CASE REPORT

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Pathological changes associated with an outbreak of colibacillosis in a commercial broiler flock

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Abstract

Escherichia coli infection was diagnosed in 5-week old broiler chickens raised intensively on a medium-sized commercial farm in Gaube-Kuje, Abuja, Nigeria. Signs of weakness, depression and inappetance with ruffled feathers and pasted vents were reportedly observed in affected birds within the flock. Detailed post mortem examinations revealed diffuse splenomegaly and hepatomegaly with multifocal greyish areas on their surfaces while the diffusely enlarged kidneys were congested with mottled pale appearance. Histopathologically, the liver of affected broilers showed diffuse congestion, multifocal coagulative necrosis and cellular infiltration. Generalized perivascular and inter-septal oedema and haemorrhage were observed in the lungs of affected broilers with generalized lymphocytic depletion within the spleen as well as locally extensive congestion and haemorrhage within the kidney, and cellular infiltration and necrosis within heart musculatures. Microbiological evaluation of liver samples yielded pure *E. coli* growth only. A diagnosis of colibacillosis, especially colisepticaemia, was made with appropriate treatment based on culture and sensitivity test result involving Levofloxacin(R). The client was consequently advised to guard against possible predisposing factors as control and preventive measures for the disease outbreak on the farm.

Keywords: Broiler chickens, Colibacillosis, Gross pathology, Histopathology, Natural infection

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Introduction

Avian colibacillosis is an infectious disease of chickens caused by *Escherichia coli* (*E. coli*) either as a primary pathogen or as a secondary pathogen (Kabir, 2010). This is because *E. coli* is a natural inhabitant of the intestinal tract of chickens, although it is capable of producing disease under certain stressful conditions such as overcrowding, poor ventilation, malnutrition, extreme temperature and immunosuppression (Kabir, 2010). It is one of the main causes of economic losses in the poultry industry (Yogaratnam, 1995; Ewers *et al*., 2003), particularly in broiler chickens (Cavero *et al*., 2009), through morbidity, lack of flock growth uniformity, lowered production, increased condemnation at slaughter plants and mortality (Gross, 1991). However, *E. coli* infection in poultry is associated with varieties of disease conditions in poultry ranging from pericarditis, perihepatitis, airsacculitis, peritonitis, salpingitis, panophthalmitis, omphalitis, cellulitis, colisepticaemia, coligranuloma to swollen-head syndrome (Wray *et al*., 1996). Although pathological investigations of natural colibacillosis are well documented (Tonu *et al*., 2011; Bhalerao *et al*., 2013; Srinivasan *et al*., 2014), information on pathological changes associated with natural colibacillosis in broiler chickens in Nigeria is scanty, hence the need for the study. There is therefore, a need for continuous pathological evaluation of field

cases of *E. coli* infection in broiler chickens in Nigeria, which is a viable part of the country's poultry industry. This study is aimed at evaluating the pathological changes associated with a natural outbreak of colibacillosis in broiler chickens in Gaube-Kuje, Abuja, Nigeria.

Case Report

History

Mortality was reported in 5-week old broiler chickens on a medium-sized commercial poultry farm in Gaube-Kuje, Nigeria as shown in Figure 1. This involved a total mortality of 691 chickens (15.80%) between day 15 and day 34 (35 weeks of age) from a stock of 4373, when the case was reported, and a detailed post mortem examination was conducted. About 55% morbidity was recorded on the farm during the course of the present disease outbreak. Birds were raised intensively on the floor in open-sided pens, whose sides were covered with tarpaulin that can be rolled up or down depending on prevailing weather conditions. History further revealed that the flock was vaccinated against Newcastle disease at day old (intraocular) and on day 18 or 3rd week of life (orally) with NCD Lasota while first infectious bursal disease (IBD) vaccine was administered orally on day 11th or 2nd week of life. Prophylactic anticoccidial treatment was done for three consecutive days between days 12 and 14 or 2nd week of life, after the IBD vaccination. Similarly, prophylactic antibiotics therapy was instituted for three consecutive days between days 2 and 8 (1st week) and between days 19 and 21 or 3rd week of age, respectively. Affected birds within the flock were reportedly depressed, weak and anorexic with pasted vents. However, there was no drastic reduction in mortality even when the entire flock was placed on Amoxy-Col WSP(R) (Amoxicillin trihydrate – 200 mg and colistin sulphate – 1,000,000 IU) and antibiotic medication @ 100 g per 150 L of drinking water for three days between days 32 and 34 before the case was reported for investigation.

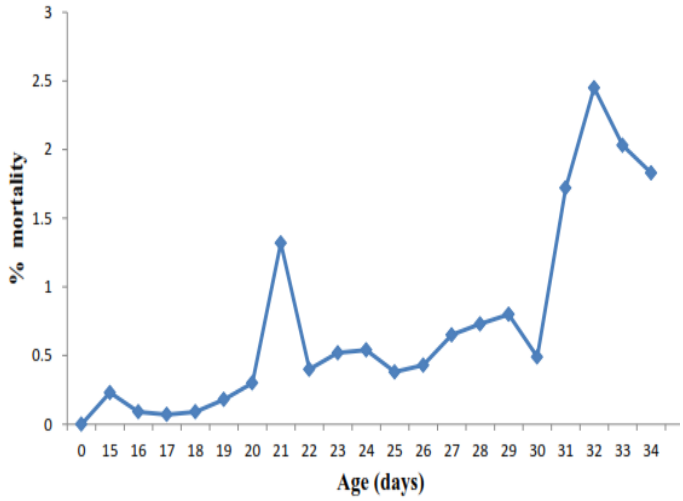

Figure 1: Daily percentage (%) mortality pattern in 5-week old broiler chickens on a medium-sized commercial poultry farm in Gaube-Kuje, Abuja, Nigeria.

Post-mortem examination and sample collection

A detailed post-mortem examination was performed on the presented carcasses. The liver, lungs, spleen, kidney and heart samples were collected for histopathological evaluation, which were initially fixed in 10% formalin prior to paraffin embedding, sectioning at 5µm and staining with haematoxylin and eosin for microscopic examination under varying magnifications according to standard procedure (Bancroft & Cook, 1994). The severity of histopathological changes in the liver, lung, spleen, kidney and heart of affected birds was determined semi-quantitatively by modifying the degree of tissue change (DTC) method described by Poleksic & Mitrovic-Tutundzic (1994). Briefly, types of histopathological changes in stages I, II and III of the degree of tissue change protocol were screened for each organ per chicken using the formula: DTC = (1 x ∑ I) + (10 x ∑ II) + (100 x ∑ III) as shown in Table 1. stage I lesions were those of

Table 1: Histopathological changes associated with the stages of the degree of tissue changes in organs of affected broiler chickens

Stages of the degree of tissue change	Histopathological changes
I	Cloudy swelling, hydropic change, hyaline change, glycogenic degeneration, fatty degeneration, amyloidosis, gout, fibrinous degeneration
II	Oedema, congestion, thrombosis, haemorrhage, cellular infiltration, haemosiderosis
III	Nuclear change (pyknosis, karyorrhexis and karyolysis), increased cytoplasmic eosinophilia, total absence of the cell, calcifications

degenerative changes and stage II lesions were those associated with vascular damage, while stage III lesions were lesions of cellular death. Degree of tissue change values were variously interpreted as mild organ damage (1– 21), moderate organ damage (22 – 50) and severe organ damage (≥ 51). Similarly, microbiological swabs were aseptically collected from livers of affected birds and preserved on ice packs. These were transported and submitted to the Microbiological Unit, Laboratory Services of University of Abuja Teaching Hospital, Gwagwalada, Nigeria for bacteriological examination according to standard procedures using cultural and morphological characteristics as well as biochemical properties for identification (Chessbrough, 2006).

Results

Gross pathology

Gross examination of the carcass of some affected birds showed diffusely enlarged liver and spleen with multifocal greyish areas on their surfaces as well as diffusely enlarged, congested and pale mottled kidneys as shown in Table 2 and Plates I – III.

Histopathology

Histopathological examination of the liver revealed the presence of diffusely congested central veins and sinusoids, multifocal coagulative necrosis, locally extensive cellular infiltrations and dilated sinusoids

Table 2: Gross lesions in organs of 5-week old broiler chickens naturally infected with *Escherichia coli* from a medium-sized commercial poultry farm in Gaube-Kuje, Abuja, Nigeria

Gross lesions	Affected organs (% incidence)				
	Liver	Kidney	Spleen	Lung	Heart
DE	100.0%	100.0%	60%	-	-
CG	-	100.0%	-	40.0%	-
MGA	80%	100.0%	40.0%	-	-

DE – diffuse enlargement; CG – congestion; MGA – mottled greyish areas

Plate I: Photograph of a liver of 5-week old broiler chicken naturally infected with *Escherichia coli* on a medium-sized commercial poultry farm in Guabe-Kuje, Abuja, Nigeria: Note the diffusely enlarged liver with multifocal greyish areas (arrows)

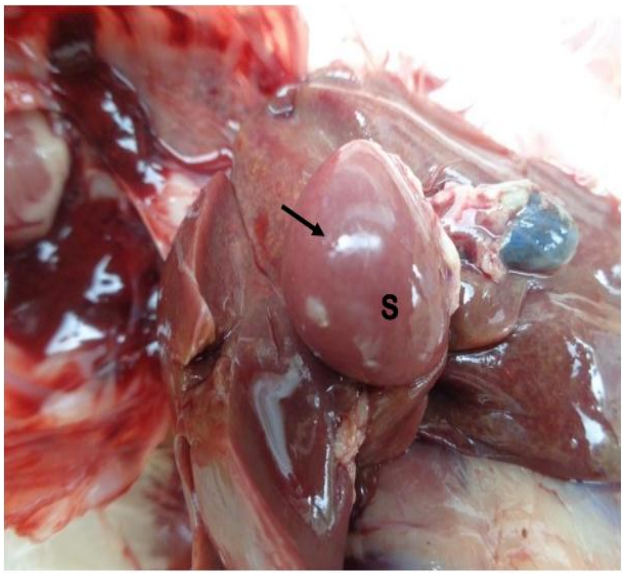

Plate II: Photograph of a spleen of 5-week old broiler chicken naturally infected with *Escherichia coli* on a medium-sized commercial poultry farm in Guabe-Kuje, Abuja, Nigeria: Note the diffusely enlarged spleen (S) with faint multifocal greyish areas (arrow)

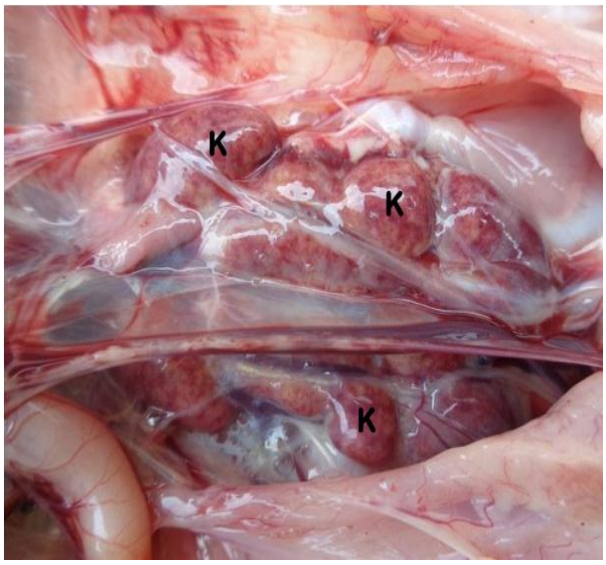

Plate III: Photograph of a kidney of 5-week old broiler chicken naturally infected with *Escherichia coli* on a medium-sized commercial poultry farm in Guabe-Kuje, Abuja, Nigeria: Note the diffusely enlarged, congested and pale mottled kidney lobes (K)

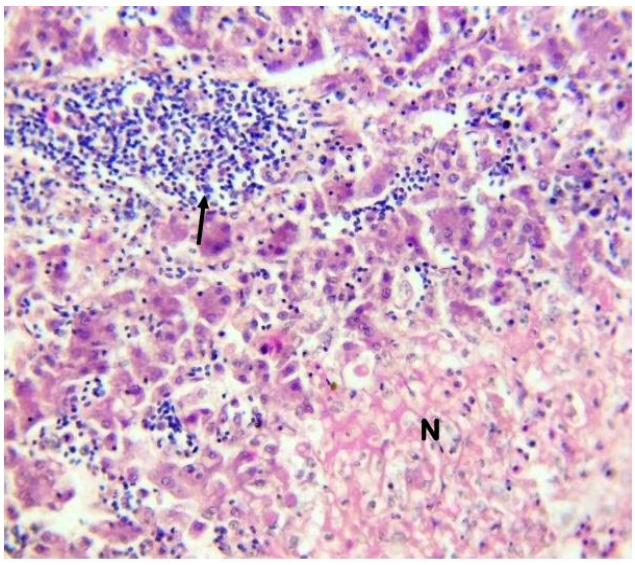

Plate IV: Photomicrograph of a liver of 5-week old broiler chicken naturally infected with *Escherichia coli* on a medium-sized commercial poultry farm in Guabe-Kuje, Abuja, Nigeria: Note the cellular infiltration (black arrow) and coagulative necrosis of hepatocytes (N). H & E stain x 307

Table 3: Histopathological changes in tissue samples of 5-week old broiler chickens naturally infected with *Escherichia coli* from a medium-sized commercial poultry farm in Gaube-Kuje, Abuja, Nigeria

DTC	Histopathological changes (% incidence)				
	Liver	Heart	Spleen	Kidney	Lung
I	-	-	-	-	-
II	CI (80.0%)	CI (40.0%)	-	CG (80.0%)	OD (60.0%)
	CG (80.0%)	-	-	HM (80.0%)	HM (60.0%)
III	-	-	LD (40.0%)	-	-
	CN (60%)	NE (40.0%)	CN (40.0%)	-	-

DTC – degree of tissue change; CI – cellular infiltration; CG – congestion; OD – oedema; HM – haemorrhage; LD – lymphoid depletion; CN – coagulative necrosis; NE – necrosis

Table 4: The degree of tissue change in tissue samples of 5-week old broiler chickens naturally infected with *Escherichia coli* from a medium-sized commercial poultry farm in Gaube-Kuje, Abuja, Nigeria

Sampled tissue	Degree of tissue change	
	Value (mean ± SD)	Interpretation
Liver	78.0 ± 23.75	Severely damaged
Heart	44.0 ± 26.94	Moderately damaged
Spleen	40.0 ± 24.49	Moderately damaged
Kidney	16. 0 ± 4.00	Mildly damaged
Lung	16.0 ± 4.00	Mildly damaged

with leukocytes. Similarly, the kidney showed locally extensive congestion and haemorrhage. Generalized perivascular and inter-septal oedema with perivascular and parabronchial alveolar haemorrhages were seen in the lungs, while generalized lymphocytic follicle depletion and locally extensive necrosis with cellular infiltration and were evident in the spleen and hearts of some affected chickens, respectively (Table 3 and Plates IV - VIII). Interpretation of calculated DTC values in the liver, lung, spleen, kidney and hearts of some of the affected birds are presented in Table 4.

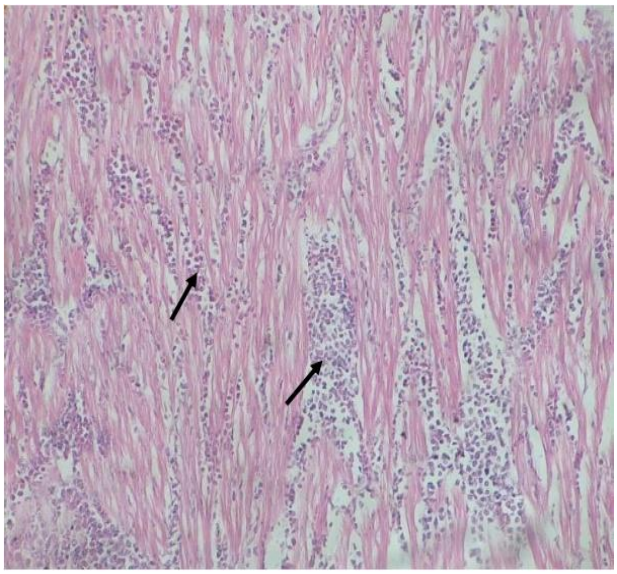

Plate Va: Photomicrograph of a heart of 5-week old broiler chicken naturally infected with *Escherichia coli* on a medium-sized commercial poultry farm in Guabe-Kuje, Abuja, Nigeria: Note the cellular infiltration of heart muscles (arrows). H & E stain x 139

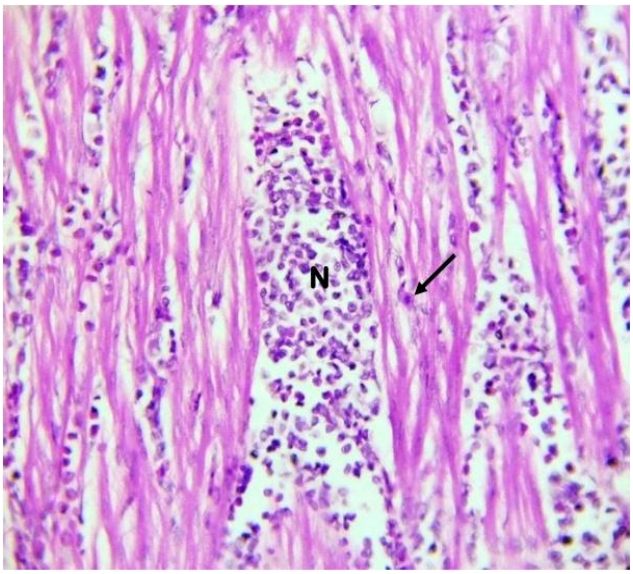

Plate Vb: (inset). Note the nucleated red blood cell (arrow) and cellular infiltration (N). H & E x 272

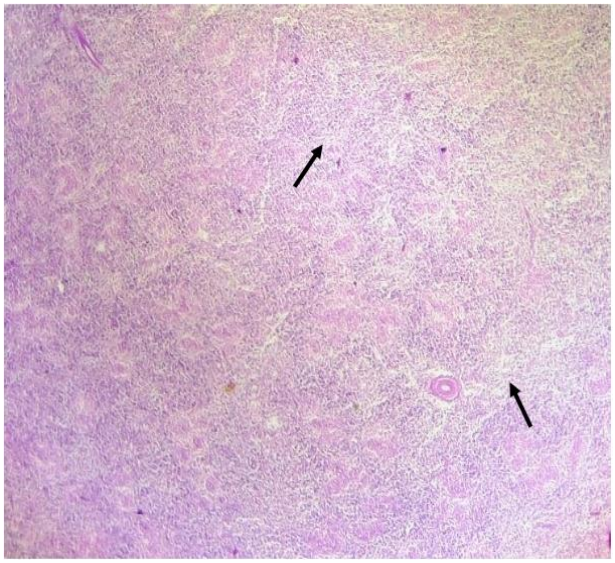

Plate VI: Photomicrograph of a spleen of 5-week old broiler chicken naturally infected with *Escherichia coli* on a medium-sized commercial poultry farm in Guabe-Kuje, Abuja, Nigeria: Note the lymphocytic follicle depletion (arrows). H & E stain x 34

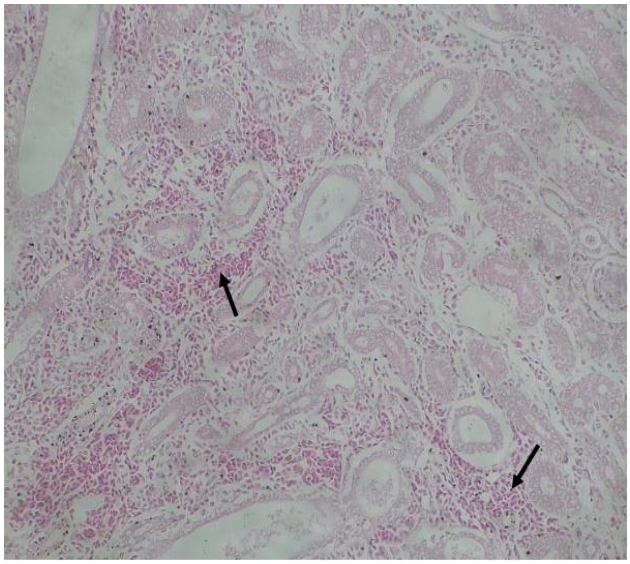

Plate VII: Photomicrograph of a kidney of 5-week old broiler chicken naturally infected with *Escherichia coli* on a medium-sized commercial poultry farm in Guabe-Kuje, Abuja, Nigeria: Note the haemorrhage within interstitial spaces (arrows). H & E stain x 131

Bacteriological culture, growth, identification and sensitivity test

Bacteriological culture of liver samples of 5-week old broilers farm in Guabe-Kuje, Nigeria yielded *E. coli* growth only from four (4) of the five (5) liver samples of affected chickens (Plate IX). Similarly, the isolated organism was a motile Gram negative rod that was urea, citrate and Voges-Praskauer negative, while being indole and beta-galactosidase positive in addition to producing yellow slant and yellow but with gas, but no hydrogen sulphide as presented in Table 5. *Escherichia coli* isolates were sensitive to levofloxacin, ciprofloxacin, nafcillin, cefataxime and tetracycline while being slightly resistant to

gentamycin, ofloxacin and completely resistant to erythromycin, cotrimoxazole, cloxacillin and clindamycin. The farm was advised to use levofloxacin ® (levofloxacin – 100 g per kg) at 10 gm per 10 L of drinking water for five days as recommended by the manufacturer. Cumulative mortality reportedly dropped to 6.59% a week after the commencement of treatment.

Discussion

The initial spike in mortality at day 21 was reportedly caused by smothering or pilling occasioned by panic response of the birds to frightening that occurred the night before, probably due to either sudden movements of rats or sudden darts of security guard`s flashlights as described by Bright & Johnson (2011). Death was due to asphyxiation occasioned by their hurdling together, overcrowding and/or piling in a corner. The second spike in mortality at day 32 was due to the underlying infection, and in spite of the fact that chickens were placed on Amoxycol WSP(R) antibiotic medication two days before the case was reported, mortality persisted. This showed lack of sensitivity to the antibiotic used to combat the condition by the client hence the timeliness of the conducted sensitivity test resulting in the recommendation and subsequent use of Levofloxacin(R). The presented clinical signs of depression, weakness and anorexia and pasted vents

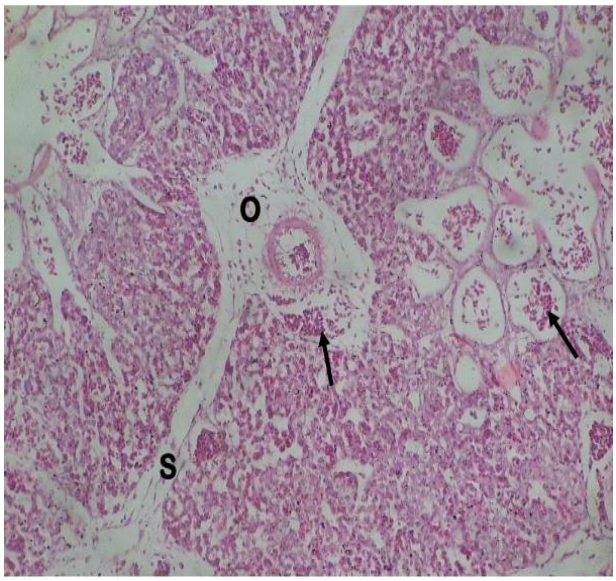

Plate VIII: Photomicrograph of a lung of 5-week old broiler chicken naturally infected with *Escherichia coli* on a medium-sized commercial poultry farm in Guabe-Kuje, Abuja, Nigeria: Note the perivascular oedema (O), interseptal oedema (S) and perivascular with parabronchi alveolar haemorrhages (arrows). H & E stain x 131

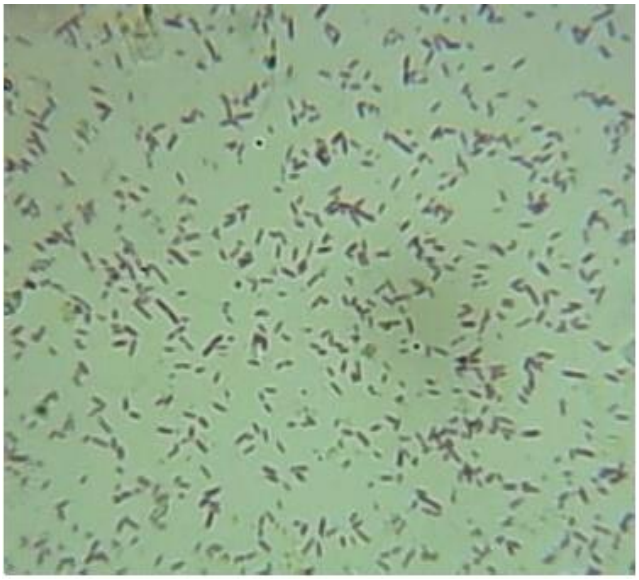

Plate IX: Isolated Gram's stained *Escherichia coli* from liver samples of 5-week old broiler chickens from a medium-sized commercial poultry farm in Gaube-Kuje, Abuja, Nigeria. Note Gram-negative short rod-shaped organisms. Gram's stain x 1000

Table 5: Characterization and identification of bacterial growth from liver samples of 5-week old broiler chcikens from a medium-sized commercial poultry farm in Gaube-Kuje, Abuja, Nigeria

Description	Indication
Gram staining	Negative
Morphology	Bacilli
Motility	Positive
Urea	Negative
Indole	Positive
Citrate	Negative
Voges-Prakauer	Negative
Beta-galactosidase	Positive
Triple sugar iron (TSI) agar	A/A Gas but no hydrogen sulphide (H_2S)
Bacterial organism	*Escherichia coli*

are non-specific depending upon the age, organs involved and concurrent diseases (Lee & Maurer, 2005). Enlarged liver (hepatomegaly) with multiple necrotic foci was also reported in the livers of broilers and layers in natural colibacillosis outbreak in Eastern Sudan (Omer *et al*., 2010). Similarly, congested and haemorrhagic kidneys have been reported (Dutta *et al*., 2013), which might be due to vascular damage caused by *E. coli* endotoxin (Srinivasan *et al*., 2014).

Histopathological evaluation showed severely damaged liver and moderately damaged spleen and heart with mildly damaged kidney, heart as well as lungs based on the DTC protocol. The observed inflammatory and necrotic lesions in the liver of affected birds might be due to *E. coli* endotoxin and vascular injury (Truscott *et al*., 1974; Thomson, 1978). The hepatic coagulative necrosis might be due to tissue hypoxia occasioned by vascular compromise (Myers *et al*., 2012). Similar focal necrosis and cellular infiltration were reported in the liver and heart of birds that came down with colibacillosis (Srinivasan *et al*., 2014). Likewise, congested central veins and sinusoids with cellular infiltration around the portal area were reported by El-Ghany & Madian (2011) in the livers of broiler chickens infected with *E. coli*. Similarly, Dutta *et al*. (2013) reported haemorrhage and degenerative tubular changes in the kidneys of *E. coli* infected pigeons. This is because when *E. coli* gets to the vascular system, most internal organs are affected (Kabir, 2010) as seen in this study. The absence of other bacterial growths apart from *E. coli* from liver samples of affected birds showed that *E. coli* was the causative agent of the outbreak as similarly reported by Omer *et al*. (2010). The fact that infectious bursal disease, Newcastle disease and infectious bronchitis antibody titres were not assayed notwithstanding, the absence of typical characteristic clinical signs associated with these common immunosuppressive diseases in this area technically ruled out a case of viral infection induced *E. coli* complication in affected chickens in this case. This is in spite of the fact that infectious bronchitis, Newcastle disease, infectious bursal disease and mycoplasma agents along with nutritional deficiencies predispose chickens to *E. coli* infection (Wray & Davies, 2001). Although *E. coli* is known to produce varieties of the disease conditions, all the described forms were not observed in this study. However, the observed pathological changes in tissues of affected birds coupled with the reported clinical manifestations were suggestive of colisepticaemia. Our findings on antibiotic sensitivity test result agreed with the report of Zinnah *et al*. (2008) on levofloxacin and ciprofloxacin but not on Tetracycline where resistance was reported.

In conclusion, the observed gross and histopathological changes were attributable to colisepticaemia based on bacteriological growth and identification of *E. coli* only during the disease outbreak in the affected broiler flock. There was a positive response to the instituted antibiotic therapy based on the sensitivity test result. Control and preventive measures should be geared towards the elimination of possible predisposing factors as *E. coli* is an opportunistic organism.

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