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Unusual Coliranuloma in very Young Japanese Quail Chick

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Abstract: A commercial Japanese Quail farmer reported death from 8 to 30% in three different flocks of 3 days old age, 3 weeks old age and 4 weeks old age of total flock size 18800. Investigation was under taken to find out the cause of death. Post mortem examination followed by bacteriological, virological and histopathological examinations revealed spontaneous colibacillosis. *E. coli* serotypes $O_{24,}$ O_6 and O_{128} were isolated in pure culture. Isolates were strong congo red binder and sensitive to enterofloxain, gentamicin and chloramphenicol. Pathological lesions also correlated well with the infection of *E. coli.* Involvement of potentially zoonotic, *E. coli* serotype O_6 in causing coligranuloma and death in 3 days old Japanese quail establishes its virulence character in Japanese quails. Strict biosecurity measures were advised to prevent transmission of public health importance *E. coli* serotype O_6 from infected Japanese quails to humans.

Key words: Japanese quails, colibacillosis, coligvanuloma

INTRODUCTION

Escherichia coli cause economically important devastating diseases in chickens and prevalent worldwide (Cheville and Arp, 1978; Margie and Lawrence, 1999; Roy *et al*., 2004). Japanese quails are being intensively used for meat production and are said to be resistant to many diseases. Very few reports of colibacillosis in Japanese quails are available in literature (Burns *et al*., 2003; Roy *et al*., 2006). Present study describes spontaneous colibacillosis and unusual coligranuloma in very young Japanese quails.

MATERIALS AND METHODS

History: In a private commercial Japanese Quail farm three flocks were maintained (1) Three days old-8000 birds (2) Three weeks old-10000 birds and (3) Four weeks old-800 birds. Mortality was recorded at 8% rate and increased to 30% within one week time. Investigation was undertaken to find out the cause of mortality and control the disease.

Necropsy and pathology: Ten dead birds from each flock were necropsied and observed for gross pathological lesions. Different tissues were collected in 10% buffered formalin, dehydrated in alcohol, embedded in paraffin and sectioned at 2-5 μm. The sections were stained with haematoxylin and eosin (H and E) and examined microscopically for histopathological lesions.

Bacteriology: Swabs were collected aseptically from heart and liver from ailing Japanese quails for the isolation of bacteria. Liver impression smears were made and stained with Giemsa stain. Swab samples were cultured on Mac Conkey's agar and sheep blood agar. Bacterial colonies were identified based on staining character, morphology and biochemical tests (Cowan and Steel, 1970). To evaluate the Congo red binding, bacteria were grown at 37°C for 24 h on tryptic soy agar supplemented with 0.02% Congo red (Sigma, USA) and 0.15% bile salt (Difco, USA). Positive colonies appeared red, whereas negative colonies were pale.

In vitro susceptibility of the bacterial isolates against antimicrobiological agents was determined by the standard disc diffusion method (Bauer *et al*., 1966). The following antimicrobial discs supplied by Hi-media laboratory (India) were used: gentamycin (30 mg), ciprofloxacin (30 mg), co-trimoxazole (25 mg), chloramphamicol (30 mg), tetracycline (30 mg), ampicillin /cloxacillin (10 mg), Nitrofurantoin (300 mg). The zone of inhibition and resistance was measured, recorded and interpreted following the recommendation of the discs manufacturer.

Serogrouping of the *E. coli* isolates were done at the National *Salmonella* and *Escherichia* Centre, Kashauli, Himachal Pradesh, India.

Virology: Different tissue samples were collected asceptically and twenty per cent (w/v) tissue homogenates were prepared in PBS, centrifuged and

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supernatants were filtered through 0.22 μm membrane filter. The filtrate was used for haemagglutination (HA) test with 1% chicken erythrocytes as described (Alexander, 1988). The filtrates were pooled and inoculated into embryonated hen's eggs for Newcastle Disease Virus (NDV) isolation as described (Alexander, 1988). Three blind passages were made for declaring the samples negative for NDV.

RESULTS

In necropsy, perihepatitis was invariably seen in all the dead birds, spleen was congested and enlarged; intestine revealed mild congestion in the duodenum; lungs were congested and consolidated. Air sacs were thickened and cloudy. In some birds haemorrhages were seen in the myocardium. Pure colonies of bacteria were isolated from all the samples. All the bacteria were identified as *E. coli* based on staining, morphological and biochemical characteristics (Cowan and Steel, 1970). *E. coli* serotype O_{24} was isolated from 4 week old age group. *E. coli* serotypes O_6 and O_{128} were isolated from 3 weeks old age group and *E. coli* serotype O_6 was isolated from 3 days old quails.

All the sero typed *E. coli* isolates were strong binder of Congo red dye and sensitive to enterofloxain, gentamicin and chloramphenicol but resistant to other drugs used in antimicrobial drug sensitivity test.

The samples were found negative for HA activity, NDV could not be isolated during 3 serial passages in embryonated hen's eggs and declared negative for NDV. Histopathological changes observed in 3 and 4 weeks old Japanese quails include: extensive necrosis in the sub capsular hepatic areas and vascular degenerative changes in the hepatocytes with granularity of cytoplasm, congestion of blood vessels and sinusoids. Lung showed congestion and haemorrhages in alveoli; infiltration of lymphocytes with fibrinous exudates. Heart showed, fibrinous pericarditis; haemorrhages in the myocardium, local degeneration of muscle fibers with infiltration of lymphocytes. Intestine revealed degeneration and necrosis of epithelium, extensive infiltration of lymphocytes in the lamina propria and sub mucosa; accumulation of necrotic debris in lumen. Kidney revealed degenerative changes in tubular epithelial cells, with interstitial haemorrhages.

Histopathological lesions in 3 days old Japanese quails include: Congestion of lung with infiltration of lymphocytes, plasma cells, fibrinous exudates and bacterial rods. Liver showed degenerative changes in the hepatocytes with congestion of blood vessels and sinusoids. Multifocal areas of necrosis observed in hepatocytes and focal granuloma with multinucleated giant cells were present (Fig. 1). Heart revealed mild myocardial haemorrhages and fibrinous pericarditis. Intestine revealed degeneration and necrosis of epithelium; extensive infiltration of lymphocytes in lamina propria and submucosa; accumulation of necrotic debris in lumen. Kidney showed degeneration of tubular epithelial cells; interstitial infiltration of lymphocytes and haemorrhages.

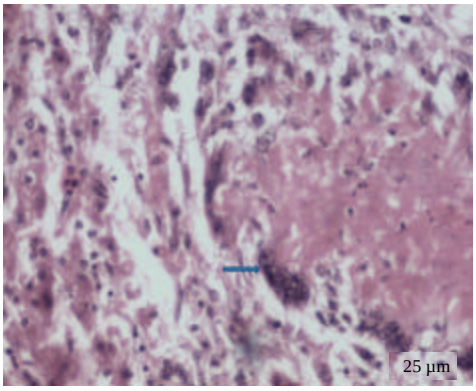

Fig. 1: Liver 3 days old-focal granulomatous reaction with multinucleated giant cells. H and E Bar = 25 μm

DISCUSSION

Colibacillosis is a worldwide problem in poultry industry (Margie and Lawrence, 1999). *E. coli* are normally present in the intestine of poultry and 10-15% of them are pathogenic (Barnes and Gross, 1997). When the bird is under stress the respiratory or intestinal epithelium are damaged and the bacteria takes an upper hand and the endotoxin they liberates cause the disease. Japanese quails used for meat purpose are said to be resistant to many diseases; however, *E. coli* was found to be responsible for cellulitis (Burns *et al*., 2003), reduced hatchability, dead-in-shell embryos, mortality (Roy *et al*., 2006).

In the present study, up to 30% mortality was recorded and dead quails showed consistent lesion of perihepatitis, enteritis, pneumonia and enlargement of spleen. *E. coli* was isolated in pure culture and serotyped as O_{24}, O_6 and O_{128}.

Since NDV is prevalent in this locality and already recorded in Japanese quails (Higgins and Wong, 1968), the possible involvement in this present study was ruled out.

In the present study, the changes observed in different organs were similar to earlier cases of spontaneous colibacillosis in broiler chickens (Nakamura *et al*., 1985). *E. coli* serotypes O_2 and O_{78} are known to be pathogenic for avian species (Hemsley and

Harry, 1965). *E. coli* serotypes O_{88} , O_9 , O_{42} were found to be involved with colibacillosis and Japanese quail mortality of which O_9 was found to be predominant and also prevalent in the hatchery environment (Roy *et al*., 2006). In the present study, *E. coli* serotypes O_{24}, O_6 and O_{128} were isolated from the Japanese quails died of colibacillosis. Earlier, *E. coli* serotype O_{24} was isolated from cases of cellulitis and colibacillosis in broiler chickens (Gomis *et al*., 2001). Virulence attribute of *E. coli* serotype O_6 has not been well established in Poultry. But zoonotic potential of *E. coli* serogroup O_6 has been established earlier (Johnson *et al*, 2008).

In the present study *E. coli* serotype O_6 was found to be responsible for mortality in 3 weeks old and 3 days old Japanese quails and the lesions were very similar to earlier description of colibacillosis in chickens (Nakamura *et al*., 1985). The significant finding is the induction of coligranuloma in 3 days old Japanese quail chicks from which *E. coli* serotype O_6 was isolated in pure culture. Induction of colibacillosis usually requires 3-5 days time and any granulomatous lesion is usually chronic in nature but in the present study, the lesion was observed in 3 days old Japanese Quail. This indicates that *E. coli* serotype O_6 is virulent enough to induce mass cellulitis reaction and granuloma in the liver of very young Japanese Quails. Treatment was suggested following antibiogram and strict biosecurity measures were advised to prevent transmission of public health importance *E. coli* serotype O_6 from infected Japanese quails to humans. This study highlights importance of *E. coli* serotype O_6 in Japanese quails and its public health importance

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