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RESEARCH ARTICLE

MULTI-DRUG RESISTANT SALMONELLA IN 13-DAY-OLD BROILER FLOCK IN SOKOTO, NIGERIA: A CASE REPORT

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ABSTRACT

Three thirteen-day-old broiler chicks were presented at the Avian Clinic of Usmanu Danfodiyo University Veterinary Teaching Hospital, Sokoto, with complaints of diarrhea and mortality observed in the flock four days prior to presentation. Upon physical examination, evidence of diarrhea was found. During flock visitation, further physical examination revealed dullness, huddling, and yellowish diarrhea, and five carcasses were discovered. Cloacal swabs were collected and submitted to the veterinary microbiology laboratory for bacterial culture, identification, and antibiotic sensitivity testing. Post-mortem examination was carried out on the carcasses, revealing lesions suggestive of Salmonella infection. Salmonella was isolated, and it exhibited resistance to all antibiotics used in the sensitivity test, including Augmentin, Gentamicin, Cotrimoxazole, Tetracycline, Cloxacillin, Erythromycin, Chloramphenicol, and Streptomycin.

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INTRODUCTION

Food safety is a critical concern in commercial poultry production. Poultry farming significantly contributes to the economies of many countries and constitutes 25 percent of local meat production in Nigeria. However, poultry productivity faces numerous disease challenges. Salmonellosis is a bacterial infection that affects both humans and animals. Chickens are one of the animal groups susceptible to Salmonellosis, and this disease is zoonotic. While Salmonellosis is prevalent worldwide, its occurrence is higher in resource-limited countries like Nigeria (1). Salmonella transmission can be both vertical and horizontal. Vertical transmission occurs when young chicks with open navels come into contact with contaminated environments before their navels fully close. Horizontal transmission takes place when an infected chicken directly contacts a healthy susceptible chicken. Clinical signs of Salmonellosis in chickens include somnolence, ruffled feathers, inappetence, thirst, yellowish diarrhea, and reluctance to move. Post-mortem findings include an enlarged bronzed liver with small necrotic foci, congestion, kidney and spleen engorgement, anemia, and enteritis in the anterior small intestine (2). The isolation and identification of Salmonella involve non-selective pre-enrichment in buffered peptone water, followed by selective enrichment in Rappaport Vassiliadis (RVS) medium, culture on Xylose Lysine Deoxycholate (XLD) Agar, and biochemical screening for Citrate, Urea Hydrolysis, and Sulfur Indole-motility.

Effective treatment of many infectious diseases faces ongoing challenges due to the emergence of antimicrobial-resistant (AMR) bacterial strains. This issue is closely linked to the widespread use and misuse of antibiotics in animal husbandry, particularly in poultry, where antibiotics are used as growth promoters, prophylactic agents, and treatment options. The production and circulation of various combined formulations of antimicrobial drugs, often misused by poultry farmers, also contribute to this problem (3). It has been reported that approximately 8,164,662 kg of antibiotics are used annually, with 70% used for non-therapeutic purposes. This overuse of antimicrobials creates significant gaps in the fight against infectious diseases (4). The aim of this work is to report Salmonella infection in a broiler chicken flock and the antibiotic susceptibility of the isolated Salmonella for effective treatment approach.

CASE REPORT

Case History: In December 2022, a client presented three 13-day-old broiler chicks to Avian Clinic of the Veterinary Teaching Hospital (VTH) Usmanu Danfodiyo University, Sokoto (UDUS). The birds were part of an initial flock of 200 and had been sourced from a hatchery in Ibadan, Oyo State, Nigeria at day old. They were housed in a deep litter facility in Sokoto, Sokoto State, and were fed a commercially formulated broiler starter feed. The client complained of mortality and diarrhea, which were first observed on day five, the mortality rate was 36%. No previous vaccinations had been done, but the client had administered multivitamin powder, Gen-dox® (Doxycycline Hydrochloride: 100 mg and Gentamicin Sulphate: 100 mg/1 gm), Oxytet® (Oxytetracycline Hydrochloride soluble powder),

and EST (Erythromycin thiocyanate 200 mg, Sulfadiazine 150 mg, Trimethoprim 30 mg/1 gm) WS powder to the birds within the period of eightdays. The client therefore decided to seek professional veterinary intervention when the condition did not improve.

Clinical Manifestation: Upon physical examination, the chicks appeared lethargic and were passing yellowish diarrhea (Figure 1). A visit to the client's house revealed that the chicks exhibited signs of dullness, with about 50% having vents pasted with diarrhea. Evidence of yellowish diarrhea was observed on the litter (Figure 2). Additionally, five more chicks were found dead, bringing the total recorded mortality rate to 38%.

Post-Mortem Findings: The five carcasses were brought to the post-mortem unit of VTH UDUS for autopsy (Figure 3). Gross lesions were primarily observed in the digestive system. These lesions included a bronze-colored liver (figure 4) and petechial hemorrhages on the jejunum (Figure 5).

Bacterial Culture and Antimicrobial Susceptibility Testing (AST)

Isolation and Identification: Bacterial isolation and identification were performed through a non-selective pre-enrichment step in buffered peptone water at 37°C for 24 hours, followed by selective enrichment on Rappaport Vassiliadis agar for an additional 24 hours at 37°C. Subsequently, the bacterial suspension was streaked on Xylose Lysin Deoxycholate (XLD) Agar. Colonies exhibited typical *Salmonella spp* characteristics on XLD. They were subjected to biochemical screening, including tests for citrate utilization, urea hydrolysis, and sulfur indole-motility. These tests were conducted and interpreted following the standard protocol outlined by (5).

Standardization of Inoculum: Bacterial inoculum standardization was achieved by preparing a direct colony suspension. The absorbance of the suspension was measured using an ultraviolet spectrophotometer at a wavelength of 620nm and adjusted to achieve an absorbance value of 0.09 (equivalent to 0.5 McFarland standard scale), corresponding to 1.5 x 10^8 cfu/ml.

Antibiotic Susceptibility Test: The antimicrobial susceptibility testing was conducted using the Kirby Bauer disc diffusion test and interpreted in accordance with the recommendations provided by the Clinical and Laboratory Standards Institute (6) in 2019. The standardized inoculum was evenly spread on Mueller Hinton agar, and multiple antibiotic discs (Oxoid, USA) were carefully placed on the plate. The plates were then incubated invertedly at 37°C for 24 hours.

AST Results: The isolates displayed resistance to all antibiotics used in the sensitivity test, resulting in a calculated multiple antibiotic resistance index (MARI) of 1.0 (8/8). The antibiotic discs included Augmentin, Gentamicin, Cotrimoxazole, Tetracycline, Cloxacillin, Erythromycin, Chloramphenicol, and Streptomycin. It's important to note that these drugs are typically effective in treating Salmonellosis.

Serology Blood samples were collected through the brachial vein from three chicks that exhibited reluctance in movement. The samples were dispensed into 2ml microcentrifuge tubes and transported to the clinical pathology laboratory of VTH for sera extraction. A hemagglutination inhibition test was conducted to routinely check for antibodies to Newcastle disease virus. The blood was centrifuged at 3,000 rpm for 3 minutes to extract the sera. The antibody titres ranged from log 2^2 to log 2^3, as shown in Table 1.

Table 1. Results of Hemagglutination Inhibition Test for Newcastle Disease

Sample	Titre value
1	$\log 2^3$
2	$\log 2^3$
3	$\log 2^2$

Sample 1: $\log 2^3$ *Sample 2*: $\log 2^3$
Sample 3: $\log 2^2$

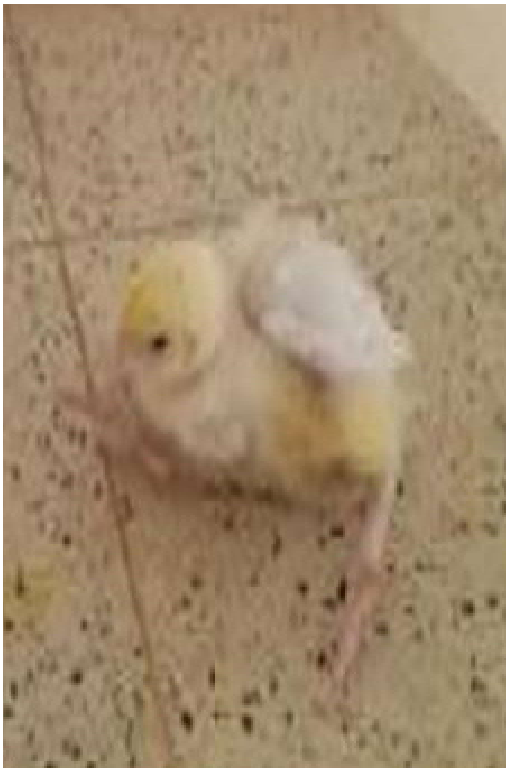

Figure 1. 13 day old broiler chick suspected with Salmonellosis appearing lethargic

Figure 2. Flock of 13 day old broiler chicks suspected with Salmonellosis huddling with evidence of diarrhea

Diagnosis Clinical signs and postmortem findings suggested Salmonellosis and Coli-septicaemia as differential diagnoses. Salmonella culture confirmed Salmonellosis, and the low antibody titre indicated a decline in maternal antibodies to the Newcastle disease virus or a decrease in antibodies following hatchery Newcastle disease vaccination.

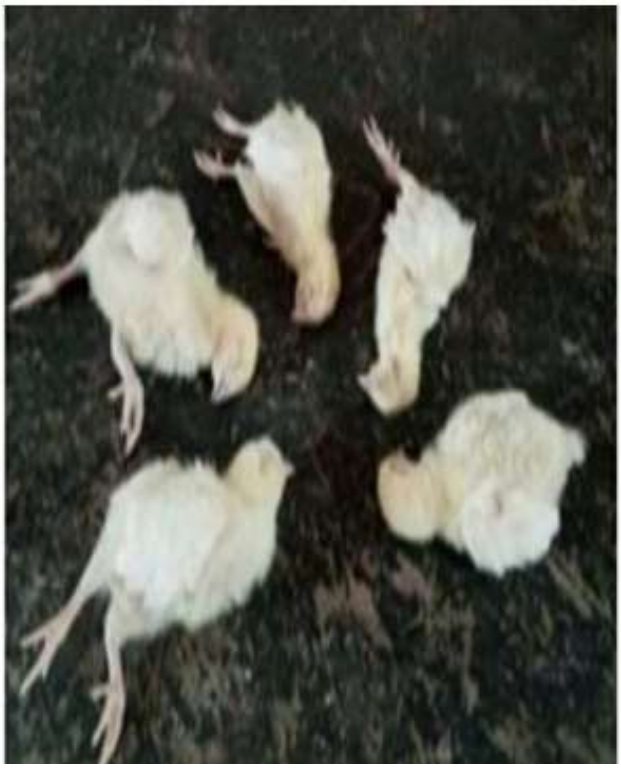

Figure 3. Carcasses of 13 day old broiler chicks suspected to have died of Salmonellosis

Figure 4. Bronze coloured liver of 13 day old broiler chick suspected to have died of Salmonellosis

Management/Treatment Immediate control measures were implemented upon the diagnosis of Salmonella. These measures included the isolation of sick birds, ensuring proper hygiene, environmental sanitation, personnel disinfection, and decontamination of the litter. Carcasses were disposed of properly, and further antibiotic administration was discontinued due to the extensive use of antibiotics and bacterial resistance observed. Sawke® (10% povidone iodine) was used for disinfection. The flock was managed symptomatically by providing adequate feed and water.

Figure 5. Petechial hemorrhage on the jejunum of 13 day old broiler chick suspected to have died of Salmonellosis

Additionally, Zeevit® Liquid Vitamin C (200 grams of Vitamin C per 1L) was administered at a rate of 1mL per liter of drinking water for five days to boost immunity. Sawke® (10% povidone iodine) was also administered at a rate of 1mL per liter of drinking water for five days for water sanitization. After the five-day therapy, clinical signs became milder, and mortality gradually decreased. The remaining birds eventually recovered.

DISCUSSION

Salmonella is an enteric pathogen that can infect almost all animals, including humans, and cross-infection is very common among birds (7). The large variety of serotypes and the very complex epidemiology of this microorganism make Salmonellosis one of the most difficult diseases to control (8). In many cases, the infection is not accompanied by clinical disease or lesions; as such, clinical signs and lesions are of little value in diagnosis. Accurate diagnosis is achieved by isolating and identifying the causative bacteria and/or detecting antibodies using serological examination (9). In this case report, the broiler chicks might have become infected with Salmonella through vertical transmission or through their navels from the hatchery and continued to spread it horizontally through feed and/or water contaminated with droppings. Similarly, (10) reported that fluff and meconium at the hatchery, feces from 3-to-7-day-old chicks, and litter at 3 and 6 weeks can be used as indicators of flock infection. (11), in research to determine constraints and adoption of practices in poultry production in Delta State, Nigeria, reported that major constraints to poultry production include the high cost of veterinary services, among other factors. This is the same situation in Sokoto State, Nigeria, where many poultry farmers opt for cheaper services from non-professionals, which leads to irrational antibiotics administration in poultry, resulting in antimicrobial resistance. In our study, the cultured Salmonella was resistant to all the antibiotics used for sensitivity testing, which we connected to the misuse of antimicrobials. Other similar works include reports by [12] on the prevalence of Salmonella associated with chick mortality at hatching A high prevalence of antimicrobial resistance in the study area was

recorded, with complete resistance to gentamicin, enrofloxacin, nalidixic acid, tetracycline, and streptomycin, and substantial resistance to triple sulfur and ciprofloxacin. Six multiple resistance profiles were recorded, with a high level of multiple resistance to quinolones. They reported that quinolone resistance has implications for veterinary and human therapy, as their misuse in poultry could lead to the emergence of resistant animal and zoonotic pathogens. (13), using processed samples of birds from hatcheries, reported that commercial day-old broiler chicks in Nigeria are colonized by multidrug-resistant coliforms (*E. coli* and *Klebsiellaspp*) and are potential reservoirs and disseminators of these organisms. Also, (14) in a study to determine the antimicrobial resistance pattern of Salmonella isolates from eggs and chicken meat in Sokoto, reported that isolates exhibited multidrug resistance against four of the antimicrobials tested, including erythromycin, sulfamethoxazole/trimethoprim, penicillin, and oxytetracycline. They attributed the increase in the rate of isolation of multiple resistant Salmonella to the use of medication at the breeder level. The majority of the isolates (93.3%) were susceptible to neomycin, which is one of the drugs commonly prescribed for the treatment of poultry diseases. The low antibody titers to Newcastle disease virus obtained after serology were probably due to waning maternal antibodies or as a result of vaccination against ND, usually carried out in hatcheries to confer immunity to the chicks, which wanes with time. It was therefore concluded that the chicks had Salmonella infection either from the parent stock or contaminated hatchery. The rational use of antibiotics in a commercial poultry farming system is the main solution to curtail the spread of these drug-resistant pathogens and its concomitant hazard to animal and human health. Neomycin and Amoxicillin should be incorporated to test for Salmonella susceptibility in order to reaffirm neomycin's efficacy and the current status of amoxicillin in the successful treatment of salmonellosis in this environment.

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