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A case report on colibacillosis outbreak in a poultry flock of laying birds in a farm in Southeastern Nigeria

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Abstract

Colibacillosis is a disease caused by *Escherichia coli*, which affects all ages and species of birds. This case report is on an outbreak of colibacillosis in a flock of 600 laying chickens in a poultry farm, which resulted in the death of about half of the birds in the flock, over a period of four weeks. Clinical signs exhibited by the layers included ruffled feathers, lethargy, persistent coughing, alterations in vocalization, respiratory distress and anorexia. Postmortem examination was carried out on some of the dead birds. Tissue samples were collected from different organs of the dead birds at necropsy for bacterial culture and sensitivity test. Blood samples were collected from the sick birds for haematology and serum biochemistry. The post-mortem findings included distended gall bladder, fluid in the thoraco-abdominal cavity, friable liver, haemorrhage in the proventriculus, cloudy air sacs and brownish intestinal contents. Bacterial culture produced colonies with green metallic sheen suspected to be *E. coli*. Sensitivity test revealed resistance to almost all antibiotics except for tetracycline and azithromycin. The haematology results showed anemia, leukocytosis, lymphocytosis and heterophilia. The serum biochemistry revealed a higher than normal serum activity of alanine aminotransferase and calcium level. Serum activity of aspartate aminotransferase and alkaline phosphatase and total bilirubin were below the reference values. Levels of serum total protein and albumin were within the reference range. The birds were treated with tetracycline and multi-vitamin and mineral supplements for 5 days, and later given *E. coli* vaccine and re-vaccinated against Newcastle disease. The birds recovered fully after treatment. Implementation of adequate biosecurity measures was advised, to prevent further recurrence.

Keywords: Colibacillosis; Case Report; Layer chickens; Haematology; Serum biochemistry; Anti-microbial resistance.

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Introduction

Colibacillosis is caused by a pathogenic variant of *Escherichia coli* (E. coli) and represents one of the most prevalent and economically detrimental bacterial diseases affecting avian species globally. The disease leads to significant morbidity, mortality and losses amounting to millions of dollars annually (Barnes *et al.,* 2013). Colibacillosis manifests as a localized or systemic infection, characterized by colisepticaemia, haemorrhagic septicaemia, coligranuloma, swollen head syndrome, omphalitis/yolk sac infection and enteritis. Factors that predispose avian species to colibacillosis include stress induced by concurrent infections such as mycoplasmosis, infectious bursal disease (IBD) virus, infectious bronchitis (IB) virus and Newcastle disease, as well as adverse alterations in environmental conditions including temperature, humidity, ammonia levels and dust present on poultry farms, nutritional inadequacies, environmental contamination, sub-optimal ventilation, and contaminated water (Kabir, 2010; Barnes *et al*., 2013). The mode of transmission of the disease include contamination of feed and water by faecal matter from infected individuals, with additional transmission by vectors such as rodents and insects (Vegad and Katiyar, 2015).

Poultry farming constitutes a significant component of agricultural systems in Nigeria, particularly within the south-eastern region, where it serves an essential function in enhancing food security and fostering economic development. This sector supplies the populace with animal protein-based food resources. According to Sorensen (2010), poultry ranks fourth among the sources of animal proteins available for human consumption in Nigeria, contributing approximately 27% to the national meat production. Poultry farming is done in substantial quantities, with chickens being the predominant species; over 50 billion chickens are raised annually to provide food, both in terms of meat and eggs (Paula, 2015). The production of eggs fundamentally entails the utilization of high quality layer birds specifically for table eggs (Ogunlade and Adebayo, 2009).

The significance of poultry farming to the Nigerian economy cannot be overstated, as it has garnered popularity among smallholder farmers who have made substantial contributions to the nation's economic landscape. In Nigeria, poultry accounts for approximately 15% of the overall annual protein intake, with an estimated consumption of around 1.3 kg of poultry products per capita per year (Ologbon and Ambali, 2012). The poultry industry has gained increased prominence in enhancing employment opportunities and animal food production in Nigeria. Layer production constitutes a crucial sector, particularly within developing nations, to satisfy household food requirements and serve as an additional source of income (FAO, 2014). In the year 2000, global egg production reached 50.4 million tons, with an estimated 53.4 million tons of table eggs produced in 2002. By 2009, an estimated 62.1 million metric tons of eggs were produced worldwide, deriving from a total laying flock of approximately 6.4 billion hens (Plantz, 2001; Memon *et al*., 2015). In 2013, the Nigerian poultry industry was valued at ₦80 billion ($600 million) and comprised approximately 165 million birds, which collectively produced 650,000 metric tons of eggs and 290,000 metric tons of poultry meat during that year (Nwuneli, 2015).

This case report presents a case of colibacillosis and possible co-infection with other diseases like Newcastle disease in a 65-weeks old laying flock of 600 Isa Brown layers, which led to mortality of 300 of the layers over a period of four weeks.

Case History and Clinical Observations

A farmer in Nsukka, Southeast Nigeria reported a mortality of 300 out of 600 layers in a 65-week-old flock over a period of four weeks. Some weeks earlier the farmer had reported suboptimal egg-laying, and diminished appetite. Clinical observation of the birds showed ruffled feathers, lethargy, persistent coughing, alterations in vocalization and respiratory distress. The birds were fed on a commercial feed (Hybrid® Layer Mash). The flock was given vaccinations against Newcastle disease as per the guidelines established by the National Veterinary Research Institute, Vom, Plateau State, Nigeria, specifically designed for laying hen prior to this incident.

Before presentation, the farmer reported treating the birds with Doxygen® (doxycycline and gentamicin), a multivitamin regimen and a deworming agent; however, this therapeutic approach did not yield any significant improvement. Subsequently, three ailing birds from the group of 300 were brought to a private veterinary clinic, with complaints of anorexia, brownish diarrhea, weakness and mouth-breathing**.**

The farm was visited and blood samples were collected from ten of the sick birds for haematology and serum biochemistry analysis. Nasal, oropharyngeal and cloaca swab samples were taken from some of the sick birds for bacteria culture and sensitivity. Fecal sample from sick birds were equally taken for faecal analysis and for culture and sensitivity. Smear from the bacterial culture was made and stained appropriately and viewed using a microscope to identify and confirm the bacteria present.

For haematology, packed cell volume (PCV**)** was done using the microhaematocrit method (Thrall and Weiser, 2002). Haemoglobin (Hb) concentration was determined by the cyanomethaemoglobin method (Higgins *et al*., 2008a). Red blood cell (RBC) counts and total white blood cell (TWBC) counts were done using a haemocytometer (Thrall and Weiser, 2002). Differential leukocyte count was done using stained blood smears made on glass slides, following the longitudinal counting method (Thrall and Weiser, 2002). The PCV, Hb. concentration and RBC counts of the sick birds were lower than the reference values, while the TWBC, lymphocyte and heterophil counts were higher than the reference values (Table 1).

Table I. Haematological profile of layer chicken that had colibacillosis, compared with reference values.

Haematology Parameters	Means ± SEM (n = 10)	Reference values*
Packed cell volume (%)	18.23± 0.21	22 – 35
Haemoglobin concentration (g/dl)	6.83 ± 0.54	07 – 13
Red blood cell count (10^6/µl)	2.18 ± 0.72	2.5 – 3.5
Total white blood cell count (10^3/µl)	14.83 ± 1.09	1.2 – 3
Lymphocyte count(10^3/µl)	9.05 ± 0.60	0.5 – 2.2
Heterophil count (10^3/µl)	4.82 ± 0.46	0.4 – 1.5
Thrombocyte count (10^6/µl)	288.88 ± 16.99	
Mean corpuscular volume (fl)	85.13 ± 1.50	80 – 130
Mean corpuscular haemoglobin concentration (g/dl)	33.54 ± 0.52	26 – 35

* Source: Nanbol *et al.* (2016).

Serum biochemistry assays were done using spectrophotometric methods. Determination of the serum activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were done following the Reitman-Frankel colorimetric method (Colville, 2002) using Randox® ALT and AST test kits respectively (Randox Laboratories Ltd., United Kingdom). Alkaline phosphatase (ALP) activity was determined by the phenophthalin monophasphate method using Randox® ALP test kit (Randox Laboratories Ltd., United Kingdom). Determination of serum creatinine was based on the modified Jaffe method (Lamb and Price, 2008) using Quimica Clinica Applicada (QCA) creatinine test kit (QCA, Spain). Serum urea determination was done by the modified Berthelot-Searcy method (Lamb and Price, 2008) using Randox® urea test kit (Randox Laboratories Ltd., United Kingdom). The determination of total serum protein levels was done following the direct Biuret method (Johnson, 2008), using Randox® total protein test kit (Randox Laboratories Ltd., United Kingdom). Serum albumin was determined based on the bromocresol green method (Johnson, 2008) using Randox® albumin test kit (Randox Laboratories Ltd., United Kingdom). Serum globulin value was calculated by subtraction of the albumin value from the total protein value and expressed in g/dl (Johnson, 2008). The serum total bilirubin was determined by the modified Jendrassik-Grof method (Higgins et al., 2008b) using a QCA® total bilirubin test kit (QCA, Spain). The serum ALT activity and serum calcium levels of the sick birds were higher than the reference values earlier reported by Nanbol *et al* (2016), while the serum AST and ALP activities of the sick birds were lower than the earlier reported reference values (Table 2).

Table 2. Serum biochemistry profile of layer chickens that had colibacillosis, compared with reference values.

Clinical Chemistry Parameters	Means ± SEM (n = 10)	Reference values *
Alanine Aminotransferase (IU/L)	14.90 ± 2.43	7 – 12
Aspartate Transaminase (IU/L)	36.37 ± 7.08	70 – 120
Alkaline phosphatase (IU/L)	26.04± 2.01	80 – 250
Total Protein (g/dl)	4.51 ± 0.72	3.6 – 7.1
Albumin (g/dl)	2.76 ± 0.07	1.2 – 3.7
Globulin (g/dl)	2.98 ± 0.14	1.5 – 4.4
Bilirubin (mg/dl)	0.21 ± 0.12	0.3 – 1.0
Urea (mg/dl)	4.30 ± 0.58	4.0 – 20
Creatinine (mg/dl)	0.61 ± 0.21	0.1 – 0.9
Calcium (mg/dl)	11.72± 1.55	4.1 – 8.0
Uric acid (mg/dl)	2.7 ± 0.82	0.6 – 9.5

* Source: Nanbol *et al*. (2016).

Postmortem Findings: Postmortem examination was carried out on three dead birds presented at the clinic. Observations at necropsy included distended gall bladder and cloudy air sacs (Figure 1), red to brown intestinal contents (Figure 2), excessive fluid content in the thoraco-abdominal cavity, congested and friable liver (Figure 3), petechial hemorrhage in the proventriculus, and greenish colour of intestinal contents (Figure 4).

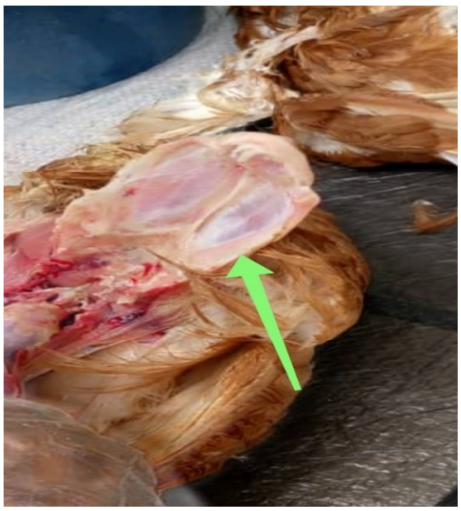

Figure 1: Carcass of layer chicken that had colibacillosis showing cloudy air sac.

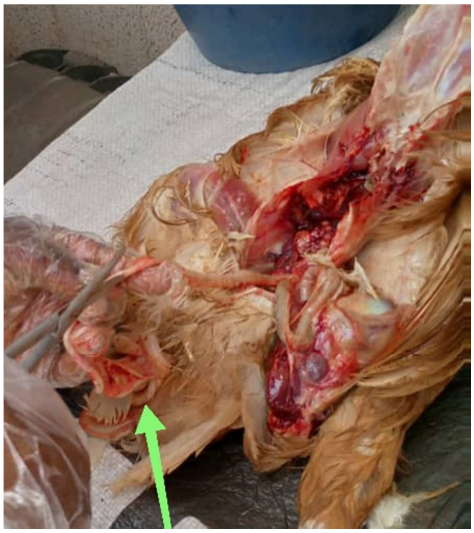

Figure 2: Brownish intestinal content of layer chicken that had colibacillosis.

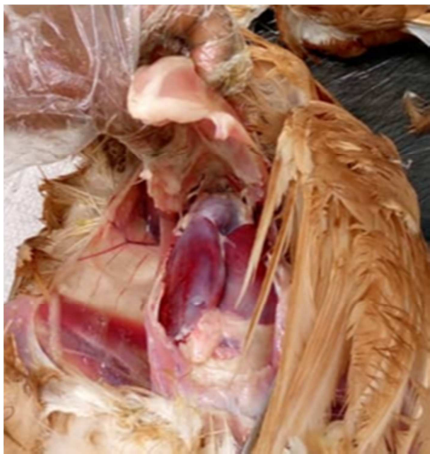

Figure 3: Presence of friable liver and excessive fluid in the thoraco-abdominal cavity of carcass of layer chicken that had colibacillosis.

Figure 4. Petechial haemorrhage on the proventriculus (green arrow) and greenish intestinal content (red arrow) on carcass of layer chicken that had colibacillosis.

Bacterial culture and Sensitivity: Bacterial culture of samples taken from the nasal, oropharyngeal, cloaca swab and fecal samples showed colonies that exhibited gram-negative characteristics, varying in morphology from mucoid forms that are small to larger specimens displaying a convex appearance ranging from white to grayish colorations. Further laboratory analyses indicated that the colonies possessed motility, demonstrated lactose fermentation, and were negative for citrate utilization and urease production, while

presenting positive results for methyl red (MR) and negative results for Voges-Proskauer (VP). Based on these findings, the bacterial species was classified as *Escherichia coli.* Further, *E. coli* was confirmed after making a smear and staining with crystal violet and Gram's iodine then decolorizing with acetone, and when viewed under the microscope, several pinkish-red rods were identified which are consistent with the morphology of *E. coli.* Antibiotic susceptibility assays demonstrated that the organism showed resistance to nearly all categories of antibiotics employed in poultry management, with the exception of azithromycin and tetracycline.

Diagnosis

Colibacillosis was diagnosed based on the clinical signs observed, the post–mortem findings, laboratory analysis and the results of bacterial culture of the organisms (*E.coli*).

The possibility of a co-infection with Newcastle disease (ND) was not completely ruled out, due to the high number of mortality and some other post-mortem lesions which pointed to ND, but knowledge of the fact that the flock was vaccinated and re-vaccinated against Newcastle disease restrained the inclusion.

Toxicity was also suspected as one of the differential diagnosis, possibly from the water source which was a shallow well, as the farmer was advised to get another source of water, until the well water was tested and found fit for consumption.

The feed and stores were examined for molds which could lead to aflatoxicosis, which was one of the differential diagnosis, however after assessment for molds the feeds were declared free of molds hence ruling out aflatoxicosis.

Treatment and Management

The flock was managed by treatment with tetracycline and vitamins-mineral supplements (Vitalyte®) administered for five days, and later vaccinated using *E. coli* vaccine. The birds responded favourably to the treatment. Improved biosecurity measures were instituted to prevent further outbreaks of colibacillosis and other bacterial or viral diseases.

Discussion

Avian colibacillosis has been reported to occur in all age groups of chicken, with a prevalence range of about (9.52 to 36.73%), and with an especially high prevalence rate in adult laying birds (36.73%) (Rahman *et al*., 2004). As was seen in this present case report, the birds presented with anorexia, and earlier reports have been documented that birds with coli-septicemia become lethargic and stop eating and drinking, and in some cases that severity of the disease may be indicated by the degree of reduced water consumption (Nolan *et al*., 2015).

Antibiotics sensitivity tests on the isolated organism showed that it was resistant to almost all the classes of antibiotics used in poultry, except azithromycin and tetracycline. The high level of resistance to almost all antibiotics indicates the severity of anti-microbial resistance in Southeastern Nigeria, which calls for more reforms and regulations in the indiscriminate use of antibiotics: farmers should be informed on the potential crisis of this situation and the general implication to global health.

The results of haematology determinations in the present case which showed that the sick birds had lower than normal PCV, Hb. concentration and RBC counts is a pointer to anaemia. The anaemia recorded in the present case report differs from earlier reports by Amin *et al* (2020), which showed no significant

alterations in the erythrocytic profile of birds with colibacillosis. There was marked leukocytosis with marked lymphocytosis and heterophilia in the sick birds. Leukocytosis is an indication of an active infection (Jain, 1986), and the heterophilia present is also most likely associated with severe bacterial (colibacillosis) infection and other possible co-infections. The lymphocytosis may be a body response to the invading organism and inflammation associated with the infectious condition (Maxwell, 1993).

The serum biochemistry results of the sick birds which showed a higher than normal serum ALT activity suggests hepatocellular damage. Earlier reports by Koynarski *et al*. (2010) have shown that colibacillosis in birds was associated with higher than normal serum ALT activity. The lower than normal serum ALP acivity recorded in the present case report contrasts with earlier reports by Amin *et al*. (2020) who documented a higher serum activity of ALP in birds with colibacillosis. The serum levels of total proteins, albumin and globulin in the present case report were all within reference ranges; this does not concur with earlier reports by Jindal *et al*. (2003) which showed that birds with colibacillosis had higher levels of serum total protein and globulins. The marked hypercalcemia and hypo-bilirubinemia recorded in the present case are also worthy of note.

The possible co-occurrence of ND with the colibacillosis in the present case report was strongly suspected, as there had been earlier reports of such co-occurrence (Bitrus *et al*., 2025).

Based on the report, observation and laboratory findings, it was concluded that the laying flock was affected by colibacillosis that led to mortality of about half of the flock across a period of four weeks, and treatment with tetracycline and multivitamins and later vaccination with *E. coli* vaccine led to recovery.

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Conflict of Interest

The authors declare that there is no conflict of interest

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