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Molecular diagnosis of chicken infectious anemia in commercial chicken in Andhra Pradesh

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Abstract
The objective of this study was to carry out molecular diagnosis of Chicken Infectious Anemia in commercial chicken. A total of 143 commercial chicken from different farms located in different districts of AP were suspected for CIA based on history, clinical signs and gross lesions. On external examination, affected birds exhibited pale comb and wattles, atrophy of all lymphoid organs, pale discoloration of bone marrow along with hemorrhages on thigh and breast muscles. Blood samples collected randomly from the birds in the suspected flocks grossly appeared as pale and watery contents. On hematological examination there was a significant decline in the Hemoglobin and Packed Cell Volume indicating severe anemia in CIA infected birds. Molecular diagnosis of CIA was performed by polymerase chain reaction (PCR) by amplifying the VP1 and VP3 genes of CIAV.

Keywords: CIAV, chicken infectious anemia, PCR, VP1, VP3

Introduction
Poultry is one of the most rapidly growing segments of the agricultural sector in India. Total poultry population in India as per the recent 20th livestock census was 851.81 million contributing to 49.01 percent of total meat production (Bharti *et al.*, 2022) [3] including 107.9 million in Andhra Pradesh (BAHS, 2019) [2]. The intensive rearing system of birds with a high density of poultry population to increase profit resulted in continuous stress over birds leading to increased susceptibility to various infections. The important viral agents that induce immunosuppression in poultry include Chicken Infectious Anemia Virus (CIAV), Infectious Bursal Disease Virus (IBDV), Marek's Disease Virus (MDV), Reo Virus, Reticuloendotheliosis Virus (REV), Avian Leukosis Viral Complex (ALVC) (Gimeno and Schat, 2018) [6].
Chicken Infectious Anemia (CIA) is a highly contagious, emerging, immunosuppressive viral disease mainly of young chickens (Natesan *et al.*, 2006) [13] caused by "Chicken Infectious Anemia Virus." CIAV belongs to the genus Gyrovirus of the family Anelloviridae that contain a negative sense, 2.3 kb circular, single stranded DNA genome (Rosario *et al.*, 2017) [14]. The disease is characterized by poor weight gain, severe anemia, aplasia of the bone marrow and generalized lymphoid atrophy (Dhama *et al.*, 2008) [5]. Subclinical infection of CIA had an adverse effect on immune status of affected chicken (Haridy *et al*., 2012) [7]. The present study was undertaken to carry out molecular diagnosis of CIA through PCR in commercial chicken in different farms located in AP.

Materials and Methods
In the present study, a total of 143 commercial chicken from different farms located in different districts of AP were suspected for CIA based on history, clinical signs and gross lesions. Blood samples from all the suspected cases were collected and tissue samples were collected in sterile tubes during post-mortems conducted at Department of Veterinary Pathology, NTR College of Veterinary Science, Gannavaram. For molecular diagnosis, tissues collected from the thymus, bone marrow, liver and spleen were stored at -20 °C.

DNA isolation and PCR

DNA was isolated from suitable tissues by using the HipurA® Mammalian Genomic DNA Purification Kit and primers were used specifically to amplify the VP1 and VP3 genes of CIAV. The primers used were i.e., F: 5' AGCCGACCCCGAACCGCAAGAA 3' and R: 5' ATCAGGGCTGCGTCCCCCAGTACA 3' for VP1 and F: 5' ATGAACGCTCTCCAAGAAG 3' and R: 5' ACTTACAGTCTTATACACCTT 3' for VP3 as described by Hiremath *et al.* (2013) [9]. The PCR conditions were standardized as per the details given in Table 1. Electrophoresis in a 1.5% agarose gel in 1 x TAE buffer (Thermoscientific) were used to detect the PCR products. The gel was visualized, and the results were documented in a gel documentation system (Biorad).

Table 1: PCR conditions for the amplification of VP1 and VP3 genes of CIAV

Primers	Initial Denaturation	Denaturation	Annealing	Extension	Final Extension
VP1	94 ºC	94 ºC	57 ºC	72 ºC	72 ºC
	4 min	1 min	1 min	2 min	8 min
		35 cycles			
VP3	94 ºC	94 ºC	57 ºC	72 ºC	72 ºC
	4 min	1 min	1 min	1 min	6 min
		35 cycles			

Results: CIA was diagnosed in 87 birds out of 143 suspected commercial chicken based on PCR (Table 2) (Fig.1).

Table 2: Details of positive samples based on PCR

S. No	No. of suspected samples	CIAV genome	No. of positive samples (%)
1	71	VP1	43 (60.5%)
2	72	VP3	44 (61.1%)
Total	143		87 (60.8%)

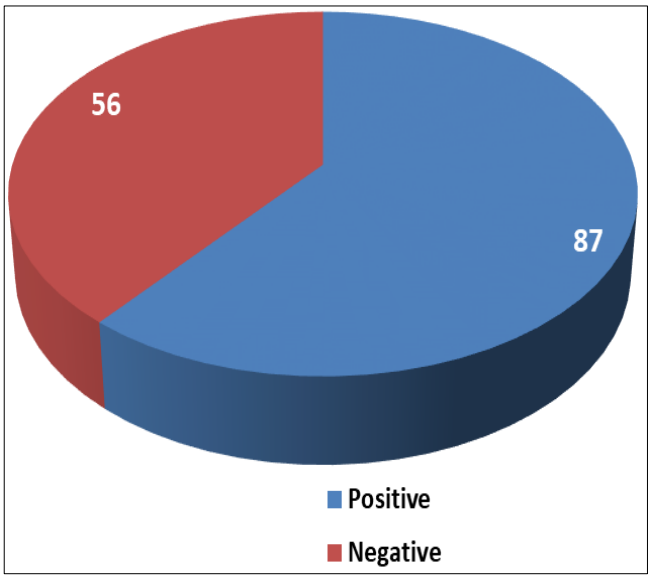


Fig 1: Pie diagram representing the positive and negative cases of CIA through PCR

Gross Pathology

Birds affected with CIA exhibited gross lesions like pale comb and wattles (Fig 2), atrophy of all lymphoid organs and patchy hemorrhages on skeletal muscles (Fig 3). Majority of affected birds revealed small, pale, shrunken thymus (Fig 4), pale pink to yellow discoloration of bone marrow of femur (Fig 5), atrophy of bursa, spleen and enlarged, friable, pale livers (Fig 6), petechiae to patchy haemorrhages on thigh muscle and breast muscles.

Hematology

Blood samples collected randomly from the birds in the suspected flocks grossly appeared as pale and watery contents (Fig. 7) when compared to blood samples collected from apparently normal chicken. On hematological examination there was a significant decline in the Hemoglobin concentration (5.56±0.29 g/dl) and Packed Cell Volume (17±0.79%) of CIA affected flock when compared to normal healthy birds indicating severe anemia in CIA infected birds (Table 3)

Table 3: Comparative haematological findings of CIA positive and CIA negative flocks

S. No	Hematological Parameter	Apparently healthy flock	CIA affected flock	P Value
1	Hemoglobin (g/dL)	11.31±0.21	5.56±0.29	$p<0.01$
2	PCV (%)	33.4±0.35	17±0.79	$p<0.01$

Fig 2: Note paleness of comb and wattles in the affected bird when compared to normal bird (left)

Fig 3: Patchy hemorrhages on thigh muscle can be observed

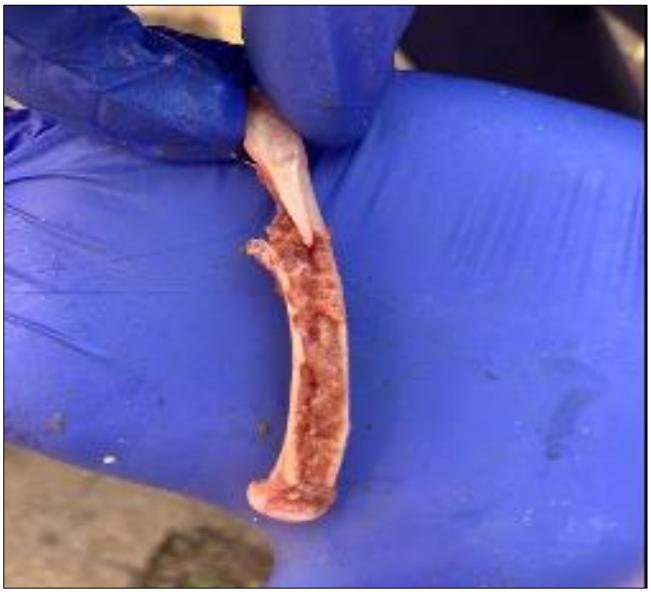


Fig 5: Pale discoloration of bone marrow in the affected bird

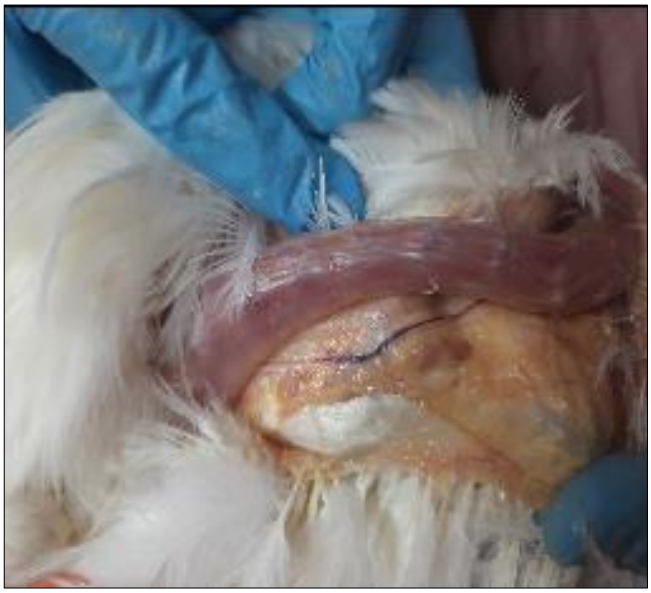


Fig 4: Severe atrophy of thymus in the CIA affected bird

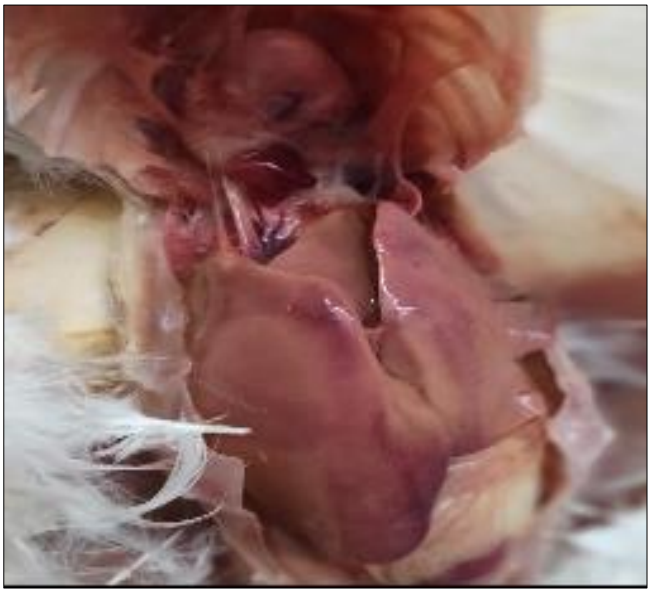


Fig 6: Note pale discoloration of liver in the affected bird

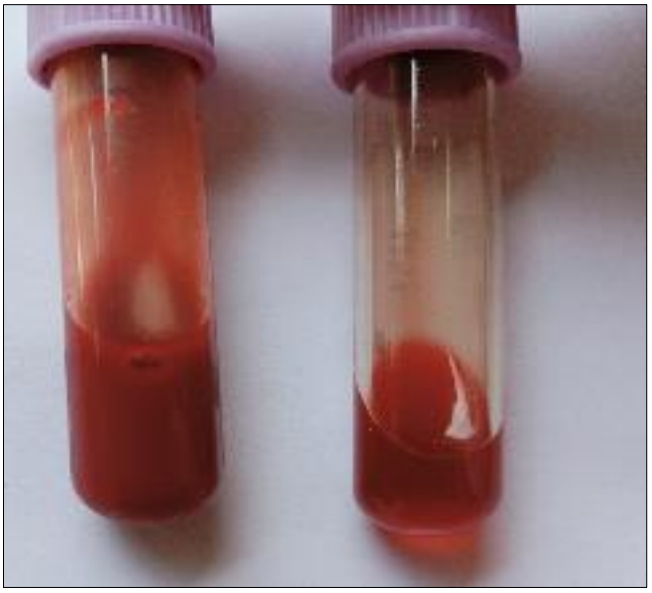
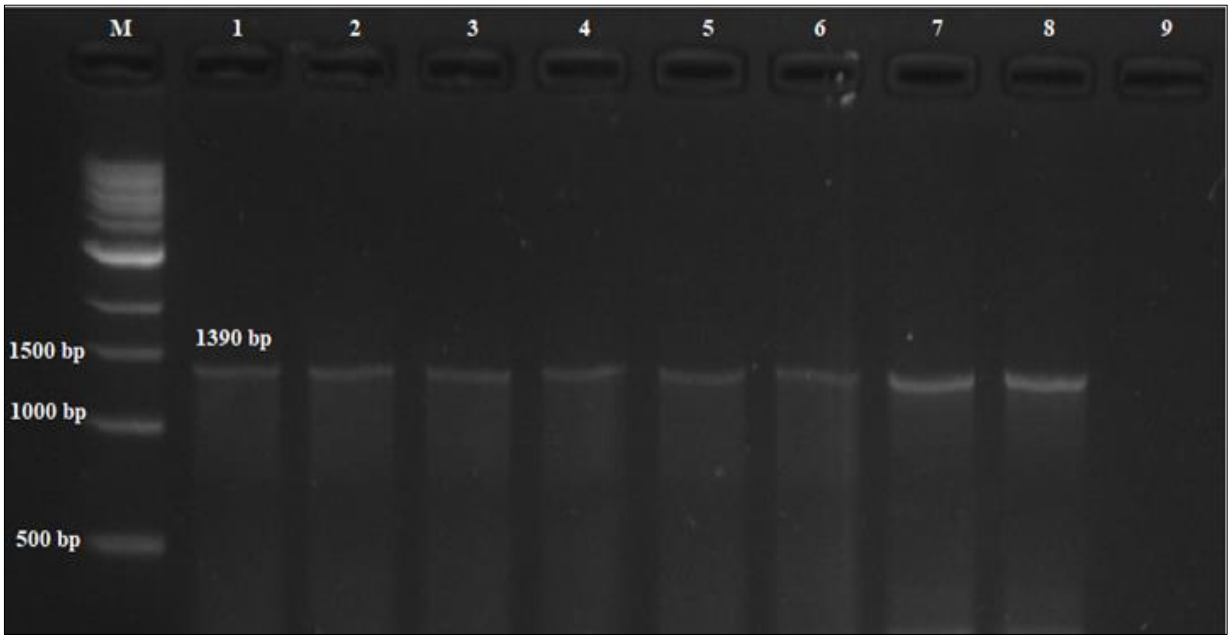
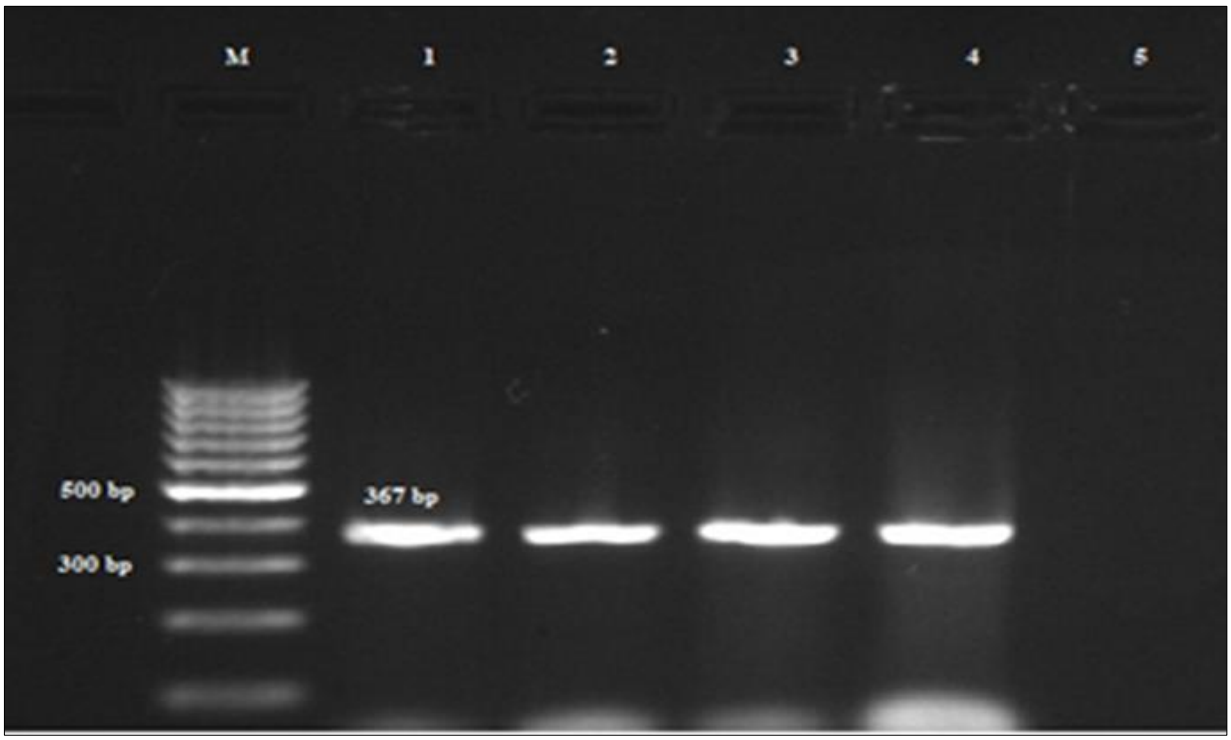


Fig 7: Note pale watery blood when compared to that of normal (left)



Lane M: Quick load 1 kb extend DNA ladder Lane 1 to 3: Thymus tissue samples
Lane 4 to 6: Bone marrow samples Lane 7: Liver tissue sample Lane 8: Spleen tissue sample Lane 9: Template DNA control

Fig 8: Agarose gel electrophoresis of amplified product of VP1 gene of CIAV



Lane M: 100 bp DNA ladder Lane 1 to 4: PCR products from thymus, bone marrow, liver and spleen tissue samples respectively Lane 5: Template DNA control

Fig 9: Agarose gel electrophoresis of amplified product of VP3 gene of CIAV

Molecular Diagnosis

In the present study, molecular diagnosis of CIA disease in commercial chicken was carried out by using primers specific for VP1 and VP3 genes of CIAV. The genomic DNA extracted from thymus, bone marrow, spleen and liver tissue samples were used for amplification and a total of 87 samples were found to be positive for CIAV. On electrophoretic analysis, amplicons of size 1390 bp for VP1 gene, 367 bp for VP3 (Fig 8 and 9) were obtained confirming the presence of CIA viral DNA in the samples.

Discussion

On post-mortem examination of CIA affected birds revealed gross lesions like atrophy of all lymphoid organs like thymus, bursa, spleen and enlarged, pale liver. The findings observed in the present study were similar to the gross findings reported by Kamdi *et al.* (2016) [11] and Chandrashekaraiah *et al.* (2020) [4] whereas Hegazy *et al.* (2010) [8] and Sonkusale *et al.* (2022) [16] observed enlarged and mottled spleen in the CIA affected chicken. Hematological findings revealed decrease in Hb and PCV values in the affected flock when compared to that of normal chicken which were in line with the reports of

Islam *et al*. (2013) [10] and Rehman *et al.* (2018) [15]. Hiremath *et al.* (2013) [9] and Andrabi *et al.* (2021) [1] confirmed outbreak of CIA by carrying out amplification of all the three genes (VP1, VP2 and VP3) of the virus obtaining similar amplicons whereas Krishan *et al*. (2015) [12] and Wani *et al*. (2015) [17] confirmed CIAV by amplifying only VP2 gene using specific primers and yielded an amplicon size of 651 bp.

Conclusion

In the present study outbreaks of CIA were confirmed based on gross lesions, hematology and PCR. The positive rate of CIA detection through PCR was 60.8% in the present study. PCR has emerged as a reliable and confirmatory diagnostic technique for the detection of CIA outbreaks in the affected flocks.

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