Pathology of Septicaemic Colibacillosis in a Guinea Fowl Breeder Flock

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ABSTRACT

The present study was undertaken to determine the cause of increased mortality in an intensively maintained 1300 guinea fowl breeder flock of 17-week-old birds during the month of August 2023. Necropsy was conducted on dead birds, and samples collected were subjected to bacteriological and pathological examinations. Affected Guinea fowl breeder flock showed the clinical signs of depression, anorexia, pasted vents and marked respiratory sounds with a cumulative mortality of 9.2 per cent over the period of two weeks. Necropsy showed good body condition, fibrinous pericarditis, congestion of lungs, greenish discolouration of liver and moderate enlargement with mottled appearance of spleen. Microbiological examination of heart blood and liver swab revealed the presence of Escherichia *coli.* The flock was successfully treated with levofloxacin (@ 10mg/kg B.wt) based on the antibiotic sensitivity test. Microscopically, fibrinous pericarditis, myocardial degeneration in the heart, vascular changes in the lungs, acute cellular degeneration and vascular changes with infiltration of inflammatory cells in the liver and lymphoid depletion and reticuloendothelial cells proliferation in the spleen were noticed. Intensively maintained guinea fowls are susceptible to *E. coli* infection, similar to that of other poultry species.

Keywords: Guinea fowl, Colisepticaemia, Pathology

INTRODUCTION

Guinea fowl is an indigenous game bird of Africa and was introduced into the Indian sub-continent during the slavery era of medieval centuries. Rich in vitamins and low in cholesterol, the guinea fowl meat is liked by consumers, and eggs can be stored longer period because of the thick egg shell (Soara *et al*., 2020). Under natural conditions, guinea fowls are resistant to diseases; however intensive system of rearing has been practiced during recent years to maximize net profit, which has predisposed them to be susceptible to multiple pathogens. Among them, *Escherichia coli* (*E. coli*) causes economic loss to these farms due to increased morbidity and mortality, reduced production performance, condemnation of carcasses and antibiotic resistance (McPeake *et al.*, 2005 and Biswas *et al*., 2006). Extensive literature was available on the prevalence and clinical manifestations of colisepticaemia in chicken, turkey, duck, geese and Japanese quail (La Ragione and Woodward, 2002). However, information on the prevalence of diseases in guinea fowl is meagre due to its resistance compared to chicken. Hence, the present study documents the clinicopathologic features observed in spontaneous septicaemic colibacillosis in a breeder flock of guinea fowl.

MATERIALS AND METHODS

During August 2023, 17-week-old guinea fowls from a flock of 1300 birds were brought to the Poultry Disease Diagnosis and Surveillance Laboratory, Veterinary College and Research Institute Campus, Namakkal for postmortem examination with the history of increased mortality. The flock owner reported that the affected birds were depressed, anorectic, with pasted vents and revealed marked respiratory sounds during night hours. Flock experienced mortality of 8 to 10 birds per day with a cumulative mortality of 9.2 per cent (120 birds) over a period of two weeks. Mortality persisted despite the entire flock being treated with antibiotics (Cotrimoxazole@ 25 mg per kg wt) in drinking water for five days before the case was brought for postmortem.

Postmortem was performed on eight dead birds. Heart blood and liver swabs collected from dead birds were placed in brain heart infusion (BHI) broth and incubated at 37°C for 24 hours, and subsequently cultured aerobically in 5 per cent sheep blood agar, MacConkey agar and eosin methylene blue agar (EMBA) for isolation of bacteria. Bacterial isolates were identified on the basis of their colony morphology, growth characteristics, sugar fermentation and other biochemical characteristics (Quinn *et al*., 2011). The bacterial isolates were tested for *in vitro* antimicrobial sensitivity test against commonly used antimicrobial agents by disc diffusion technique on Muller-Hinton agar (Bauer *et al*., 1966).

Pooled organ samples of trachea, lung, spleen, proventriculus, caecal tonsil and kidney collected from dead birds were subjected to the haemagglutination test for the detection of Newcastle disease virus (NDV) (Balachandran *et al*., 2014) and infectious bronchitis virus (IBV) (Villarreal, 2010). Random serum samples collected from 10 recovered birds of the affected flock were examined by haemagglutination inhibition test for the presence of antibodies to NDV and IBV and by enzyme linked immunosorbent assay for the *Mycoplasma gallisepticum* and *Mycoplasma synoviae* antibodies.

After external and internal examinations of tissues and organs for gross lesions, materials for histopathology were collected from the heart, lung, liver, spleen and kidney and fixed in 10 per cent neutral buffered formalin. After fixation, samples were embedded in paraffin, sectioned at 5 *µm* thickness with a rotary microtome followed by routine staining with hematoxylin and eosin. The slides were mounted with DPX mountant solution and covered with coverslips for histopathological examination.

RESULTS AND DISCUSSION

On postmortem examination, all the dead birds were in good body condition. Pectoral muscles were dark red in appearance (congested). In the heart, the epicardium was congested and covered with a thin, greyish white layer. Tracheal lumen contained mucoid exudate, and the mucosa was congested. Thoracic and abdominal airsacs were cloudy and thickened (Fig. 1). Lungs were severely congested and edematous with patches of consolidation. Liver was moderately enlarged and showed greenish discolouration (Fig. 2). Kidneys were congested with petechial hemorrhages on its surface. Spleen was moderately enlarged and congested with multiple greyish white foci (Fig. 1). The widespread morphological changes observed in visceral organs of the guinea fowl concur with subacute polyserositis observed earlier in chicken (Nakamura *et al*., 1985; Srinivasan *et*

al., 2014). In *E. coli* infections, two types of lesions occur depending on the age of birds affected. In younger birds with little resistance to infection suffer from acute septicemia and die in a fulminating course, while older birds as observed in the present case, were resistant and survived the initial septicemic stages and developed serosal lesions due to subsequent bacterial colonization (Nakamura *et* al., 1985).

Cultural examination of heart blood and liver swabs from dead birds producedlactose fermenting pink coloured round, smooth and glistening colonies on Mac Conkey's agar and black metallic sheen colonies on EMB agar. Light microscopic examination of Gram's-stained culture revealed Gram-negative, pink colored, single or pairedshort rod shaped organisms. The isolates were found to be motile with 'hanging drop' preparation under the microscope and fermented dextrose, lactose, maltose and mannitol with the production of acid and gas, but did not ferment inositol. Based on the cultural characters of the colonies, cellular morphology of the organism and biochemical reactions the isolates were identified as *E. coli* (Quinn *et al.*, 2011).

The Guinea fowls may acquire infection from a contaminated environment via the respiratory tract, feed and water or from other carrier birds. In the birds reared under an intensive system of housing, the commonest source of *E. coli* might be its own intestinal flora, which produces the diseases under favourable conditions. *E. coli* is a normal inhabitant of the poultry intestinal tract, with up to 10^6 of these bacteria per gram of intestinal contents. Approximately 10 to 15 per cent of intestinal *E. coli was* considered to be potential pathogens and it may be excreted as a result of stress induced by high flock density (Nolan *et al*., 2013). Poultry house dust may contain 10^5 to 10^6 *E. coli* per gram and these bacteria persist for long periods, particularly during low temperatures. In the present study, birds are kept in a deep litter system, which favours excessive dust formation and the microorganisms adhered to this dust. Inhalation of this contaminated dust leads to initial colonization of *E. coli* in the respiratory tract, followed by crossing and penetration into the mucosa of air sacs, then multiplication in the blood stream and visceral organs which might induce lesions and clinical signs in the affected birds (Levine *et al*., 1983). Virological examination of tissue samples was found to be negative for NDV and IBV infections. Heamagglutination inhibition titer for NDV, IBV antibodies and ELISA value for Mg and Ms antibodies were found to be within the normal range. Traditionally, *E. coli* has been considered a secondary pathogen requiring predisposing factors such as viral infections, including IBV, NDV and avian metapneumovirus, mycotoxins, poor ventilation and hygiene, which compromise the mucosal barrier and aid in bacterial attachment to the respiratory tract resulting in infections (Nolan *et al*., *loc cit*). However, in the present study, *E. coli* was isolated without obvious predisposing factors, which indicates that the colisepticaemia in the investigated flock might have occurred as a primary infection.

In the present study, antibiotic sensitivity pattern of *E. coli* isolates revealed sensitivity to amikacin, levofloxacin enrofloxacin and azithromycin, moderately sensitive to ciprofloxacin and gentamicin and resistant to penicillin, erythromycin, co-trimoxazole, amoxicillin and chlortetracycline. Lower sensitivity of the isolates towards the co-trimoxazole might be the cause for persistent

mortality in the investigated flock; hence, timely conduction of sensitivity tests is essential (Darwish *et al*., 2013). Based on the drug sensitivity pattern, the flock was treated with levofloxacin @ 10 mg per kg body weight in the morning and evening drinking water for five consecutive days resulted in improvement in the health of affected birds in terms of reduced respiratory sounds as well as mortality and increased feed intake.

Histopathologically, lungs showed congestion in interlobular septa, pulmonary oedema, haemorrhage in parabronchi and bronchopneumonic changes with infiltration of inflammatory cells predominantly heterophils (Fig. 3). The pericardium was thickened with fibrinous exudation and infiltration of degenerated heterophils (Fig. 4). Liver showed degeneration of hepatocytes in the form of cellular swelling, congestion of blood vessels, hemorrhages Kupffer cells hyperplasia and moderate hypercellularity with mononuclear cells infiltration around portal areas (Fig 5). The changes in the liver might be due to the response and reaction to detoxification and excretion of endotoxins. Spleen exhibited congestion, haemorrhages, disruption of white pulp architecture due to moderate lymphoid depletion, multiple variably sized confluent areas of fibrinoid necrosis with bacterial aggregates and reticular cells hyperplasia (Fig. 6). Kidneys showed varying degrees of congestion and haemorrhages in the interstitial tissue, accompanied by mild degeneration of tubular epithelium. Overall pathological lesions of colisepticaemia observed in the present study were more or less similar to those reported by other workers in the spontaneous cases of other poultry species (Srinivasan *et al.,* 2014).

CONCLUSION

The observed gross and histopathological changes were attributable to colisepticaemia, and it was confirmed by bacteriological culture and identification of *E. coli* in the affected guinea fowl flock. In order to minimize the losses and enhance productivity in guinea fowl, determination of the cause of mortality and appropriate therapeutic management is essential. In the present study, a positive response to the instituted antibiotic therapy was noticed since it was chosen after the sensitivity test result.

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Fig. 1: Thickened thoracic air sac and cloudy and enlarged spleen with mottled appearance

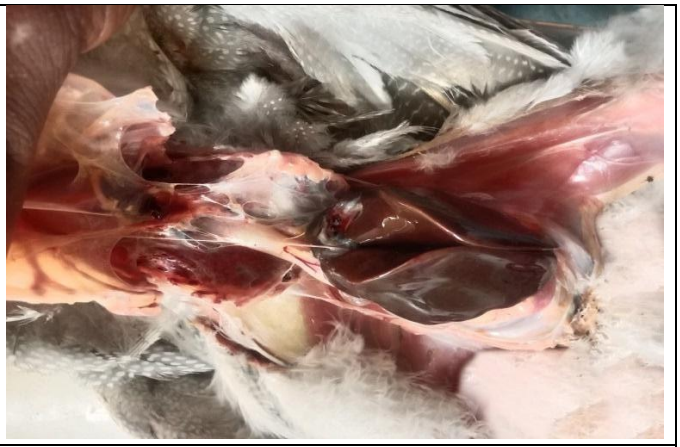

Fig. 2: Enlarged liver with greenish discolouration

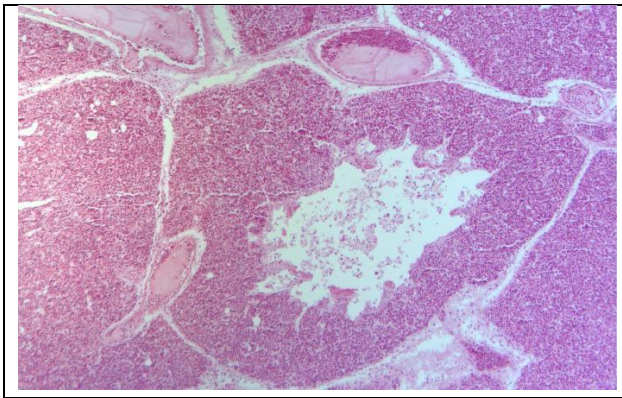

Fig. 3: Lung showing congestion, haemorrhage and oedema in interlobular septa and parabronchial lumen (H&E x 100)

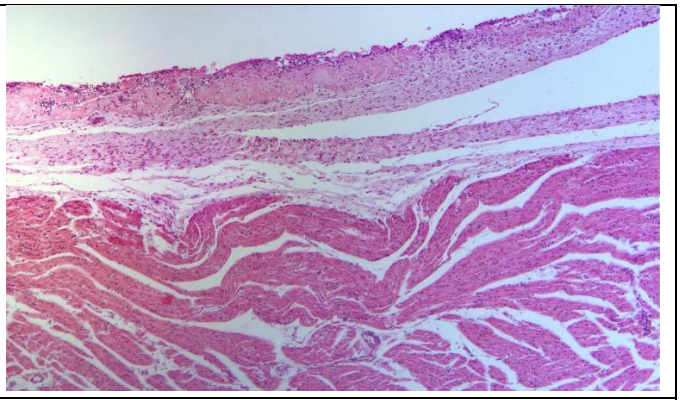

Fig. 4: Pericardium showing fibrinous exudate with infiltration of degenerated heterophils (H&E x 40)

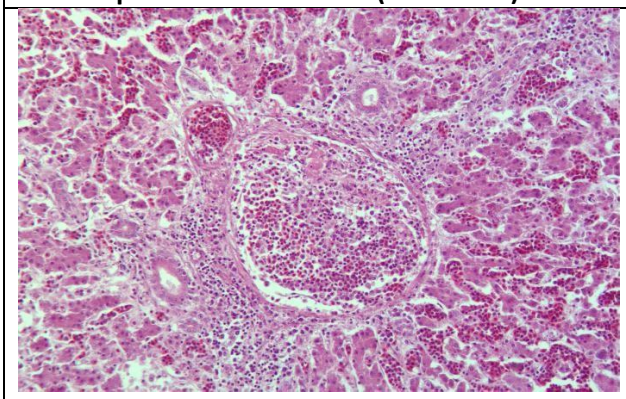

Fig 5. Liver showing degeneration of hepatocytes, congestion of blood vessels, haemorrhages and infiltration of mononuclear cells around portal areas (H&E x 100)

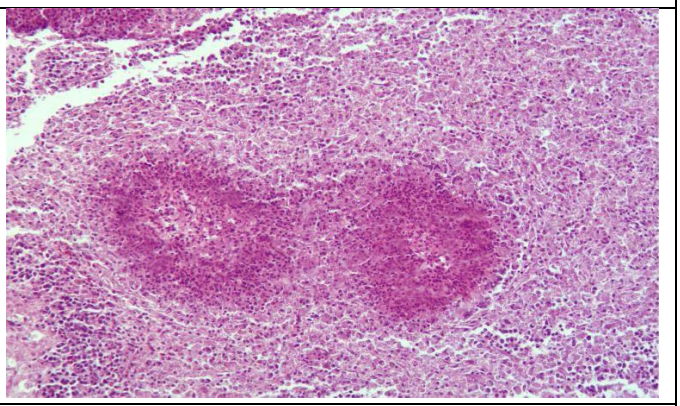

Fig 6. Spleen showing lymphoid depletion, fibrinoid necrosis with bacterial aggregates and reticular cells hyperplasia (H&E x 100)