CASE REPORT

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Outbreak of Newcastle disease with colibacillosis in seven-week-old pullets: Diagnosis and management

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Abstract

Newcastle disease (ND) is a fatal viral disease that affects a wide variety of avian species and is considered an impediment to the development, survival and productivity of the poultry industry. *Escherichia coli* is a Gram-negative, non-spore-forming, enteric rod that often complicates viral infections in poultry. This case report described the diagnosis and management of an outbreak of suspected Newcastle disease and colibacillosis in 7-week-old pullets in a commercial poultry farm in Jos, Plateau State, Nigeria. The clinical case was diagnosed and treated based on results of clinical history and diagnostic work-up including high mortality, coughing, nasal discharge, pale comb, distended ureters, peritonitis and diffused petechial haemorrhage on the intestinal mucosa, low protective antibody titre (1:1 (0 Log2), and isolation of *Escherichia coli* from sampled organs. Based on these results, the case was diagnosed as Newcastle disease (ND) with colibacillosis. The remaining birds that showed signs of weakness and respiratory distress were treated with a recommended dose of Penprovit ® (Penicillin + Streptomycin + Vitamins) at 100g/100 litres of drinking water X 1/52 (+3 days). Polidine ® (Iodine solution) was also administered (1mL/2 litres of drinking water X1/52). Ten days post-treatment, peak mortality dropped from 70 to zero. These results demonstrate that a combination of diagnostic work plans, including clinical history, postmortem examination, microbial culture, and antibiotic susceptibility testing, as well as haemagglutination inhibition test (HI), is reliable for the diagnosis and treatment of natural concurrent infections associated with Newcastle disease and colibacillosis.

Keywords: Colibacillosis, Laboratory diagnosis, Newcastle Disease, Outbreak, Poultry

Introduction

The Nigerian agricultural sub-sector remains the major source of livelihood and employment despite its declining contribution to the country's foreign exchange earnings (Ekiri *et al*., 2021). A significant proportion of Nigerians (65%) hugely depend on agriculture as their economic mainstay. Additionally, agriculture contributes to about 34.8% of the Gross Domestic Product (GDP) and 38% of non-oil sources of foreign exchange earnings (OIE, 2017). The poultry and livestock production value chain are making important contributions to Sustainable Development Goals, through provision of protein sources and potentially improving climate change (OIE, 2017). Poultry is the most commercialized sector of the agricultural livestock value chain in Nigeria. The enterprise is expanding and has seen increased investment and Nigeria, is arguably one the largest producer of poultry and poultry product in the African continent, after South-Africa, Morocco and Egypt (Nkukwana, 2018).

Despite these huge gains, the poultry value chain is faced with several challenges, most notably, viral and bacterial diseases such as Newcastle Disease (ND) and colibacillosis, which lead to severe reduction in production and economic losses to farmers and stakeholders. ND is an infectious viral disease of domestic, caged and wild avian species worldwide. The disease is characterised by a high morbidity, mortality, and a sharp drop in egg production and quality, causing a devastating economic loss in the poultry sector (Ekiri *et al*., 2021). ND is clinically manifested by gastrointestinal haemorrhage and ulcers, commonly seen in chickens, and severe atrophy and depletion of lymphocytes in the lymphoid organs (Ekiri *et al*., 2021). Because of the severe economic consequences of outbreaks of virulent ND in commercial poultry, the disease is reportable to the World Organization for Animal Health (*Office International des Epizooties* (OIE), (OIE, 2017). Local or village chickens in Nigeria are rarely vaccinated against any disease and they serve as a reservoir of infection for commercially-produced chickens, thereby being an important limiting factor in the development of commercial poultry production and the establishment of trade links (Ekiri *et al*., 2021). Avian colibacillosis is worldwide in distribution and also causes high mortality in poultry of any age (Abalaka *et al*., 2017). Clinical signs include retarded growth in affected birds, decreased egg production, and egg quality. Diagnosis is mostly based on the clinical signs of the disease, isolation and identification of the causative agent, *Escherichia coli (E. coli)* from tissue samples of infected birds (Abalaka *et al*., 2017). In this case report, we describe the clinical signs, laboratory diagnosis and management of an outbreak of a suspected case of ND and with concurrent colibacillosis in seven-week-old pullets in Jos, Plateau State, Nigeria.

Case Presentation

Four carcasses of seven-week-old pullets were presented at the Avian and Aquatic Clinic, Veterinary Teaching Hospital (VTH), University of Jos, Nigeria. The farm had a flock size of 500 pullets procured from a local hatchery primarily for commercial purposes. The birds were fed with commercial feed (Vital feed ®), and water were provided from untreated well water. Prior to presentation at the VTH, the mortality started after three days of live ND vaccine (Lasota) administration. The farmer observed that the birds were coughing, followed by the mortality of 50 birds within five days. The farmer consulted a Veterinary drug vendor, where he was advised to administer Doxy-gen®, vitamin C, Sawke® (10% iodine) and Royal solution® (anti-viral preparations) in the birds' drinking water, and this was done without laboratory confirmation and before presentation to the VTH. On the day of presentation at VTH, four dead birds were submitted for post-mortem investigation. The farm experienced significant economic losses due to the high mortality rates, reduced growth rates, and increased treatment costs. A review of the farm's vaccination schedule revealed that although ND vaccination had been administered, it was not sufficient to prevent the outbreak, suggesting the possibility of a more virulent NDV strain or vaccine failure.

Pathological examination and sample collection

Post-mortem

Post-mortem (PM) was done on the four carcasses presented, and gross lesions were identified and recorded. At the time of PM, the differential diagnoses included Vitamin A deficiency/hypovitaminosis A, colibacillosis, and Newcastle disease. Gross lesions included pale comb (¼) and empty crops (¾). The ureters were distended with urates (4/4), pericarditis with slight hepatic congestion (4/4), foamy and slightly caseous peritonitis (Plate I).

Histopathology

Tissue samples of the affected organs; liver, kidney, and spleen were collected and fixed in 10% neutral buffered formalin. The tissue samples were processed as described by Baker *et al.* (2000). Briefly, the samples were dehydrated in varied concentrations of alcohol, cleared in xylene and saturated in paraffin wax. Tissue samples were then incubated in a vacuum at 60 °C, embedded in plastic embedding rings, and sectioned into 5-µM sections with the aid of a microtome. Next, the samples were deparaffinized with xylene and rehydrated in graded concentrations of alcohol, and stained with haematoxylin and eosin and viewed under the light microscope at X40 objective. Histopathologic findings

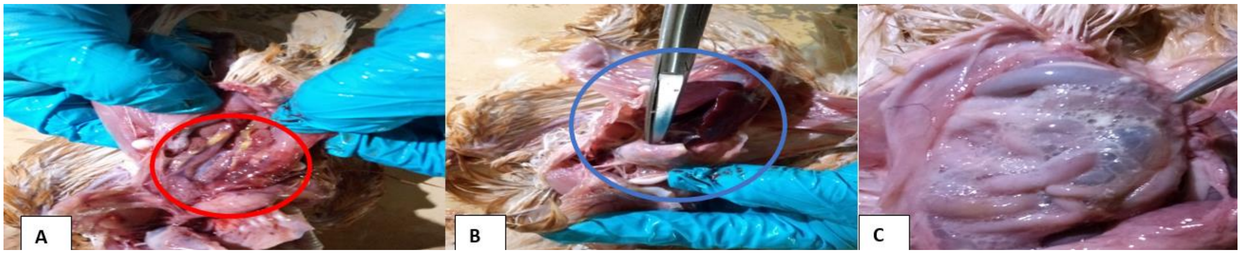

Plate I: (A) Nephritis with ureters distended with urates (red circle); **(B)**: Pericarditis with hepatic congestion (Blue circle); **(C)**: Foamy and slightly caseous peritonitis

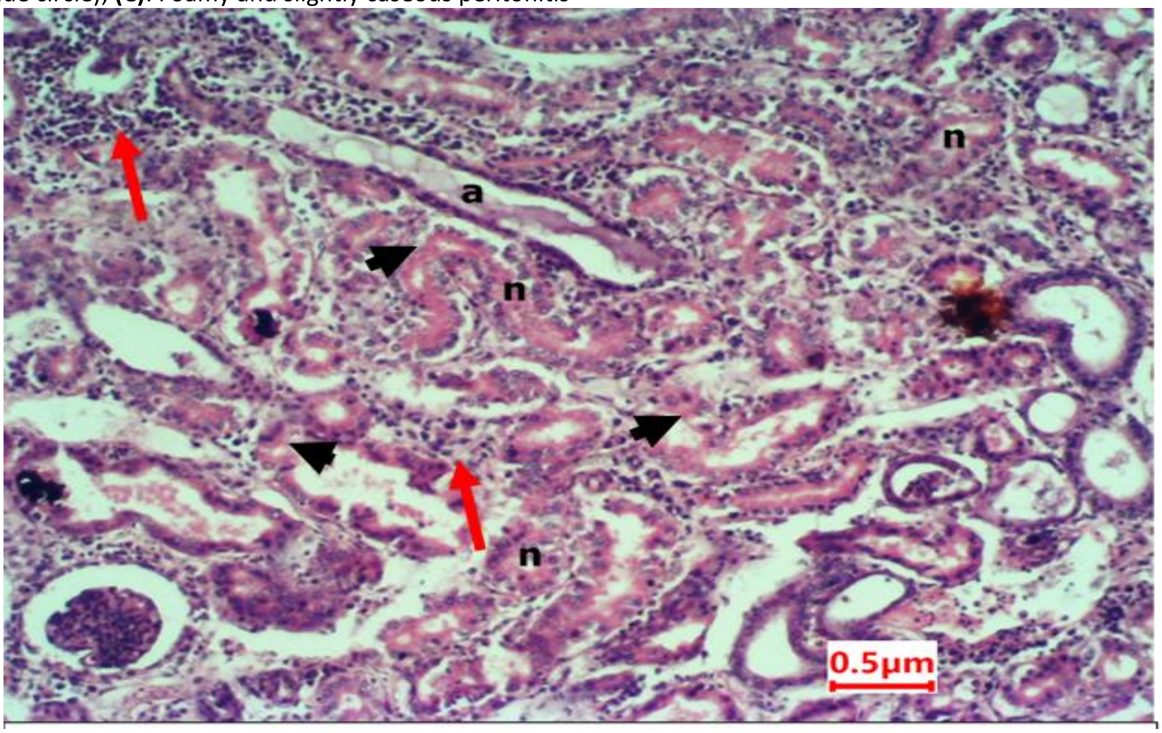

Plate II: A chicken kidney showing diffused interstitial inflammatory cellular infiltrations (arrow), necrosis of tubular epithelium (arrow head), and presence of necrotic material(n), and crystals (a) within the tubules, H&E X 100 magnification

revealed diffused inflammatory cellular infiltrations in the kidney interstitium, coagulative necrosis of tubular epithelium and crystals within the tubules (Plate II). The liver showed severe congestion of blood vessels, diffused cellular infiltrations and coagulative necrosis of hepatocytes (Plate III).

Bacteriological examination

Phenotype isolation and identification of *Escherichia coli (E. coli)* using routine bacteriological techniques was conducted on tissue samples of the liver, lungs, spleen, and kidney.

Homogenised tissue samples were cultured on 7% horse blood agar (BA) and MacConkey agar (MCA) (HIMEDIA, Maharastra, India), the cultures that showed pink lactose-fermenting colonies on MCA, were identified on eosin methylene blue (EMB) agar (Plate IV), and purified on nutrient agar. The cultures were Gram-stained to examine their cellular morphology (pink, slender, short rods). The identification of *Escherichia coli* was also done through biochemical methods (catalase test, Triple sugar iron, indole test, oxidase and citrate test). A positive indole reaction with Kovac's reagent, acid over acid with gas production on triple sugar iron (TSI), and a negative citrate test indicated *E. coli* culture. Additionally, direct antibiotic susceptibility testing was performed as described by She (2019) using locally prepared antibiotic discs [Oxytetracycline (250 µg), Tylosin (250 µg), Colistin (480 IU), Streptomycin (300 µg), Gentamicin (250 µg), and Penstrept (150 IU/300 µg)] on nutrient agar plates seeded with cultures of *E. coli*. The outcome showed that the *E. coli* isolates were resistant to oxytetracycline, streptomycin, tylosin and colistin while susceptible to gentamicin and penstrept® (penicillin-streptomycin).

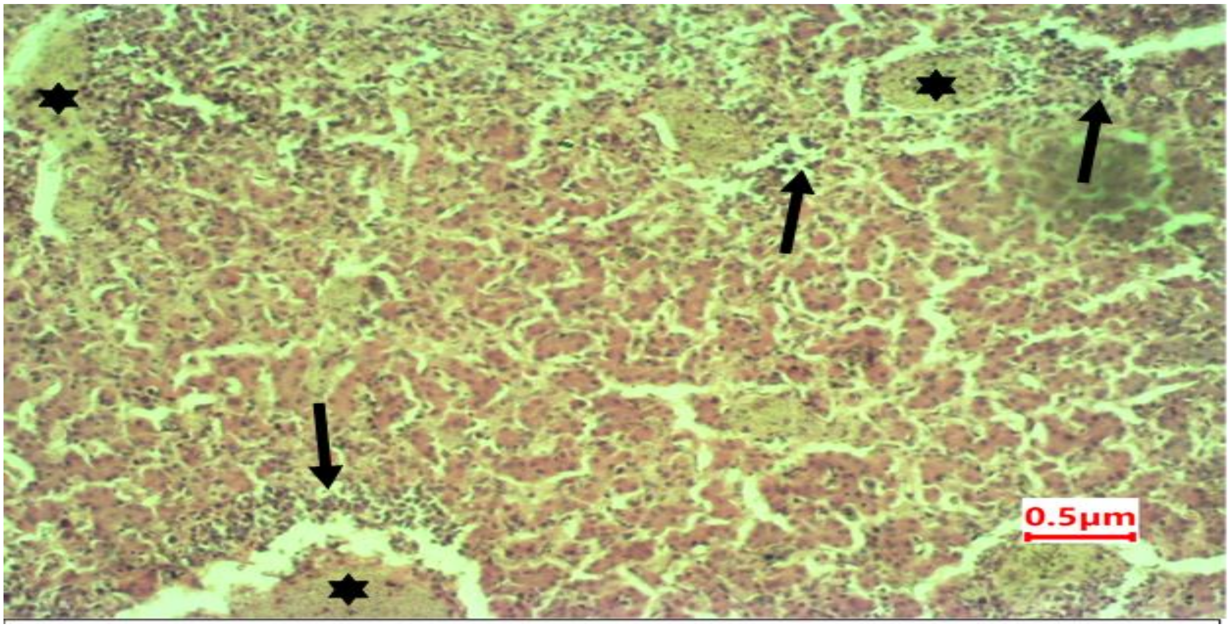

Plate III. Liver showing severe congestion of blood vessel (asterisk), diffused cellular infiltration (arrows), H &E X100

Serological evaluation of NDV antibody titre using Haemagglutination inhibition test

The haemagglutination inhibition (HI) test is based on the principle that the viral haemagglutinin can elicit agglutination of chicken red blood cells, which can be inhibited by specific antibodies. The HI test conducted in this case report was as described by World Organisation for Animal Health. Serum samples analysed for Newcastle disease antibodies by haemagglutination inhibition (HI) test showed that 14 out of 16 samples (87.5%) had a titre of 1:1 (0 Log2) being below 1:16(4Log 2) which is the minimum protective ND antibody titre against the lethal effect of the field ND virus. The minimum and maximum titres being 1:4096 (12 log 2) and 1:512 (9log 2), while the modal titre is 1:1. The geometric mean titre (GMT) 21/16 (1.31), and the minimum protective titre is 1:16. These unusually high titres were commonly seen in birds that had not received any inactivated or live mesogenic ND vaccine, hence, it is indicative of infection titres. With 87.5% of samples with no detectable ND antibody titres, this indicates that most birds were not protected against Newcastle disease.

Plate IV: Characteristic greenish metallic sheen of *Escherichia coli* on Eosin Methylene Blue agar

Treatment and outcomes

Based on the results of clinical history, postmortem investigation, bacterial culture, and serology, the case was diagnosed as Newcastle disease virus with concurrent colibacillosis. The remaining birds were treated with Penprovit® (Penicillin + Streptomycin+ vitamins) at 100g/100 litres of drinking water X 1/52 (+3 days). Also, Polidine® (Iodine solution) 1mL/2 litres of drinking water X1/52 was also administered. Ten days post-treatment, mortality pattern had dropped from an average of 42 mortalities within the first 3 days of treatment to zero mortality by day 10 (Figure 1).

Discussion

Newcastle disease (ND) with concurrent *Escherichia coli* (*E. coli*) infections poses a significant challenge to poultry producers worldwide (Abalaka *et al*., 2017; Ekiri *et al*., 2021). The poultry production value chain is threatened by outbreaks of infectious diseases such as ND, infectious bursal disease (IBD), and Highly Pathogenic Avian influenza (HPAI) diseases that are characterised by high mortality and morbidity (Abalaka *et al*., 2017; Badau *et al*., 2024). In this case report, in addition to the 50 deaths recorded before the case was presented to the VTH, a total of 179 birds died during the 10-day treatment period, with approximately 70 fatalities on the second day and none on the tenth day. Morbidity and mortality associated with ND could lead to a reduction in production, profitability, and long-term financial viability of the poultry production value chain. This is more worrisome, especially in Low- and Middle-Income Countries (LMICs) where there is a high rate of vaccination failures due to inadequate cold chain facilities, lack of access to veterinary care, lack of active monitoring of vaccine seroconversion in birds, and a lack of effective implementation of farm biosecurity measures (Edward & Ogbonna, 2023). Furthermore, even though the birds were vaccinated with live attenuated vaccines, yet were susceptible to ND as observed in the lack of seroconversion using the HI test. This agreed with the work of Sajo *et al*. (2023), who reported a severe form of ND in vaccinated birds which continuously shed the virus in faeces. Also, the endemic nature of NDV could be a result of the emergence of newly identified virus sub-genotypes (Alsahami *et al*., 2018). While viral culture remains the gold standard for isolation and confirmation of NDV, the use of serologic tests like haemagglutination inhibition (HI) and enzyme-linked immunosorbent assay (ELISA) tests gives promising outcomes. These tests are relied upon for the diagnosis of ND, estimating the seroprevalence of ND in poultry, and determining protective antibody titres in birds. This underscores the importance of laboratory diagnosis in accurately determining causal relationships with disease occurrence.

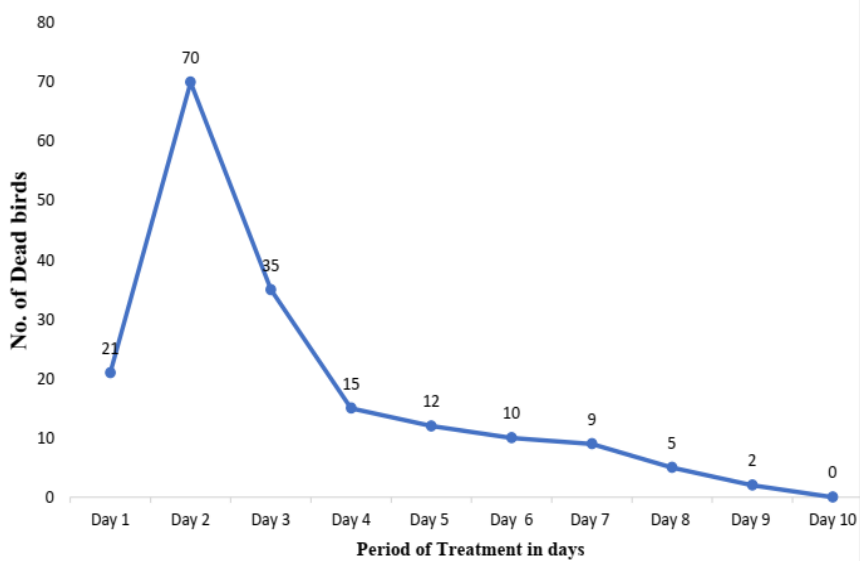

Figure 1: Ten-day mortality pattern of pullets showing a drop in bird mortality after commencement of treatment

Gross lesions observed in this case report included respiratory-associated lesions, distended ureters, cloudy air sacs, slightly mottled spleen, congested caecal mucosa, and diffused petechial haemorrhage on the enteric mucosa. While there is no particular lesion that is characteristic of ND, petechial haemorrhages on the intestinal mucosa, and congested caecal mucosa have been reported to be associated with acute stages of viscerotropic velogenic ND virus infections (Badau *et al*., 2024). The findings in this case report were consistent with histopathologic changes of ND in poultry.

In this case, the farmer used untreated well water on the farm. This could be the source of the secondary bacterial infection implicated in the disease condition because *Escherichia coli* is a common indicator for faecal contamination of water and is known to exacerbate other viral disease conditions, especially when the host immune system is compromised. The *Escherichia coli* identified in this case though not confirmed as pathogenic could be nephropathogenic, leading to severe kidney damage and complicating the clinical picture. Early intervention with appropriate antibiotics, combined with supportive care and vaccination, can reduce mortality and improve recovery, but prevention remains crucial. To prevent future occurrence, the farmer was advised to sanitize bird's drinking water with Isochlor® (55% sodium dichloroisocyanurate) at 1 tablet per 1,000L of water and to ensure the implementation of strict biosecurity measures, including proper feed storage, limiting unauthorised access to the farm, regular cleaning, and disposal of litters, flock health monitoring, separation of sick birds, and good personnel hygiene protocols. Prevention and control of poultry diseases require comprehensive biosecurity, vaccination, and good management practices. Additionally, birds that survived were treated with Penprovit ® (Penicillin + Streptomycin + Vitamins) at 100g/ 100L of drinking water for 10 days. This drug combination is therapeutically effective against many Gram-positive and Gram-negative bacteria like *E. coli*. The use of this drug combination was the result of direct antimicrobial susceptibility test conducted, which informed the antibiotic of choice to use. The farmer was advised to always ensure day-old chicks are screened for maternal antibodies against infectious bursal disease, Newcastle disease, and other bacteria that could be vertically transmitted. Additionally, strict biosecurity should be maintained in the poultry farm, including routine treatment of water with Isochlor® (55% sodium dichloroisocyanurate) at 1 tablet/1,000L of drinking water, and always seek professional advice when birds show signs of illness. A major limitation of this report is the absence of molecular characterization of the identified pathogens (Newcastle disease virus and *Escherichia coli*), which would have accurately identified the circulating strains in the flock. Molecular assays are robust, sensitive and specific. This case report highlights the need for improved diagnostic methods and management practices to mitigate the impact of Newcastle disease and colibacillosis on the poultry industry.

In conclusion, this case underscores the importance of active disease monitoring, robust vaccination protocols, and biosecurity measures in preventing outbreaks of ND complicated by *E. coli*. A comprehensive management plan incorporating vaccination, rapid therapeutic intervention, and strict farm hygiene can significantly reduce the impact of such infections.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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