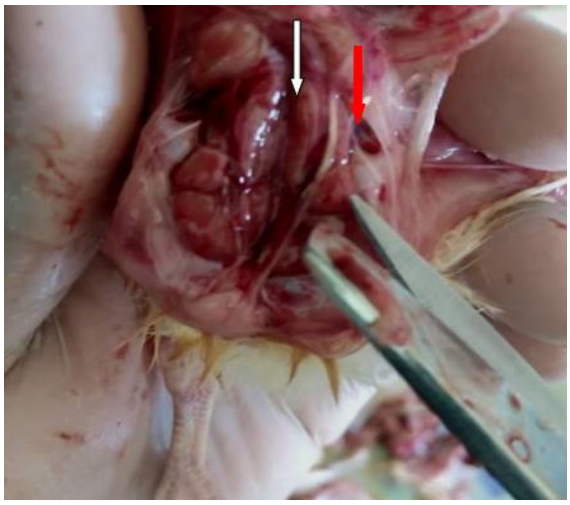

Plate II: Marked nephritis with hemorrhage (white arrow) and slightly distended ureter (red arrow) in 2 weeks old chick

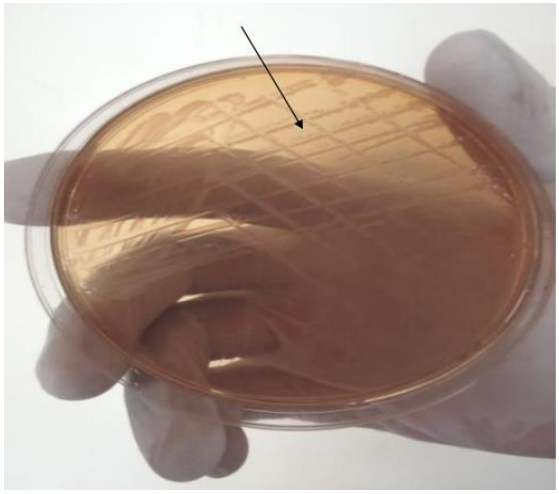

Plate III: Growth of non-lactose fermenting bacterial colonies on MacConkey agar

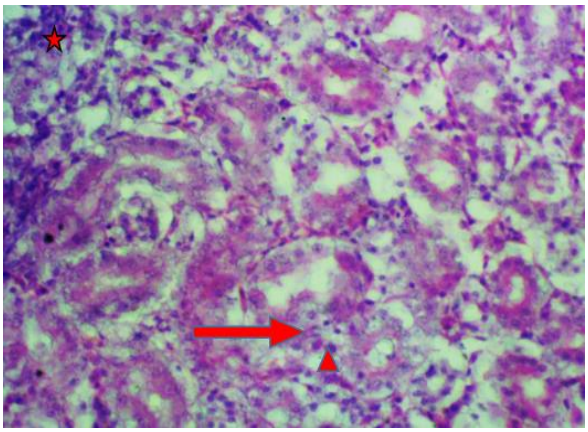

Plate IV: Photomicrograph of kidney from 2 weeks old pullets: Severe vacuolation (arrow) and necrosis of tubular epithelia cells (arrow head) and interstitial infiltration with heterophils (asterisk) ×40. H & E

were picked and added to a drop of sterile water on a clean glass slide to make a smear. This was allowed to air-dry before adding absolute methanol to fix it. Crystal violet stain was applied on the smear for 1 minute then, rinsed off before addition of Lugol's iodine for 1 minute. The Lugol's iodine was then washed off and acetone added for 10 seconds to decolorize. This was also washed off under running tap water before application of neutral red as counterstain for 1 minute before rinsing off and stained smear allowed to air-dry before viewing under the microscope at x 100 (oil immersion).

A few drops of 1% oxidase reagent (N, N, N, N-tetramethyl-p-phenylenediamine dihydrochloride) were added to soak a piece of filter paper in a petri dish. With the edge of a clean glass slide, a few colonies were taken and smeared on the oxidase reagent-soaked filter paper and observed for 15 seconds for color change and there was none (oxidase negative). The catalase test was done by use of 3% hydrogen peroxide. With the aid of an inoculating loop, a few colonies of the organism were taken and brought in contact with a drop of 3% hydrogen peroxide on a clean glass slide to observe for bubble formation. It was positive for catalase. Slants of simmon citrate agar and triple sugar iron agar were also inoculated with broth of the organism and incubated at 37°C for 24 hours. Other sugar

fermentation tests like those of galactose and maltose were done by placing the sugar discs on phenol red agar plate that had been seeded with broth of the organism and allowed to be incubated for 4 to 6 hours to check for fermentation (yellow zone formation around the sugar disc when positive). The bacterial isolate that grew from streaked tissue inocula was differenciated on MacConkey agar as tiny, non-lactose fermenting, translucent colonies (Plate III). This organism was subjected to several biochemical tests for identification (Table 1) and the outcome of these were checked in the manual for identification of medical bacteria (Cowan, 1974) which identified the organism as *Salmonella* Enteritidis (Table 1).

Table 1: Biochemical tests for bacterial identification

Gram reaction	Gram-negative rods
Oxidase	-
Catalase	+
Citrate	+
Methly red	+
Voges proskeur	-
TSI Agar	*R/Y, H_2S
Indole	-
Urea	-
Motility	+
Starch hydrolysis	-
Growth on blood agar	+
Hemolysis	-
Growth on MacConkey agar	+
Glucose fermentation	+
Galactose fermentation	-
Maltose fermentation	+
Lactose fermentation	-
Identification	*Salmonella* Enteritidis

Keys
*R/Y, H_2S Means red slant, yellow butt and production of hydrogen sulphide from Triple sugar iron agar.
\+ means positive
\- means negative

Histologically, in the kidneys there were severe vacuolation and necrosis of tubular epithelia cells and interstitial infiltration with heterophils (Plate IV). In the liver, there were severe disorganization of hepatic cords, infilteration with inflammatory cells and mild necrosis of hepatocytes (Plate V). In the lungs, there was severe congestion and diffuse hemorrhages (Plate VI). There was cellular infiltration within the lamina propria of small intestine as well as stunting and blunting of the villi with presence of necrotic debris and fibrin (Plate VII).

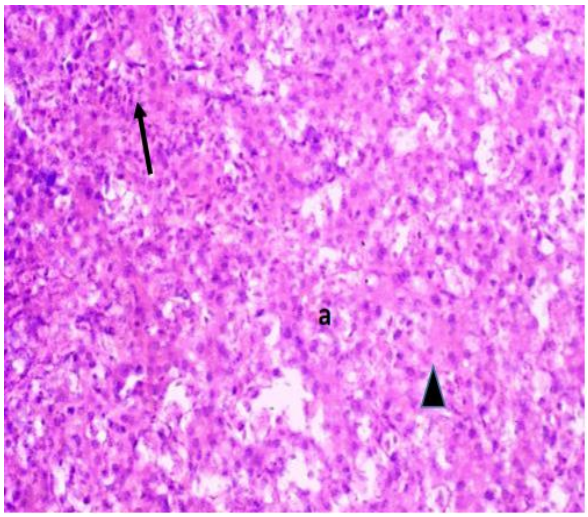

Plate V: Photomicrograph of liver from 2 weeks old pullets: Severe disorganization of hepatic cords, edema (**a**), infiltration with inflammatory cells (arrow) and mild necrosis of hepatocytes (arrow head) ×40. H & E

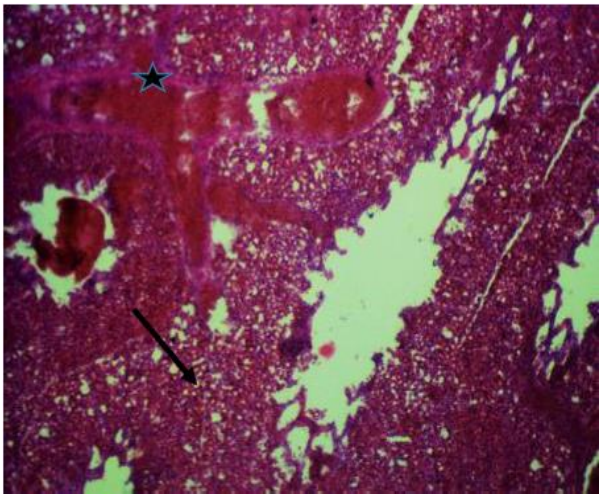

Plate VI: Photomicrograph of lung from 2 weeks old pullets: Severe congestion (black asterisk) and diffuse haemorrhages ×10. H & E

Management

The client was advised to commence treatment with pure streptomycin (Strepa®, North China Pharmaceutical Co. Ltd, Shijiazhuang, China.) via drinking water at 40 mg/kg body weight, for 7 days which was later extended for another 3 days.

Discussion

The result of the antibiotic susceptibility test showed that the isolate was most susceptible to streptomycin, then penstrep® (penicillin + streptomycin) and enrofloxacin. The diameter of zone of inhibition of streptomycin was 35mm, while that of enrofloxacin was 25mm. The mortality pattern during treatment with streptomycin showed that by the 4th day of administration of streptomycin via drinking water, there was a more significant reduction in mortality pattern when compared with earlier administered enrofloxacin to which the bacterium was also susceptible as mentioned earlier. Several factors could be responsible for what appears as an *in vivo* failure of enrofloxacin in this case. This could be related to the quality of the brand of enrofloxacin, dose applied by the farmer or the chemical nature of the drinking water. If the water is hard with calcium and magnesium ions being over 50 ppm, the bivalent cations therein will chelate the drug and reduce bioavailability, hence poor response to treatment (Sumano *et al*., 2004).

Table 2: Antibiogram of *Salmonella* Enteritidis isolated from tissues of pullets

Sensitive	Intermediate	Resistant
Streptomycin	Gentamicin	Colistin
Penstrep®	Furaltadone	Oxytetracycline
Enrofloxacin	Tylosin	

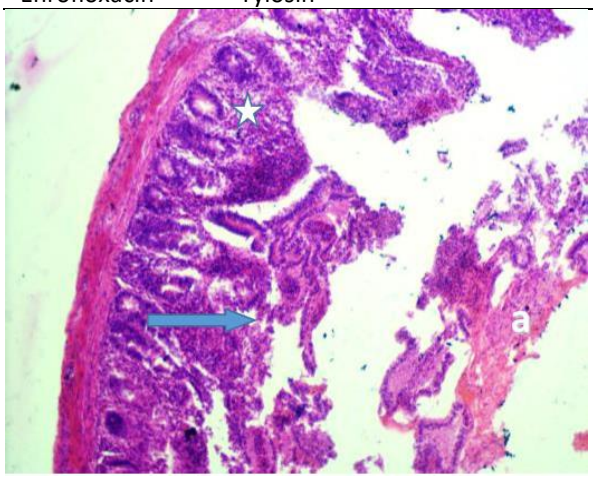

Plate VII: Photomicrograph of small intestine from 2 weeks old pullets: Stunting and blunting of the villi with necrotic debris and fibrin (**a**). with cellular infiltration within the laminal propria (asterisk) and haemorrhages (arrow) ×10. H & E

It is also documented that aminoglycosides like streptomycin (which is bactericidal in action) are poorly absorbed from the gastro-intestinal track (Brander *et al.,* 1991). This infers that such a drug would be of no systemic therapeutic value via oral administration. Contrary to this, the observation in this case revealed a significant reduction in daily mortality of chicks from 87 to 0 on day 10 of treatment. It is possible that enteritis which was observed (grossly and histologically) could have enhanced absorption of streptomycin, hence the response to treatment.

The histological findings in the liver pointed to a major economic effect of this disease in birds as it would result in poor feed conversion of birds, hence a delayed onset of maturity and egg production. The multi-organ damage potential of this pathogen as shown from the histopathology of the lungs and kidneys could be a strong reason for the high mortalities observed (Barrow, 2000).

Table 3: Daily mortality pattern in pullets infected with *Salmonella* and treated with 40 mg/kg streptomycin via drinking water

Days	Mortality pattern of chicks
1	75
2	62
3	58
4	20
5	13
6	7
7	4
8	5
9	2
10	0

Salmonella Enteritidis that is isolated from tissues of dead birds is also shed through faeces of sick live birds and this will contaminate the drinking water, feed and litter. In order to break the cycle of infection on the farm, the farmer was advised to commence drinking water sanitation, using a chlorine-based preparation, Isochlor® (55% sodium dichloro isocyanurate, Ceva Polchem Private Ltd, Pune, India) at 1 tablet per 1,000 litres of water and ensure change of litter after treatment. The use of inactivated *Salmonella* Enteritidis vaccine was also suggested, considering the fact that the etiologic agent of this disease outbreak is of public health importance and eggs that will be produced by these birds will be sold for human consumption.

For farmers who make their poultry feeds, it has been shown that *Salmonella* spp could contaminate raw materials that are either of animal or plant protein (Davies & Wales, 2010). This implies that to control salmonellosis, the aspect of feed sanitation through use of organic acids or heat while pelleting feed, should be considered as part of a multi-pronged approach needed, not just at the commercial level poultry, but at parent stock poultry farms from where there could be vertical transmission of infection to day-old chicks. This will be of benefit to the farmers and safeguard the health of the public that consume the farm produce.

Conflicts of Interest

The authors declare no conflict of interest.

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