The Pharma Innovation Journal 2022; SP-11(8): 360-364

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(8): 360-364

www.thepharmajournal.com
Received: 08-06-2022
Accepted: 11-07-2022

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Pathology of chicken infectious anemia with concurrent infections in chickens of Chhattisgarh

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Abstract
Poultry industry is constantly exposed to various immunosuppressive agents such as viruses, mycotoxins and environmental stress. Chicken Infectious Anemia is one of the important immunosuppressive viral agents caused by circular single stranded *Circovirus* which leads to increased susceptibility to concurrent infection and heavy economic losses in terms of mortality and reduced growth performance. During present study, mortality of 10 to 18% due to Chicken infectious anemia in thirteen, 5-12weeks old commercial layer and cockerel farms located at Balod, Durg, Rajnandgaon and Raipur district of Chhattisgarh was noticed. Increased mortality of 15 to 24% was noticed in the chickens with concurrent infections. Characteristic clinical signs of anaemia, typical pale bone marrow and thymic atrophy were suggestive of CIA. Generalized lymphoid atrophy was the most constant and characteristic lesion found in CIA affected birds. Concurrent infections with one or more infections due to Gangrenous dermatitis, Coccidiosis, Colibacillosis and Salmonellosis were noticed in nine flocks. Clinical samples of thymus collected from thirteen flocks were confirmed as CIA by PCR amplification of 419bp of VP2 gene of virus. The clinical signs, gross and histopathological findings along with PCR amplification of VP2 gene confirmed the outbreak of CIA in commercial layers and cockerels.

Keywords: CIA, chickens, pathology, molecular diagnosis, concurrent infections

Introduction
Poultry industry is constantly been exposed to various immunosuppressive agents such as viruses, mycotoxins and environmental stress which further leads to secondary bacterial and viral infections and vaccination failure resulting into severe economic losses. The important viral diseases involving immunosuppression includes Chicken Infectious Anemia virus (CIA), Infectious Bursal Disease (IBD), Marek's Disease (MD), Reovirus, Avian Leukocis and Reticuloendotheliosis (Umar *et al.*, 2017, Gimeno and Schat, 2018) [15, 6].

Chicken Infectious Anemia caused by a Circovirus was first identified in the year 1979 as a new viral disease in young chickens (Yuasa *et al.*, 1979) [17]. Circoviruses are small, non-enveloped icosahedral viruses characterized by circular single stranded DNA genomes. The genome codes for three viral proteins (VP1, VP2 and VP3) from single major transcript of 2.0 kbp size of three overlapping reading frames (ORF1, 2 and 3). VP1 and VP2 are the main targets for neutralizing antibodies (Dhama *et al.*, 2008) [5].

CIA is a highly contagious disease of young chickens characterized by aplastic anemia, generalized lymphoid atrophy, stunted growth, hemorrhages on subcutaneous tissue, breast and thigh muscles, immunosuppression, drop in haematocrit values, watery blood and morbidity and mortality of up to 100 and 60% respectively (Dhama *et al.*, 2008; Schat and Santen, 2008; Wani *et al.*, 2013) [5, 14, 16]. With generalized lymphoid atrophy and immunosuppression, the disease is commonly complicated by secondary bacterial, viral, protozoan, mycotic infections or parasitic infestations (Bakshi *et al*., 2016) [1].

Polymerase chain reaction (PCR) has been considered as the most useful tool for confirmatory diagnosis of many viral diseases including CIA. The highly specific and conserved genes of putative scaffold viral protein VP2 are generally employed using thermal cycler for this purpose (Islam *et al.*, 2013) [8].

In India, the disease has long been suspected on the basis of clinical manifestations and lesions, virus detection by immunoperoxidase test and polymerase PCR (Islam *et al.*, 2013) [8]. Disease has been reported from poultry flocks of some states of the country and included in the list of emerging and important viruses that are a severe threat to the Indian poultry industry (Bhatt *et al.*, 2011, Wani *et al.*, 2013, Gowthaman *et al.*, 2014) [2, 16, 7].

Hence, present investigation was carried out to investigate pathological features and molecular detection of CIA complicated with concurrent infection in Chhattisgarh.

Materials and Methods

Outbreaks of CIA in commercial layer and cockerel farms with capacity of 3000 - 8000 birds were observed in Balod, Durg, Rajnandgaon and Raipur district of Chhattisgarh. Total thirteen farms where mortality was suspected due to CIA were visited and information about age, flock size and mortality was collected.

Clinical signs and gross pathology: The ailing birds were examined for clinical signs, if any. Dead birds were subjected to detailed post mortem examination and gross pathological lesions were recorded.

Histopathology: Tissue samples of thymus, bursa of Fabricius, spleen, liver, kidney, bone marrow and skin were collected in 10% buffered formalin and processed for histopathological study by paraffin embedding technique. Sections were cut at 4-5µ thickness and stained with routine haematoxylin and eosin (H and E) staining (Luna, 1968) [9].

Detection of CAV by PCR: Tissue samples of thymus was also collected from the birds showing gross lesions suspected of CIA and preserved at - 20ºC for detection of VP2 gene of CAV by PCR. Viral DNA from tissue homogenate was extracted using HiGenoMB® genomic DNA Purification Kit (Himedia) as per the manufacturer's instructions. The VP2 gene of CAV from field samples were detected by using the forward primer 3' CTA AGA TCT GCA ACT GCG GA 5' and reverse primer 3' CCT TGG AAG CGG ATA GTC AT 5' to amplify CAV virus specific 419 bp fragment (Ottiger, 2010) [12]. For amplification, 3µl of DNA was incubated in total volume of 20 µl reaction mix containing 10 µl PCR master mix, 1µl of each forward and reverse primer and 5 µl of nuclease free water. PCR was carried out following initial denaturation at 95 °C for 3 min and then 30 cycles at 94 °C for 1 min, 60 °C for 1 min, and 72 °C for 1 min and a further extension at 72 °C for 10 min. The PCR products were separated in 1.5% agarose-gel and visualized in Geldoc (Biorad).

Cultural isolation of co-infected bacteria: Samples collected aseptically during post-mortem were used for culturing as per the standard protocols (Markey *et al.*, 2013) [10]. Various media/broth such as eosin methylene blue (EMB) and MacConkey lactose agar (MLA) (for *E. coli*), MLA and xylose-lysine-deoxycholate (XLD) agar (for *Salmonella* spp.) and mannitol salt agar (for *Staphylococcus* spp.) were used for cultural isolation of bacteria. Further, bacteria were confirmed by gram staining.

Microscopic examination of oocysts of *Eimeria* spp: Microscopic examination of intestinal / caecal or faecal content of chickens showing the lesions suspected of coccidiosis was carried.

Results and discussion

Mortality of about 10-18% due to CIA in 5 to 12weeks old commercial layers and cockerels were recorded at 13 farms located at Balod, Durg, Rajnandgaon and Raipur district of Chhattisgarh. Wide prevalence of CIA in India (Bhatt *et al.*, 2011; Wani *et al.*, 2013; Gowthaman *et al.*, 2014; Baksi *et al.*, 2016) [2, 16, 7, 1] has been well documented. However, increased mortality of 15 to 24% was noticed in the chickens with concurrent infections during the present study. Exacerbated clinical presentation with increased mortality has also been reported with concurrent infections (Chandrashekaraiah *et al.*, 2020) [4]. Affected birds showed signs of anemia, generalized weakness, depression, pale comb (Fig.1), wattles and shank, stunted and reduced growth rate and decreased feed intake and water consumption. These clinical signs are in accordance with Dhama *et al.*, (2008) and Wani *et al.*, (2013) [5, 16].

Gross pathology: Gross lesions observed in all the commercial layer and cockerel farms were typically of CIA. Carcass of the birds appeared pale and anemic. Bone marrow of femur appeared fatty, yellowish or pink coloured in most of the birds of affected flock. Lesions consisted of markedly atrophied thymus glands (Fig. 2) and enlarged, pale liver, kidney and spleen. Bursal atrophy was observed in small percentage of birds in few outbreaks. In addition to the above mentioned lesions, birds showed subcutaneous and intramuscular hemorrhages in pectoral and thigh region (Fig. 3). The CIA affected birds also showed gross lesions of concurrent infections such as fibrinous pericarditis, perihepatitis, pneumonia and air saculitis in *E. coli* (Fig 4), necrotic foci on liver in Salmonellosis and hemorrhagic enteritis and typhilitis in intestinal and caecal coccidiosis (Fig. 5 and 6). Blue wing lesions and gangrenous type of dermatitis were also noticed in few cases (Fig. 1). These finding are in agreement with Chandrashekaraiah *et al.*, (2020) [4].

Histopathology: CIA affected bird revealed variety of the lesions. Thymus showed moderate to severe atrophy with lymphoid depletion in cortex and medulla (Fig.7). The cortical depletion of lymphocytes leads to thinning of cortex which appeared similar to medulla. These observations are in consensus with the findings of Rimondi *et al.*, (2014) [13] and Chandrashekaraiah *et al.*, (2020) [4]. Bone marrow showed severe hypoplasia or atrophy of the hematopoietic tissues in the marrow and medullary sinuses with few mature erythrocytes or completely devoid of bone marrow cells (Fig. 8). Bursa of Fabricius showed mild atrophy of the lymphoid follicles due to lymphocytolysis, hydropic degenerative changes, multiple areas of necrosis, cystic bursal follicles and fibrous tissue proliferation. Spleen showed various degrees of lymphocytic depletion in white pulp, proliferation of macrophages and reticular cell hyperplasia with increased juvenile arteries. Similar findings have also been reported by Chandrashekaraiah *et al.*, (2020) [4] indicating immunosuppressive effect of the virus. These changes may be attributed due to destructive effect of the virus to hematopoietic and lymphopoietic tissues leading to impaired immune response.

Kidney showed degenerative changes along with haemorrhages. Liver revealed degenerative and fatty changes in hepatocytes, necrotic areas and periportal leucocytic infiltration. The lesions observed in the present study are in agreement with Narayani and Ghosh (2018) [11].

CIA affected birds with concurrent infections revealed variety of lesions based on agents involved. In gangrenous dermatitis, affected skin showed necrotic areas and inflammatory changes with infiltration of bacteria, heterophils and lymphocytes. While in *E. coli*, pneumonia is characterized by congestion, hemorrhages and infiltration of heterophils,

lymphocytes and macrophages in the wall of bronchi and alveoli. Fibrinous exudates with large number of heterophills was observed in liver and heart leading to perihepatitis and pericarditis respectively. In Salmonellosis, multifocal areas of necrosis and leucocytic infiltration were noticed in liver. Further, congestion, hemorrhages, loss of mucosal epithelium along with presence of oocysts and various stages of *Eimeria* spp in intestine and caeca was evident in coccidiosis. These pathological lesions were in agreement with Narayani and Ghosh (2018) and Chandrashekaraiah *et al.*, (2020) [11, 4].

Polymerase chain reaction (PCR): Tissue samples of thymus collected from thirteen commercial layer and cockerel flocks were confirmed as CAV by PCR. Amplification of VP2 gene of CAV revealed 419bp product for all thirteen layer and cockerel flocks (Fig. 9). These findings are in accordance with Gowthaman *et al.*, (2014) [7] and Chandrashekaraiah *et al.*, (2020) [4] who also detected CAV in thymus collected from birds**.** This probably indicated that CAV targets lymphoid progenitor cells in the thymus. PCR technique has been routinely used for diagnosis of CIA and detecting CAV in clinical samples without necessitating virus isolation. Hindrance for virus isolation is that, most of very virulent field isolates do not replicate in common tissue culture, whereas for virus neutralization test, the field strains need to be adapted to grow *in vitro* (Chandrashekaraiah *et al.*, 2020) [4].

Cultural and microscopic examination: Among the 13 outbreaks of CIA, 9 outbreaks showed concurrent infection with one or more infections which includes Gangrenous dermatitis due to *Staphylococcus* spp. (5 cases), *E. coli* (3 cases) and Salmonellosis (1 case) on the basis of cultural examination and Coccidiosis (4 cases) on the basis of microscopic examination of oocysts.

Haemolytic zone around the colonies on blood agar and typical metallic yellow colour colonies on mannitol salt agar medium were suggestive of *Staphylococcus* spp. leading to gangrenous dermatitis. Colorless, translucent, smooth and raised colonies on MLA indicative of lactose non-fermenter organisms and jet black colony on XLD were suggestive of Salmonellosis. Pink color colonies (lactose fermenter organisms) on MLA and metallic sheen on EMB were indicative of *E. coli.* Further, microscopic examination of intestinal/caecal or faecal material revealed oocycts and various stages of *Eimeria* spp. indicative of coccidiosis. The present findings of concurrent infection in CIA are agreement with Bougiouklis *et al.*, (2007) [3] and Chandrashekaraiah *et al.*, (2020) [4].

The clinical signs, gross and histopathological findings along with PCR amplification of VP2 gene suggested the outbreak of CIA in chickens of Chhattisgarh. Typical pale, yellowish changes in the bone marrow and thymic atrophy confirm the CAV infection beside other means of diagnosis. Lymphoid atrophy is the most constant and characteristic lesion that can be found in CAV infected birds. Concurrent infections with Gangrenous dermatitis, Colibacillosis, Salmonellosis and Coccidiosis occurred due to immunosuppression brought about by CIA virus by the direct and indirect action on lymphoid organs such as bone marrow, thymus, spleen and bursa.

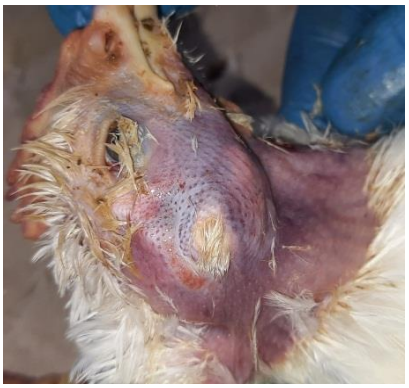

Fig 1: Pale comb due to CIA and concurrent gangrenous dermatitis in neck & face area

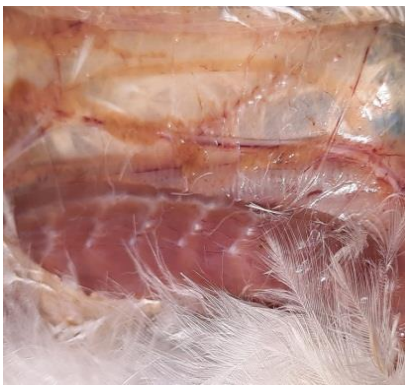

Fig 2: CIA: Thymus atrophied

Fig 3: CIA: Intramuscular haemorrhages on thigh area

Fig 4: Concurrent Colisepticaemia: showing fibrinous pericarditis and perihepatitis

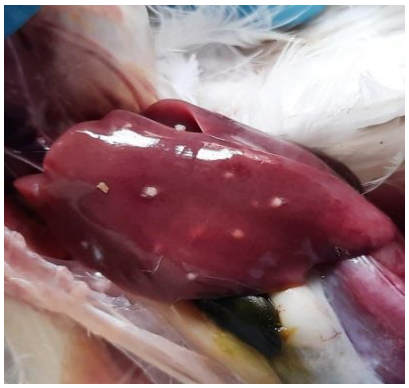

Fig 5: Concurrent Salmonellosis: Showing necrotic foci on liver

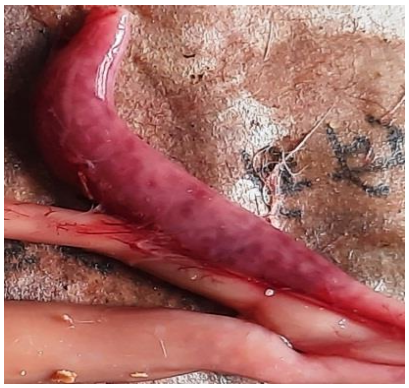

Fig 6: Concurrent caecal coccidiosis: Showing haemorrhagic typhilitis

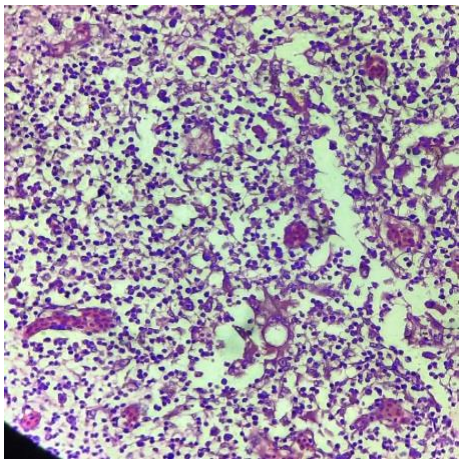

Fig 7: CIA: Thymus showing depletion of lymphocytes (H&E, 10x**)**

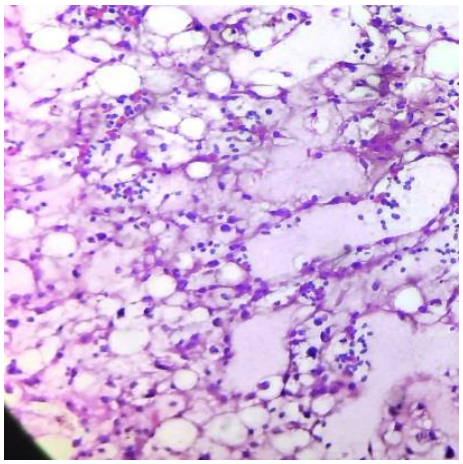

Fig 8: CIA: Bone marrow showing hypoplasia of hematopoietic tissues as replaced by adipose tissue (H&E, 40x)

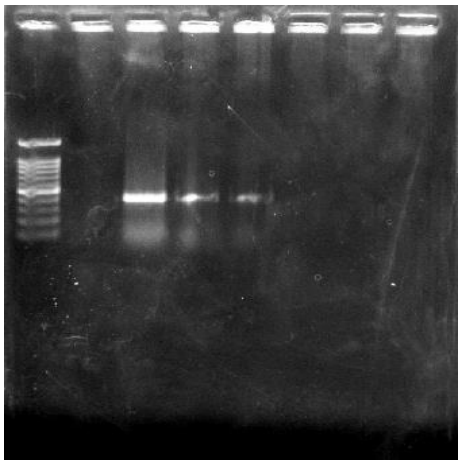

Fig 9: Agarose gel photograph showing amplified PCR products of CAV. Lane 1: 100bp DNA ladder, Lane 2: Negative control, Lane 3,4,5: positive field sample (419bp of VP2 gene of CAV)

References

1. Baksi S, Savaliya BF, Rao N, Khan M. Sero-Prevalence of Chicken anaemia virus (CAV) in layer poultry flocks in different parts of India. J Poul. Sci. and Tech. 2016;4(3):39-42.
2. Bhatt P, Shukla SK, Mahendran M, Dhama K, Chawak MM, Kataria JM. Prevalence of chicken infectious anaemia virus (CIAV) in commercial poultry flocks of northern India: A serological survey. Transbound. Emerg. Dis. 2011;58:458-460.
3. Bougiouklis PA, Sofia M, Brellou G, Georgopoulou I, Billinis C, Vlemmas I. A Clinical Case of Chicken Infectious Anemia Disease and Virus DNA Detection in Naturally Infected Broilers in Greece. Avian Dis. 2007;51(2):639-642.
4. Chandrashekaraiah B, Karamala S, Narayanaswamy HD, Karumuri NK, Nagaram VK. Pathology of chicken infectious anemia (CIA) with concurrent infections. J of Entom and Zoology Stud. 2020;8(2):519-524.
5. Dhama K, Mahendran M, Somvanshi R, Chawak MM. Chicken infectious anemia virus: an immunosuppressive pathogen of poultry-A review. Ind. J Vet. Pathol. 2008;32:158-167.
6. Gimeno IM, Schat KA. Virus-Induced Immunosuppression in Chickens. Avian Dis. 2018;62(3):272-285.
7. Gowthaman V, Singh SD, Dhama K, Barathidasan R, Srinivasan P, Mahajan NK. Molecular characterization of chicken infectious anemia virus isolated from commercial poultry with respiratory disease complex in India. Adv. in Animal and Vet. Sci. 2014;2(3):171-176.
8. Islam NU, Saleemi MK, Khan MZ, Butt SL, Khan A, Javed I. Molecular diagnosis and pathology of chicken infectious anemia in commercial white leghorn layer flocks in Pakistan. Pak. Vet. J. 2013;33(3):378-381.
9. Luna AG. Manual of histology and special staining technique. 3rd Ed, 1, Armed Force Institute of Pathology-Magraw Hill Book Co, London, 1968.
10. Markey B, Leonard F, Archambault M, Cullinane A, Maguire D. Clinical Veterinary Microbiology. 2nd Ed, 1, Mosby Elsevier, Edinburgh, 2013.
11. Narayani A, Ghosh RC. Studies on pathogenicity of Chicken infectious anaemia virus isolated from poultry flock of Chhattisgarh. Ind. J of Animal Res. 2018;8(3):361-366.

12. Ottiger HP. Development, standardization and assessment of PCR system for purity testing of avian viral vaccine. *Biologicals*. 2010;38:381-388.
13. Rimondi A, Pinto S, Olivera V, Diborbora M, Perez-Filgueira M, Craig MI. Comparative, histopathological and immunological study of two field strains of chicken anemia virus. Vet. Res. 2014;45:1-12.
14. Schat KA, Santen VL. Diseases of Poultry, 12th ed, 1, Blackwell publishing, Ames- USA, 2008, 211-235.
15. Umar S, Munir M, Ahsan U, Raza I, Chowdhury M, Ahmed Z. Immunosuppressive interactions of viral diseases in poultry. World's Poul. Sci. J. 2017;73(1):121-135.
16. Wani MY, Dhama K, Barathidasan R, Gowthaman V, Tiwari R, Bhatt P, Mahajan NK, *et al*. Molecular detection and epidemiology of chicken infectious anaemia virus in India. South Asian J Exp. Biology. 2013;3(4):145-151.
17. Yuasa N, Taniguchi T, Yoshida I. Isolation and some characteristics of an agent inducing anemia in chicks. Avian Dis. 1979;23:366-385.