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Original Research

Pathology and Molecular Diagnosis of Chicken Infectious Anemia in Commercial Layers of Chhattisgarh

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Abstract

Poultry is constantly exposed to various immunosuppressive agents such as viruses, mycotoxins, and environmental stress. Chicken Infectious Anemia (CIA) caused by a Circovirus is one of the very important viral diseases. During the present study, mortality of 15 to 25% due to Chicken infectious anemia in eleven, 6-11weeks old commercial layer flocks located at Durg, Rajnandgaon, and Raipur districts of Chhattisgarh was noticed which were not vaccinated against CIA. Characteristic clinical signs of anemia indicated by pale comb and wattle, yellowish changes in the bone marrow, and thymic atrophy were suggestive of CIA. Generalized lymphoid atrophy was the most constant and characteristic lesion found in CIA-affected birds. Clinical samples of thymus collected from eleven commercial layer flocks were confirmed as CIA by PCR amplification of 419bp of the VP2 gene of the virus. The clinical signs, gross and histopathological findings along with PCR amplification of the VP2 gene confirmed the outbreak of CIA in the commercial layer.

Keywords: CIA, Commercial Layers, Molecular Diagnosis, Pathology

Introduction

Chicken infectious anemia (CIA) is a highly contagious disease of young chickens characterized by severe anemia, generalized lymphoid atrophy, stunted growth and increased mortality (Todd, 2004; Dhama *et al.,* 2008). The causative agent of the disease is *Chicken Anaemia Virus* (CAV), belonging to the genus *Gyrovirus* of the family *Circoviridae*. The CAV is one of the smallest, 23–25 nm in size, non-enveloped, icosahedral having a 2.3 Kb circular single-stranded DNA genome. The genome codes for three viral proteins (VP1, VP2, and VP3) from a single major transcript of 2.0 kb size of three overlapping reading frames (ORF1, 2, and 3). VP1 and VP2 are the main targets for neutralizing antibodies. CAV-induced immuno-suppression has gained considerable economic importance around the poultry globe in recent years (Dhama *et al.,* 2008).

CAV-infected birds develop profound immunosuppression in presence of concurrent infection with other viruses such as *Marek's disease virus* (MDV), *Fowl adenovirus* (FAV), and *Reoviruses* leading to synergistic effects of both agents which further causes decreased immune response against several vaccine viruses, resulting in vaccination failures (Toro *et al.,* 2006; Dhama *et al.,* 2008).

In India, the disease has long been suspected on the basis of clinical manifestations and lesions, virus detection by immunoperoxidase test, polymerase chain reaction (PCR), and isolation (Khanna, 2010). The 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 (Natesan *et al.,* 2006; Praveen *et al.,* 2008; Bhatt *et al.,* 2011; Wani *et al.,* 2013; Gowthaman *et al.,* 2014). Hence, present investigation was undertaken to confirm CAV by PCR technique in commercial layers of Chhattisgarh showing sings and lesions suggestive of CIA.

Materials and Methods

Outbreaks of CIA in 6-11 weeks old commercial layer farms with a capacity of 5000-10000 birds were observed in the Durg, Rajnandgaon, and Raipur districts of Chhattisgarh. Total of Eleven 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 the thymus, bursa of Fabricius, spleen, liver, kidney, and bone marrow were collected in 10% buffered formalin and processed for histopathological study by paraffin embedding technique. Sections were cut at 5-6 µ thickness and stained with routine haematoxylin and eosin (H and E) staining (Luna, 1968).

Detection of CAV by PCR

Tissue samples of the thymus were also collected from the birds belonging to 11 layer farms which showed gross lesions suspected of CIA and preserved at - 20ºC for detection of the 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 specific 419 bp fragment (Ottiger, 2010). For amplification, 3µl of DNA was incubated in the total volume of 20 µl reaction mix containing 10 µl PCR master mix (2x), 1µl of each forward and reverse primer (10 pmol), 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).

Results and Discussion

Mortality of about 15-25% due to CIA in 6 to 11 weeks old commercial layers were recorded at eleven farms located

at Durg, Rajnandgaon and Raipur district of Chhattisgarh which were not vaccinated against CIA. Affected birds showed signs of anemia, generalized weakness, depression, pale comb, wattles (Fig.1), 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).

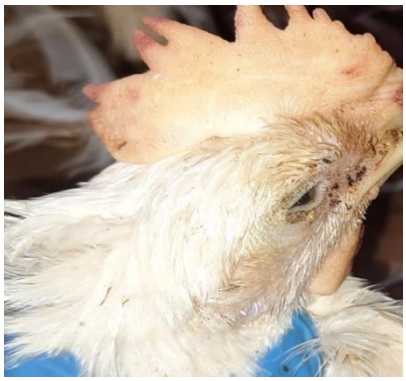

Fig.1: Pale comb and wattle

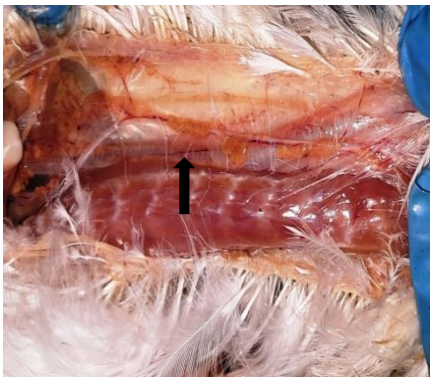

Fig.2: Thymus atrophied

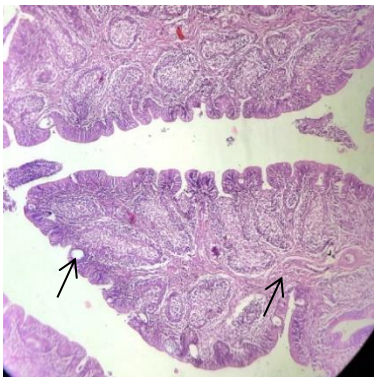

Fig. 3: Cystic bursa with depletion of lymphocytes and proliferation of fibrous tissue

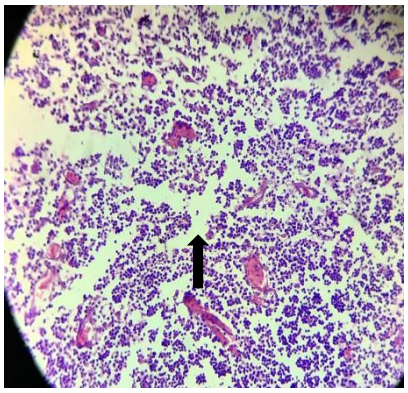

Fig.4: Thymus showing depletion of lymphocytes (H&E, 10x)

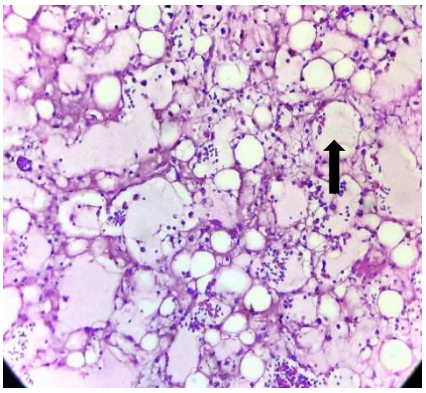

Fig.5: Bone marrow showing hypoplasia of hematopoietic tissues as replaced by adipose tissue (H&E, 40x)

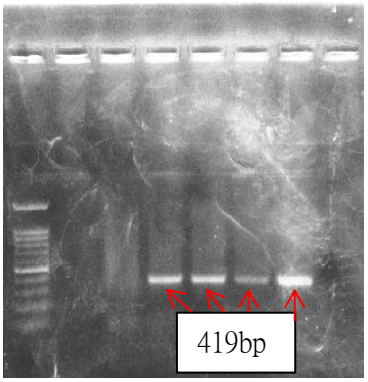

Fig. 6: Agarose gel photograph showing amplified PCR products of CAV. Lane 1: 100bp DNA ladder, Lane 2, 3: Negative control, Lane 4,5,6,7: positive field sample (419bp of VP2 gene)

Gross Pathology

Gross lesions observed in all the commercial layer farms were typical of CIA. The carcass of the birds appeared pale and anemic. Bone marrows of the femur appeared pale, yellowish, or pink-coloured in most of the birds of the affected flock. Gross lesions included severe atrophy of all thymic lobes (Fig. 2) and bursa of Fabricius and enlarged, pale liver, kidney, and spleen. Some of the birds showed subcutaneous and intramuscular hemorrhages in the pectoral and thigh region in addition to the above-mentioned lesions. These findings of lesions noticed during the investigation are in agreement with Chandrashekaraiah *et al.*, (2020).

Histopathology

Bursa of Fabricius showed mild to severe atrophy of the lymphoid follicles due to lymphocytolysis, hydropic degenerative changes, multiple areas of necrosis characterized by pyknotic and karyorhexic changes in the nuclei of lymphocytes, cystic bursal follicles and fibrous tissue proliferation (Fig. 3). Thymus showed moderate to severe atrophy with lymphoid depletion in cortex and medulla (Fig. 4). These observations are in consensus with the findings of Rimondi *et al.*, (2014) and Chandrashekaraiah *et al.*, (2020).

Bone marrow showed severe hypoplasia or atrophy of the hematopoietic tissues with increased adiposity in the marrow and medullary sinuses and had few mature erythrocytes or was completely devoid of bone marrow cells

(Fig. 5). Spleen showed various degrees of lymphocytic depletion in the white pulp, the proliferation of macrophages and reticular cell hyperplasia with increased juvenile arteries. Similar findings have also been reported by Chandrashekaraiah *et al.,* (2020) indicating the immunosuppressive effect of the virus. These changes may be attributed due to the destructive effect of the virus on hematopoietic and lymphopoietic tissues leading to impaired immune response.

The liver revealed mild to moderate degenerative and fatty changes in hepatocytes, necrotic areas, and periportal leukocytic infiltration. The kidney showed degenerative changes along with severe hemorrhages. The lesions observed in the present study are in agreement with Narayani and Ghosh (2018).

Polymerase Chain Reaction

Tissue samples of thymus collected from eleven commercial layer flocks were confirmed as CAV by PCR. Amplification of the VP2 gene of CAV revealed 419bp product for all eleven-layer flocks (Fig. 6). These findings are in accordance with Gowthaman *et al.,* (2014) who also detected CAV in thymus collected from birds**.** Similarly, the prevalence of CIA in India (Bhatt *et al.,* 2011; Wani *et al.,* 2013; Baksi *et al.,* 2016) and other parts of the World such as Switzerland (Hoop *et al.* 1992), Mexico (Ledesma *et al*., 2001), Israel (Davidson *et al.,* 2004) and China (Yao *et al.,* 2019) has been well documented by PCR assay. PCR technique has been routinely used for the diagnosis of CIA and detecting CAV in clinical samples without necessitating virus isolation. The hindrance to virus isolation is that most of the very virulent field isolates do not replicate in common tissue culture, whereas for the virus neutralization test, the field strains need to be adapted to grow *in vitro* (Chandrashekaraiah *et al.,* 2020).

Conclusions

The clinical signs, gross and histopathological findings along with PCR amplification of the VP2 gene suggested the outbreak of CIA in commercial layer farms of Chhattisgarh. In fact, typical pale, yellowish changes in the bone marrow and thymic atrophy confirm the CAV infection besides other means of diagnosis. Lymphoid atrophy is the most constant and characteristic lesion that can be found in CAV-infected birds.

Contribution by authors

All the authors contributed equally to writing the manuscript. The final manuscript was read by all others and consented to publication.

Conflict of Interests

There is no conflict of interest.

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