RESEARCH ARTICLE

VP3 Gene Analysis and Histopathological Investigation of Chicken Infectious Anaemia Virus in Poultry Flocks of Andhra Pradesh

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Abstract

Chicken infectious anaemia (CIA) is a significant economic concern affecting poultry, particularly young chicks and is marked by poor weight gain, anaemia, immunosuppression, and production losses. This research focused on the molecular detection of the VP3 gene, histopathological examination and phylogenetic analysis of chicken infectious anaemia viruses (CIAVs) in suspected commercial poultry flocks. Samples were collected from 75 birds across 22 commercial poultry flocks during necropsy. Tissue samples including thymus, bone marrow, liver and spleen were collected in phosphate buffer saline (PBS) and 10% formalin for molecular detection and histopathological studies, respectively. Samples were subjected to PCR targeting the VP3 gene producing an amplicon size of 367 bp. Among 22 flocks, 16 were found positive for the VP3 gene. The DNA from four different flocks tested positive, was sequenced and phylogenetic analysis revealed that all four isolates clustered within the same clade, grouping with strains from diverse regions in India, as well as strains from China, the USA, Malaysia, and vaccine strains. Histopathological studies revealed moderate degree of lymphocytosis, bone marrow atrophy, hepatic congestion and periportal fibrosis. The study will enhance the understanding of circulating CIAV genotypes in India and help in developing effective control and prevention strategies.

Key words: Chicken infectious anaemia, CIA virus, Histopathology, Phylogenetic analysis, Polymerase chain reaction.

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Introduction

Chicken infectious anaemia (CIA) is a notable viral disease impacting poultry, first identified in Japan in 1979 (Yuasa *et al*., 1979). It presents considerable health challenges for poultry populations worldwide (Chandra *et al*., 2001; Schat andvan Santen, 2003; Balamurugan and Kataria, 2006; Dhama *et al*., 2008). The causative agent, Chicken infectious anaemia virus (CIAV), is classified within the Gyrovirus genus of the Circoviridae family. CIAV is a small, non-enveloped virus with a circular, single-stranded DNA genome of about 2298-2319 nucleotides, encoding three proteins: VP1 (the capsid protein), VP2 (a scaffold protein) and VP3, known as apoptin, which triggers apoptosis in immune cells of chickens. CIAV is highly contagious and resilient, spreading through vertical transmission and contaminating specific pathogen-free eggs. CIAV specifically targets the thymus impeding T lymphocyte maturation and impairing cell-mediated immunity. This lymphocyte depletion causes immunosuppression, heightening vulnerability to various bacterial and viral infections, lowering vaccine effectiveness and impacting overall production performance (Ganar *et al*., 2017). This study was aimed to diagnose CIAV in poultry flocks in Andhra Pradesh through PCR targeting the VP3 gene, along with a phylogenetic analysis of field strains based on VP3 gene sequences.

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Materials and Methods

Sample Collection

The tissue samples were collected aseptically from 75 birds across 22 different commercial poultry flocks (13-layer flocks

and 9 broiler flocks) around Gannavaram (Andhra Pradesh, India) showing signs of anaemia, poor performance, dullness, and postmortem findings of regressed thymus, pale bone marrow, haemorrhages, atrophied spleen and high mortality rates. The samples including liver, thymus, bone marrow and spleen were collected in phosphate-buffered saline for molecular detection, transported on ice to the laboratory of Microbiology Department, NTR College of Veterinary Science, SVVU, Gannavaram, and stored in a -20°C refrigerator. Subsequently all the samples were examined for the presence of CIAV-DNA using polymerase chain reaction.

Viral DNA Isolation and Amplification of VP3 Gene by PCR

Tissue samples from each farm were pooled individually, and DNA was extracted using DNAZOL® Reagent following the manufacturer's instructions. The extracted DNA was then used as a template for PCR analysis targeting the VP3 gene. To amplify the gene oligonucleotide primers CIAV VP3-F (5'-ATGAACGCTCTCCAAGAAG-3') and CIAV VP3-R (5'-ACTTACAGTCTTATACACCTT-3') were used (Hiremath *et al.*, 2013). The PCR reaction was carried out in a buffer containing 1.5 mM $MgCl_2$, 200 µM of each dNTP, 10 pmol of each primer and 1.0 Unit of Taq polymerase with a total reaction volume of 25 µL. PCR conditions included an initial denaturation at 94°C for 4 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 56°C for 1 min and extension at 72°C for 1 min, concluding with a final extension at 72°C for 10 min. The amplified PCR products were resolved on a 1.5% agarose gel and visualized using a Bio-Rad gel documentation system.

Nucleotide Sequencing and Phylogenetic Analysis

The PCR products from four field isolates - CIAV-C, CIAV-M, CIAV-S and CIAV-VK that tested positive for the VP3 gene were sequenced at Barcode Biosciences Private Limited in Bangalore, Karnataka, India. Nucleotide sequences were manually edited in BioEdit software by aligning them with reference CIAV strain sequences obtained from GenBank. Phylogenetic analysis was performed using MEGA 11 and Clustal W, and a Neighbor-Joining tree was constructed with a bootstrap value of 1000 replicates.

Histopathology

During the post-mortem examination of birds suspected to have CIAV, tissue samples from the liver, thymus, bone marrow and spleen were collected separately in 10% formalin for histopathological analysis. The samples were processed using the standard method for Hematoxylin and Eosin (H&E) staining.

Results and Discussion

Detection of CIAV by PCR

Out of the 22 farms tested, 72.7% (16 farms) were positive for the VP3 gene using VP3 F and VP3 R primers, with a 367 bp amplicon confirmed on agarose gel (Fig. 1). Among these, 5 out of 9 broiler farms (55.5%) and 11 out of 13 layer farms (84.6%) were found positive. Similarly, Andrabi *et al.* (2021) identified CIAV in 26% of poultry farms in Punjab, with higher detection rates in layer farms (84.6%) than broilers (55.5%). This supports the present finding that layer farms have a higher CIAV positivity rate.

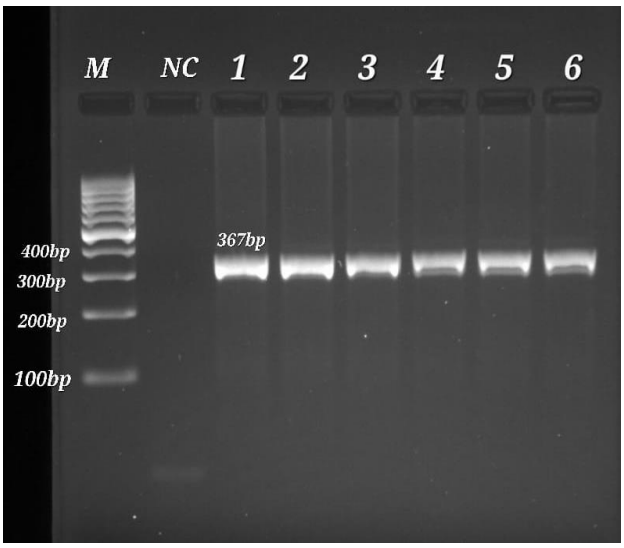

Fig. 1: Amplification of CIAV VP3 gene (367 bp). Lane M: 100 bp DNA ladder, Lane NC: Negative control, Lane 1: Positive control and Lane 2-6: Samples positive for CIAV.

Phylogenetic Analysis of CIAV VP3 Gene Sequences

The nucleotide sequences of the VP3 gene, named as CIAV-C, CIAV-M, CIAV-S, and CIAV-VK, were sequenced and submitted to GenBank, received the accession numbers PQ059476, PQ059477, PQ059478 and PQ059479, respectively.

Nucleotide BLAST analysis of the VP3 gene showed that local CIAV isolates (CIAV-C and CIAV-S) had 100% similarity with several global strains including Del-Ros/USA/vaccine, Gujarat-India, TANUVAS-India, Nagpur-India and strains from the USA, China, Japan, and Argentina with the lowest similarity (98.9%) observed with a China strain. Isolates CIAV-M and CIAV-VK showed 99.7% and 99.4% similarity with these strains with a minimum similarity of 98.3% and 98.9%, respectively. Phylogenetic analysis (Fig. 2) indicated that all four local isolates clustered with strains from India, China, the USA, Malaysia, and vaccine strains (Cux-1, Del-Ros) highlighting strong conservation of VP3. Consistent with this study, Hiremath *et al.* (2013) and Andrabi *et al.* (2021) also reported high VP3 gene homology (97.3-100%) with Indian and international strains suggesting minimal genetic divergence among VP3 sequences worldwide.

Histopathology

Histopathological analysis of tissues from CIAV-infected samples showed notable microscopic changes (Fig. 3-8) particularly in the bone marrow, where moderate atrophy was observed with extensive fatty degeneration and stem

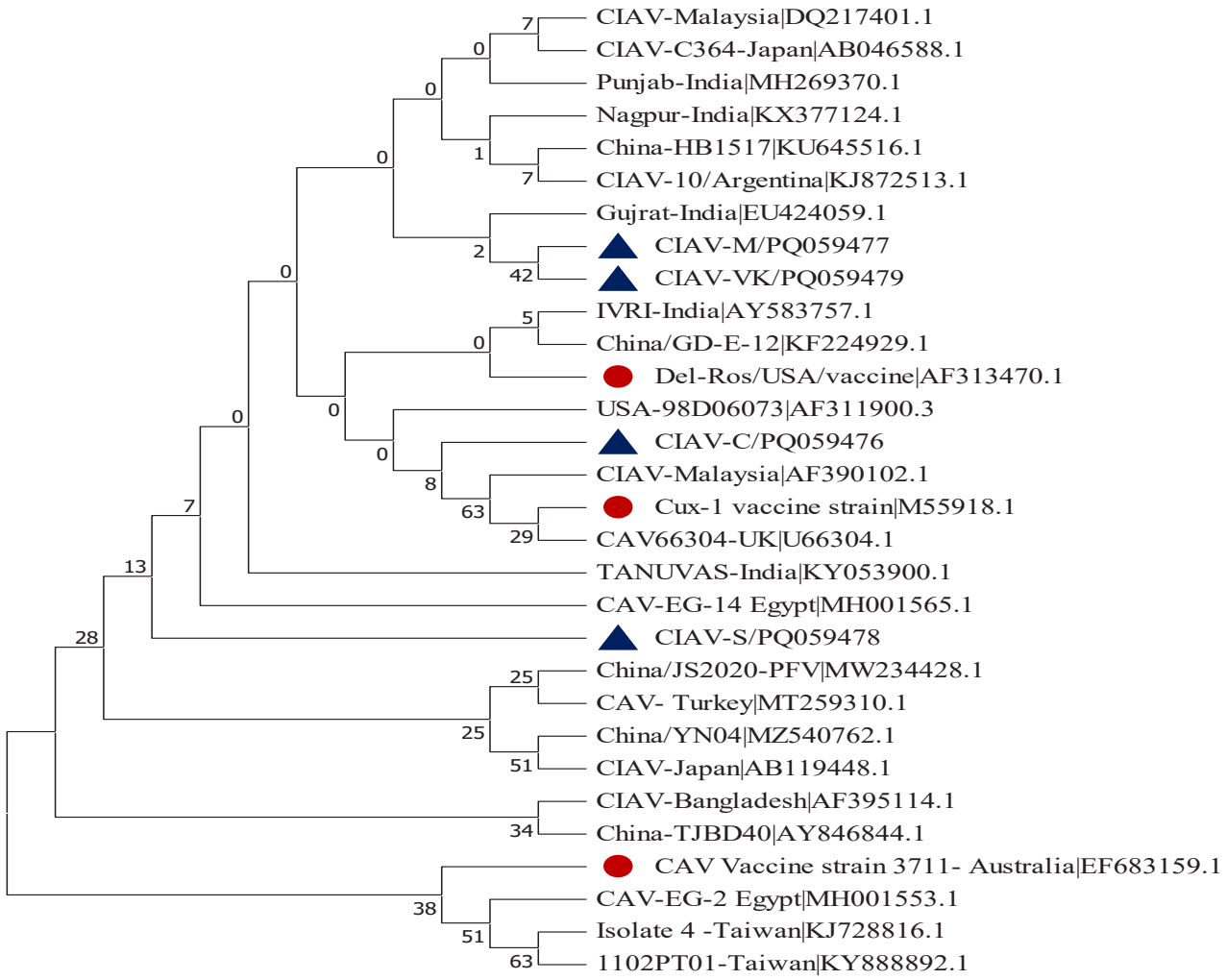

Fig. 2: Phylogenetic tree based on multiple sequence alignment of VP3 gene nucleotide sequences by Neighbour-Joining Method.

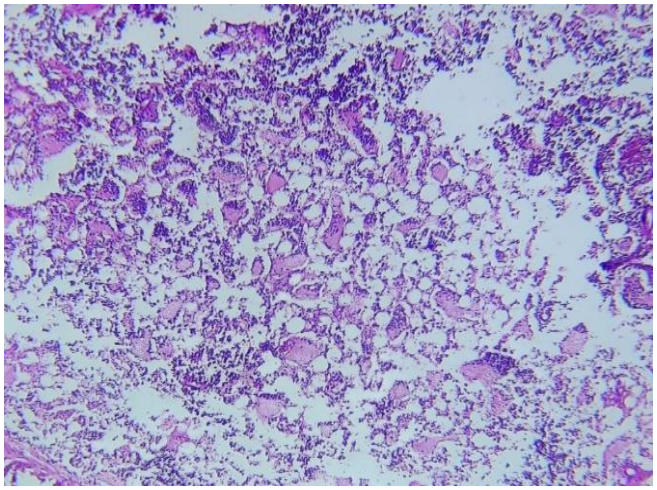

Fig. 3: Histopathological changes in bone marrow showing moderate degree of bone marrow atrophy.

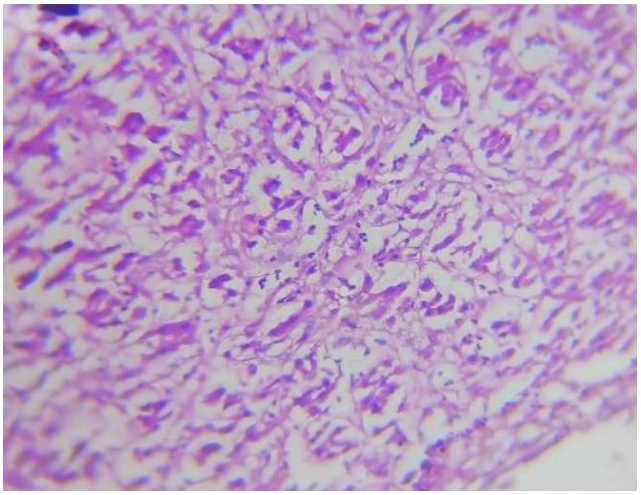

Fig. 4: Histopathology of liver showing focal necrosis

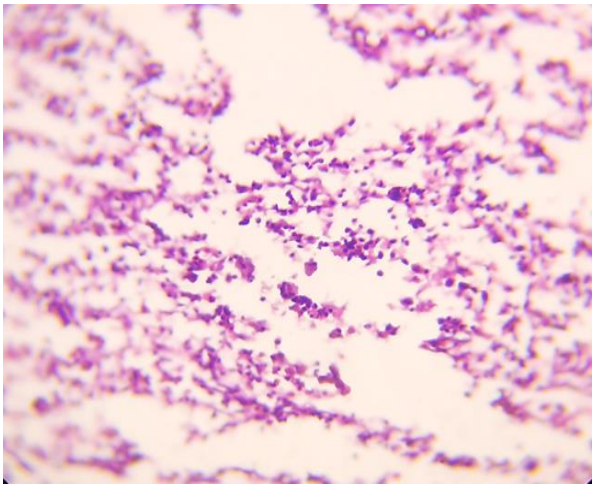

Fig. 5: Histopathology of spleen showing depletion of RBC's and lymphocytolysis

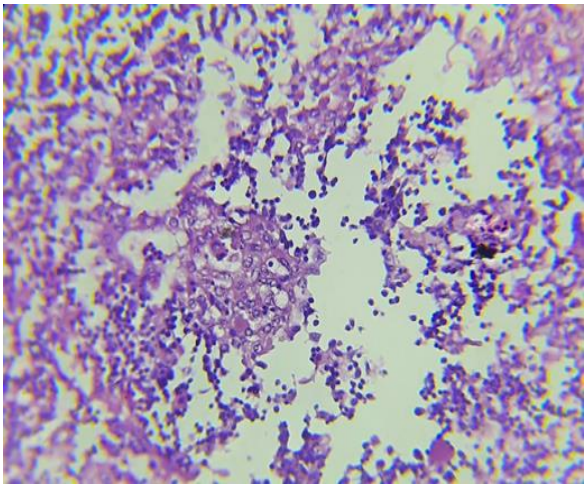

Fig. 6: Histopathology of thymus showing lymphocytolysis

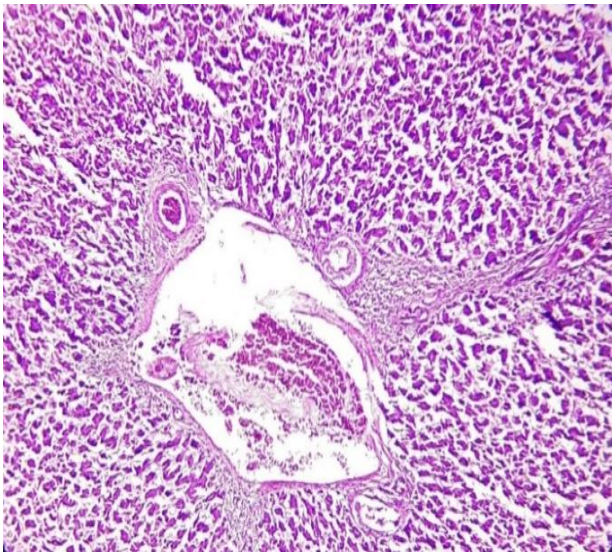

Fig. 7: Histopathology of liver showing bile duct proliferation

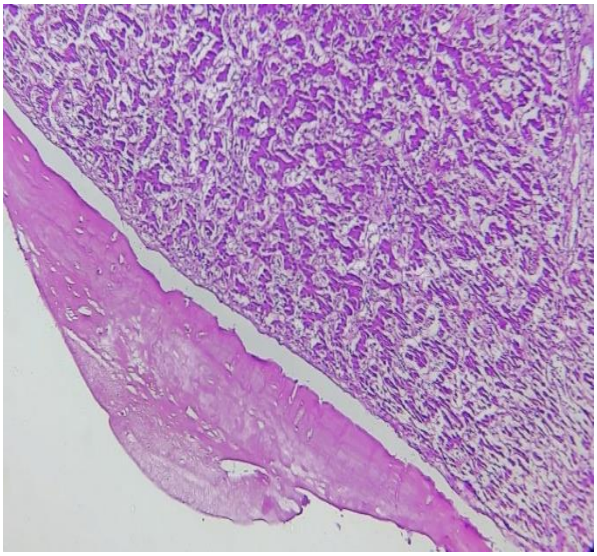

Fig. 8: Histopathology of liver showing perihepatitis

cell loss. The spleen showed mild lymphocytolysis, while the liver exhibited focal necrosis, vascular congestion, periportal fibrosis, bile duct proliferation and perihepatitis. The thymus displayed focal lymphocytolysis and reticuloendothelial cell proliferation. The findings were consistent with those of Hegazy *et al.* (2010), Haridy *et al.* (2012) and Castano *et al.* (2019), who reported similar observations, including lymphoid depletion in the thymus and spleen as well as hypoplasia of the bone marrow. The histopathology confirmed CIAV infection.

Conclusion

The research revealed that the circulating CIAV in Andhra Pradesh shows a significant potential for spread and contains motifs typical of highly virulent viruses suggesting its high pathogenicity. The VP3 gene nucleotide sequences phylogenetic analysis revealed that the local CIAVs grouped with established genotypes as well as strains from China, USA, Malaysia and vaccine strains. This observation implies that the VP3 gene in these circulating strains likely lacks genetic diversity which may suggest that any differences in virulence are not related to this particular gene. This data is essential for developing effective disease control strategies and management practices to reduce economic losses and address vaccine inefficacy related to CIA in commercial poultry.

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