Pathomorphology and Ethnoveterinary Herbal Intervention in Mixed Infection of Infectious Bronchitis, Colibacillosis and Aflatoxicosis in Giri Raja Chicken

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Abstract

A mixed infection of infectious bronchitis with colisepticaemia was recorded in a dual purpose (Giri Raja) chicken at an organized farm in Thanjavur District of Tamil Nadu. Twenty seven week old (898) Giri Raja breeder chicken showed clinical signs of anorexia, staggering gait, gasping and sudden collapse. Morbidity and mortality were 31.74% and 8.77% respectively. The gross and histopathological examination of vital organs revealed lesions suggestive of Infectious bronchitis and aflatoxicosis. The reproductive organs showed egg bound conditions, haemorrhages in the ovaries. Compounded feed was positive for aflatoxin B_1 (75 ppb). Based on laboratory analysis, the present outbreak was suggestive of a combined infection of infectious bronchitis with concurrent infection of Colibacillosis and aflatoxicosis. The disease was controlled by treating with enroflaxacin, colistin sulphate, and preparation of vitamin A, D3, E, selenium and arginin. Fresh plant materials such as Phyllanthus niruri, Cuminum cyminum, Allium sativum, Moringa oleifera leaves, Curcuma longa mixture were given concurrently for all the affected birds orally twice daily.

Keywords: *Infectious bronchitis, Pathomorphology, Chicken, Etnoveterinary medicine*

Introduction

Infectious bronchitis virus (IBV) is prevalent in all countries with an intensive poultry industry, with the incidence of infection approaching 100% in most locations. The virus is belonging to group 3 coronavirus (Saif, 2003). Infectious bronchitis virus (IBV) is an acute, highly contagious disease which can have a significant effect on egg production and egg quality. Increasingly IBV is being seen in the field as a cause of poor peak production, egg quality and hatchability. Colibacillosis is a widespread diseases resulting in economic losses (Barnes *et al*., 1997). Aflatoxin B_1 is a hepatoxin causes toxic hepatitis, immunosuppression (Ahamad and Vairamuthu, 2001). Avian colibacillosis starts as a respiratory infection (air sacculitis) frequently followed by generalized infections which manifested by perihepatitis, pericarditis, and septicemia (Ewers *et. al*., 2003). Clinically apparent *E. coli* infection is generally indicative of immunosuppression in poultry (Mc Gruder and Moore 1998). *E. coli* infection damaged the immune systems of the chickens including

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lymphocyte depletion in both bursa and thymus (Nakamura *et al* 1986 and 1990). Ethno-Veterinary medicinal practice is in primary veterinary health care for sustainable livestock production and to help in conservation of medicinal plants. In order to control various poultry diseases and there by prevent high mortality rates, ethno-veterinary medical (EVM) practices are widely used by farmers in India (Punniamurthy, 2011). In the present disease outbreak investigation, detailed study on pathomorphology and ethnoveterinary herbal intervention in combined infection of infectious bronchitis, colibacillosis and aflatoxicosis in Giri Raja chicken was carried out.

Materials and methods

Flock History

Disease investigation was conducted in an organized farm. Clinical signs, mortality and morbidity was recorded. Necropsy on five bids were done. Necropsy was performed. Liver impression smear was stained with Leishman‘s stain. Feed sample was sent to Central University Laboratory, TANUVAS, Chennai for analysis mycotoxin if any. Twenty seven week old (898) Giri Raja breeder chicken maintained in concrete houses in deep litter system (vaccinated against Marek’s disease, Ranikhet disease and infectious bursal disease) showed clinical signs of anorexia, staggering gait, gasping and sudden collapse. Morbidity and mortality were 31.74% and 8.77% respectively. In house one and three waterers were placed near to the side walls and the birds try to sit on the wall and subsequently jumping on the waterers which cause spillage of water. The side walls of the house was 3 to 4 feet height which is higher than standard (2 feet). Ammonia levels were high in these two sheds. In house three 10 per cent of the birds showed the snicking of the head and tracheal rales. Other birds were apparently normal. Mishapened eggs were noticed in house one and three. At 27 weeks of age, mortality in one house rose sharply due to the culling of birds showing greenish to whitish diarrhea and sudden death. These signs eventually spread to the other houses and were preceded by upper respiratory signs reported as a snick. Serum filter paper HI test for Ranikhet disease, visceral organ culture, Antibiotic sensitivity test, and cultural examination of the feed samples were done at Poultry disease diagnostic laboratory, Namakkal. Samples of the liver, heart, kidney, lungs, spleen, bursa of Fabricius from the birds were collected, processed routinely and stained with hematoxylin and eosin (H&E).

The disease was controlled by treating with enroflaxacin, colistin sulphate, preparation of vitamin A, D3, E, selenium and arginin .For every 10 birds, fresh plant materials such as *Phyllanthus niruri* - 25g, *Cuminum cyminum* - 25 g, *Allium sativum* - 5 pulp, *Moringa oleifera* leaves- 25 g, C*urcuma longa* -15g were ground in mixy. The mixture was mixed with 25 g of jaggery and was given three time a day for 4 days either given orally or with feed. The freshly prepared herbal mixture was given concurrently for all the affected birds.

Results

Feed Analysis

The feed samples were sent to pharmacovigilance laboratory for animal feed and food safety for analyzing the toxins. The result showed compounded feed was positive for aflatoxin B_1 (75 ppb).
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Gross findings

Post mortem examination was carried out on live (one) and three (dead) birds. Externally the dead birds revealed greenish to whitish faecal material around the vent region. Condition of the carcasses was good, trachea showed mild to moderate congestion with mucus exudates. Air sacs were cloudy and thickened. Moderate to severe whitish thin layer in pericardium, liver and peritoneum were observed in birds. Lungs were with patches of reddish brown firm areas. In few birds epicardium showed petechial hemorrhages. Liver was congested, friable consistence and the gall bladder was distended with greenish yellow bile. In one bird, gizzard and proventriculus junction showed diffused hemorrhages. Ovarian follicle were mishappened and congested. Oviduct was congested with mucus exudates. In a few birds, egg bound condition was observed. Kidney appeared congested/ pale, with enlargement with or without cooked appearance. The ureter showed whitish deposition of uric acid. Meningeal blood vessels were congested.

No HA activity was noticed with the above samples after neutralization with New castle disease virus infectious bronchitis virus antiserum. But in kidneys (1:16 to 1: 512), spleen (1:8 to 1: 16) and trachea (1:04 to 1:32) HA activitiy was recorded after neutralization with infectious bronchitis virus antiserum

Serum filter paper HI results

Serum samples from shed 1, 3 and ailing birds revealed RD HI titre of 16 and less and than 16 with GM values of 8.0.Filter paper samples from shed No. 1, 2 and 3 showed RD titre of 16 and less than 13 with GM values of 9.2. Same samples are also subjected to IB HI titre. It revealed IB HI titre of 64 in two samples and the remaining samples showed 32 and below 32 with GM values of 24.3.

Histopathology

Liver showed multifocal mononuclear cells, diffuse micro-vacuolar degeneration (Fig.1), acinar transformation of hepatocytes bile duct hyperplasia and diffuse mild periductular fibrosis. Glissions capsule revealed thickening with subacute fibrinous perihepatitis The upper respiratory tract reveled subacute air-sacculitis, Trachea and bronchi revealed large plugs of inspissated exudate. Lungs revealed mild congestion and haemorrhages (Fig.2). Kidneys revealed severe inter tubular congestion and hemorrhages, swelling of renal tubules (Fig.3.) severe tubular degeneration, multifocal aggregation of mononuclear cell (MNC) were observed. .Mucosa of trachea infected showed degenerative changes in epithelial lining cells and activation of goblet cells. Lamina propria of trachea revealed severe hemorrhages.

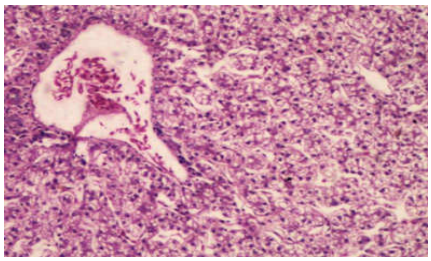

Fig. 1: Liver showed diffuse moderate vacuolation of hepatocytes HE X100X

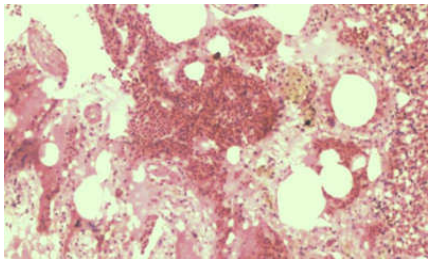

Fig. 2: Lungs revealed diffuse congestion and haemorrhage HE 100X

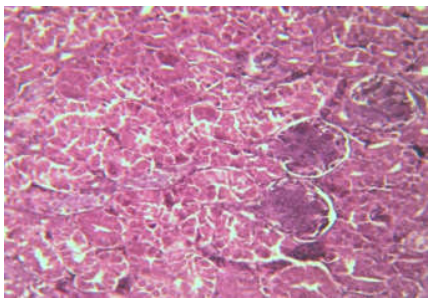

Fig. 3: Kidney revealed swellinbg of renal tubulesHE 400X

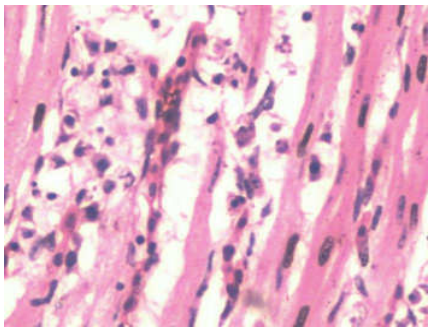

Fig. 4: Heart revealed mononuclear cell infiltration HE 400X

Pericardium showed severe subacute fibrinous pericarditis and peritonitis Most of the chickens recorded as having died from IBV and *E. coli* infection had pericarditis and many of these had peritonitis; it was unusual to find chickens with peritonitis but not pericarditis.

Diagnosis

Based on the clinical signs, lesions and laboratory results the outbreak was diagnosed as mixed infection of infectious bronchitis and aflatoxicosis.

Antibiotic Sensitivity Test

E. coli isolates showed ABST of the organism revealed sensitivity to Gentamicin, Cephalexin and Chloramphenicol and intermediately sensitive to Enrofloxacin and Ciprofloxacin and resistant to Amoxacilin, Norfloxacin, Co-trimaxazole and Oxytetracyaline.

Treatment

The disease was controlled by treating with. For first two days days enroflaxacin, colistin sulphate, preparation of vitamin A, D3, E, selenium and arginin was given through water. However, after investigation of the disease, as per herbal therapeutic advise that all l the birds were fed with mixture of fresh plant materials such as *Phyllanthus niruri, Cuminum cyminum, Allium sativum, Moringa oleifera* leaves and C*urcuma longa.* The mixture was mixed with 25 g of jaggery and was given three time a day for 4 days either given orally or with feed. All the affected bird healthy and taken feed normally from next day of treatment gradually.

Discussion

Poultry production plays an important role in providing valuable proteins, poverty alleviation and economic development. Despite great potential and opportunities, poultry production is threatened by many disease outbreaks, these diseases are the major constrains for developing the poultry industry (Ewers *et al*., 2003). To establish poultry farms, the incidence of the disease should be considered for prevention and control. E.Coli, as opportunistic pathogens, could have further augmented mortality in these immunosupressed chickens as a consequence of aflatoxicosis and Infectious Bronchitis due to vaccination failure.

Colibacillosis was reported by many workers (Omer *et al*., 2008; Vandekerchove *et al*., 2004c; Yang *et al*., 2004; Saenz *et al*., 2003; White *et al*., 2000). Therefore, the disease is considered one of the principal causes of mortality and morbidity in poultry, responsible for high economic losses to poultry industry worldwide (Ewers *et al*., 2003; Gomis *et al*., 2001; Allan *et al*., 1993).

In the present study, outbreak of aflatoxicosis, colibacillosis and infectious bronchitis was encountered in deep litter system of rearing. Morbidity related to the clinical signs was estimated to be about 31.74% morbidity and 8.77% mortality. However, the findings disagree with Omer *et al*. (2008) who reported 1.8% mortality rate of the disease in layers and 1% in broilers.

Ahamad *et al*. (2009) recorded aflatoxin B_1 in different feed samples from these poultry farms ranging between 10and 215 ppb. In the present study the higher mortality rate was observed in the Giriraja chickens, this could be attributed to the housing condition where, birds were reared in deep litter system. From the study the clinical manifestations were observed in the affected birds considered as typical signs of colibacillosis in addition to, inflammatory changes such as pericarditis, salpingitis and airsaculitis were seen in the autopsied birds considered as pathognomonic lesions of the disease, this in agreement with Omer *et al*. (2008) and Landman and Cornelissen (2006) who reported similar clinical signs associated with colibacillosis. Sudden death of the birds, these findings were corborated with the findings of Vandekerchove *et al*. (2004b), who reported acute mortality of colibacillosis in laying hens without prior clinical signs of the disease. Pathomorphological changes on immune organs such spleen, bursa of Fabricius, liver were in accordance with Ahamad and Vairamuthu (2001).

In this study *E. coli* was isolated from heart blood and liver swabs of the sick chickens. Since, the isolation of the organisms were obtained as pure form from these organs and absence of other bacteria, this emphasized that *E. coli* act as the primary causative agent of this outbreak. However, only two isolates were obtained from heart blood and liver swabs and failed to isolate the organisms from other organs, this might be due to pre-treatment with antibiotic of such cases, these findings were in agreement with Omer *et al*. (2008) who obtained only one

isolate from chickens pre-treated with antibiotics. The results from this study indicate that outbreaks of colibacillosis are not necessarily associated with IBV or NDV infections, this similar previous study conducted by Vandekerchove *et al*. (2004b).

Some IBV strains either caused nephrosis /nephritis in young birds or else contributed to urolethiasis in layers (Cowen et al., 1987). Four of our birds showed gross renal urate deposition and histological lesions of subacute nephritis with or without nephrosis and haemorrhagic tracheitis in this outbreak. Changes accompanied with kidney infection with IBV were documented. The serum ions content were affected by the change in electrolyte balance in the kidney and the intake of ions in feeds. The major change in electrolyte balance in the kidney is the increased output of sodium in the urine, which is associated with diuresis (Condron and Marshall, 1985). The ability to reabsorb sodium in infected birds might have damaged, which subsequently led to the low sodium content in the blood. Both the intracellular and renal luminar potassium concentration in IBV-infected birds were lower than those of normal birds (Condron and Marshall, 1991). The higher potassium content in the blood in infected birds might possibly be a consequence of the leakage of potassium from damaged cells and secretory damage. This explain the sever watery feaces observed in groups inoculated with variant IBV isolates as this watery feaces result from polyurea.

Concerning histopathological lesions in trachea with haemorrhagic trcheitis with edema, sloughing of mucosa and degeneration of epithelial cells and activation of globlet cells, which agreed with the findings of Cavanagh and Naqi (2003). The lamina propria was characterized by massive infiltration by lymphoid inflammatory cells, a result that have been already reported by Cavanagh and Naqi (2003) and Mahgoub et al. (2010) as features which can take place in trachea of birds infected with IBV.

From this study it was observed that there were many factors might play an essential role in the occurrence of this outbreak, such as bad management system and hygiene measures as mentioned by Vandekerchove *et al*. (2004a). Factors like presence of rodents and flies in the poultry farms, can act as vectors of the disease. Furthermore, distances between poultry farms may have a little importance in transmission of bacterial diseases. Since, the outbreak has been reported in the hot summer season (May) the environmental factors possibly have a role in this case (Aielo, 1998). These factors might be the exact cause of this outbreak.

Control and prevention of poultry diseases especially colibacillosis, antimicrobial agents are administered to chickens via food and water. This practice also improves feed efficiency and accelerates weight gain (Bower and Daeschel, 1999). However, the treatment of whole flocks with antimicrobials for disease prevention and growth promotion has become controversial practice (Van den Bogaard and Stobberingh, 1999;

Witte, 1998). Therefore, this practice is reported to have caused high resistance to antimicrobial agents in both normal flora and pathogenic organisms (Allan *et al*., 1993; Amara *et al*., 1995). There is also concern that antimicrobial use in food animals can lead to the selection of antimicrobial resistant zoonotic enteric pathogens which may then be transferred to people by the consumption of contaminated food or by direct animal contact (Caudry and Stanisch, 1979; Turtura *et al*., 1990).

In this study almost all *E. coli* isolates showed ABST of the organism revealed sensitivity to Gentamicin, Cephalexin and Chloramphenicol and intermediately sensitive to Enrofloxacin and Ciprofloxacin and resistant to Amoxacilin, Norfloxacin, Co-trimaxazole and Oxytetracyaline.

Moreover, inappropriate use of antibiotics, resistance transfers among different bacteria and possible cross resistance between antibiotics used in poultry (Allen *et al*., 1993). The major factors responsible for antimicrobial resistance in bacteria and immunosuppression are antibiotic use, crowding poor sanitation and contamination of mycotoxin in the feed and feed ingredients therefore, these factors are explain the high degree of resistance in *E. coli* isolates, this in agreement with these studies (Van den Bogaard and Stobberingh, 1999; Ahamad *et al*., 2009). Furthermore, this might be explained by the fact that antimicrobial use and misuse have contributed to the emergence and spread of antimicrobial resistant microorganisms (Levy, 1994).

The course of treatment recommended included segregation of affected birds, change of poultry sheds, change in feed, liver tonics, multivitamins and mineral supplements. In the present study the ethenoveterinary herbal medicine showed effective control of the disease. Curcumin in the *Curcuma longa* has been shown to have antioxidant in skin (Phan *et al*.,2001) and antiviral activity (Araujo and Leon, 2001). The antiproliferative effect is mediated partly through inhibition of protein tyrosine kinase and c-myc mRNA expression and the apoptotic effect may partly be mediated through inhibition of protein tyrosine kinase, protein kinase C, c-myc mRNA expression and bcl-2 mRNA expression (Chen, and Huang, 1998) and to block constitutive NF-.B activation by down regulating IKK. in a manner similar to the neem leaf subfractions (Aggarwal *et al*., 2005). *Moringa oleifera* leaves as a source of plant growth factors(s), antioxidants, beta carotein, vitamin C and various glycosinolates and their degraded products for possible use as antioxidant, anticarcinogenic and antipest agents Medicinal plants still play a vital role in the primary healthcare of livestock and poultry in India. Hopefully, this study offers a model for studying the relationship between plants and livestock farmers within the contexts of a traditional medical system. (Punniamurthy, 2005, 2006, Punniamurthy *et al*., 2009). The purpose of standardizing ethnoveterinary herbal practice adopted in this case reduced the mortality to some extent.

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