



Pathological studies on chicken infectious anaemia and concurrent infections in layer flocks of Namakkal, Tamil Nadu

SEDENEINUO SUOHU¹✉, G A BALASUBRAMANIAM¹, A ARULMOZHI¹,
 T R GOPALAKRISHNAMURTHY² and A RAJA³

Veterinary College and Research Institute, Namakkal, Tamil Nadu 637 002 India

Received: 21 October 2023; Accepted: 24 July 2024

ABSTRACT

Chicken Infectious Anaemia (CIA) is an economically important avian viral disease and causes severe economic loss due to vaccination failure and makes the birds more susceptible to secondary infections. Huge mortality is being recorded while CIA occurs concurrently with other bacterial and viral diseases. The present study aimed to study the incidence, pathology and disease pattern of Chicken Anaemia Virus (CAV) and its concurrent infections. A total of 60 layer poultry flocks aged 6 to 55 weeks with the history suggestive of CAV and concurrent infections were investigated. Out of 60 flocks, 46 were found to be positive for CAV out of which 17 had concurrent infections of viral and bacterial diseases. Among them, MD was found to be positive in 6 flocks and the remaining 11 flocks showed gangrenous dermatitis in which CAV was co-infected with various combination bacterial agents, viz. *Clostridium perfringens*, *Staphylococcus aureus* and *Escherichia coli*. CAV with concurrent infections caused more intense pathological changes as compared to CAV alone. The organs showed prominent lesions of severe enlargement and nodules in concurrent infection of CAV with MD, whereas in CAV + GD, organs showed more vascular changes like congestion as compared to individual infection of CAV alone. Pleomorphic lymphocyte infiltration and fibrinous exudate were observed in organs of CAV co-infection with MD. Bacterial clusters were observed in the organs of CAV + GD indicating septicemia due to immunosuppression by CAV.

Keywords: Chicken infectious anaemia, Concurrent infections, Gangrenous dermatitis, Marek's disease, PCR

Chicken infectious anaemia is an economically important viral disease of poultry which was first reported in Japan in 1979 and causes considerable health problems in the poultry flocks worldwide. CIA has worldwide distribution which is of enormous significance and has attained the status of emerging and immunosuppressive disease affecting chickens (Kamdi *et al.* 2020). The etiological agent is chicken anaemia virus (CAV) of genus *Gyrovirus* under family *Anelloviridae* having circular single stranded DNA genome of 2.3 kb in size. CAV is a potent immunosuppressive agent and affects primarily the young chicks. The disease is characterized by severe anaemia, poor weight gain, generalized lymphoid atrophy with concomitant immunosuppression. The immune system gets damaged by CAV, thereby increasing susceptibility of the host to various viral diseases like Marek's disease and fowl adenovirus and secondary bacterial infections like *Clostridium perfringens*, *Staphylococcus aureus* and *Escherichia coli* (Umar *et al.* 2014).

Present address: ¹Veterinary College and Research Institute, Tamil Nadu Veterinary and Animal Sciences University, Namakkal, Tamil Nadu. ²Poultry Disease Diagnosis and Surveillance Laboratory, Namakkal, Tamil Nadu. ³Madras Veterinary College, Chennai, Tamil Nadu. ✉Corresponding author email: iamsedesuohu@gmail.com

It is an immunosuppressive disease in which the virus has tropism for the haemocytoblasts of the bone marrow and lymphoblast in the thymic cortex. The destruction of the erythroid and myeloid progenitors in the bone marrow results in severe anaemia, eventually leading to depletion of the erythropoietic and leukopoietic cells. CAV induced immunosuppression results from destruction of T lymphocyte precursor cells consequently leading to susceptibility and enhancement of pathogenicity of secondary infections and suboptimal antibody responses. The effects of CAV are multifactorial and depresses vaccine immunity and production performances under field conditions (Francesco *et al.* 2021).

The present study was designed to study the disease pattern of CIA and other concurrent diseases in relation to the incidence and pathological changes which occur due to the immunosuppressive nature of CIA.

MATERIALS AND METHODS

Flock history: Sixty commercial layer flocks with a flock size of 2,000 to 50,000 chickens were investigated in and around Namakkal District of Tamil Nadu, India. Dead and ailing birds suspected for CAV infection from chick to adult layer stage, i.e. 6 to 55 weeks of age were examined. Detailed data regarding breed and strain of chicken, flock

size, age, method of rearing and vaccination schedule were collected.

Postmortem examination: Detailed necropsy was conducted on the birds suspected for CAV and concurrent infections brought to the Department of Veterinary Pathology and PDDSL (Poultry Disease Diagnosis and Surveillance Laboratory, Namakkal) and the gross lesions were documented.

Cytological examination: Bone marrow smears were prepared from femur by impression method. The cytological smears were air dried, fixed in methanol and subsequently stained with Giemsa stain.

Histopathological examination: The samples such as thymus, bone marrow, spleen, bursa of Fabricius, liver, kidney, skin and muscles were collected in 10% neutral buffered formalin for histopathological studies. The paraffin embedded sections were cut at 5 µm thickness and stained with haematoxylin and eosin (H&E) for histopathology and phloxine tartrazine stain was used for detection of viral inclusion bodies (Bancroft and Gamble 2008).

Confirmation of viral etiology by molecular method

Chicken infectious anaemia (CIA): DNA extraction and purification was done using the QIAamp® DNA Mini Kit (Qiagen). Thermal cyclor conditions were 95°C for 3 min (Initial denaturation) followed by 35 cycles of 94°C for 1 min (Denaturation), 60°C for 1 min (Annealing), 72°C for 1 min (Extension) and 72°C for 10 min (Final extension). The amplification of VP2 gene targeting 419 bp amplicon was done by using the following specific primers for CAV. Details of the primers used in the study are given in Table 1.

Marek's Disease virus (MDV): For molecular detection of Marek's disease, two genes namely *meq* gene (1081 bp) and 132 bp tandem repeat gene (434 bp) were targeted. The thermal cyclor conditions were 94°C for 2 min (Initial denaturation) followed by 35 cycles of 94°C for 1 min (Denaturation), 60°C for 1 min (Annealing), 72°C for 1 min (Extension) and 72°C for 10 min (Final extension).

Fowl adenovirus (FAdV): The thermal cycling conditions for PCR were 95°C for 2 min, 35 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s followed by a cycle of 72°C for 5 min. The following primer sequence was used to detect hexon gene (897 bp) of FAdV.

Isolation and identification of bacterial agents

Cultural tests: The suspected samples/swabs from birds

were collected for identification of bacterial agents. The samples were inoculated into brain heart infusion broth and agar and then streaked on to selective agar plates viz. Mannitol salt agar (MSA) and Eosin-methylene blue agar (EMB) to identify *Staphylococcus aureus* and *Escherichia coli* respectively. The samples were inoculated into skim milk media and streaked into egg yolk agar for identification of *Clostridium perfringens*. The plates were incubated under aerobic and/or anaerobic condition at 37°C for overnight. The organisms were identified based on colony morphology, growth and biochemical characteristics (Markey *et al.* 2013). Bacterial organisms were identified by various biochemical tests such as indole, methyl red, Voges-Proskauer, citrate and tube coagulase tests.

RESULTS AND DISCUSSION

Incidence of CIA and concurrent infections: In the present study, 60 flocks were investigated for CIA infection (Table 2). Out of 60, CIA was found to be positive in 46 flocks among which 29 flocks showed individual infection of CIA and the remaining 17 flocks were co-infected with viral (6 flocks) and various bacterial agents (11 flocks) (Table 3). The concurrent infection of CAV with MDV was observed in 6 flocks (13.04%) (Yao *et al.* 2019).

Likewise, coinfection of CAV with gangrenous dermatitis caused by various bacterial pathogens was observed in 11 flocks. The major bacterial pathogen was *S. aureus* which was observed in all the 11 flocks. CAV along with *Clostridium perfringens* and *S. aureus* in 6 flocks followed by CAV with *S. aureus* and *E. coli* in 3 flocks and CAV with *S. aureus* in 2 flocks. The concurrent infections recorded in the present study is in agreement with the reports of Chandrashekaraiiah *et al.* (2020) and Arulmozhi *et al.* (2021).

The immunosuppression caused by CAV results in vaccination failure leading to concurrent infection of CAV + MD. Likewise, the invasion of opportunistic bacteria present on the skin due to immunosuppression leads to CAV + gangrenous dermatitis (Arulmozhi *et al.* 2021).

Age-wise incidence: Though CAV and concurrent infections was found in 6 to 55 weeks of age, the majority outbreaks were recorded in 11-15 weeks age group (39.13%) followed by 6-10 weeks age group (30.44%) (Bhatt *et al.* 2011). The more susceptibility of birds in these age groups to CAV may be due to rapid growth rate and pre-laying stress.

Table 1. Details of primers used in the study

Target gene	Primer sequence	Base pair	Reference
VP2 gene	Forward: 5'CTAAGATCTGCAACTGCGGA 3' Reverse: 5'CCTTGGAAGCGGATAGTCAT-3'	419 bp	Ottiger 2010
<i>Meq</i>	Forward: 5'GGCACGGTACAGGTGTAAAGAG3' Reverse: 5'GCATAGACG ATGTGCTGCTGAG3'	1081 bp	Gong <i>et al.</i> 2013
132 bp tandem repeat	Forward: 5' TACTTCCTATATAGATTGAGACGT 3' Reverse: 5'GAGATCCTCGTAAGGTGTAATATA 3'	434 bp	Becker <i>et al.</i> 1992
FAdV	Forward: 5' CAARTTCAGRCAGACGGT 3' Reverse: 5' TAGTGATGMC GSGACATCAT 3'	897 bp	Ottiger 2010

Table 2. Commercial layer flock details for Chicken anaemia virus (CAV) and concurrent infections confirmation

Farm no.	Flock size	Age (wk)	Clinical signs	Etiology detected by PCR and culture
1.	6,000	13	Pale discoloration of skin, comb, wattles	CAV
2.	4,000	12	Pale discoloration of skin, comb	CAV
3.	4,000	12	Emaciation, dullness, anorexia	CAV
4.	20,000	8	Moist exudative, edematous lesions in wings, thighs	CAV + <i>S.aureus</i> + <i>E.coli</i>
5.	10,000	16	Dullness, anorexia	Negative
6.	3,000	20	Pale discoloration of skin, emaciation	CAV
7.	31,000	12	Pale discoloration of skin, comb, wattles	CAV
8.	5,000	19	Emaciation, dullness, anorexia	CAV
9.	13,000	17	Emaciation, dullness	Negative
10.	2,500	13	Sudden death	Negative
11.	5,000	12	Pale discoloration of skin, emaciation	CAV
12.	5,500	9	Pododermatitis	CAV + <i>S.aureus</i>
13.	14,500	11	Pale discoloration of skin, emaciation	CAV
14.	2,000	13	Emaciation, dullness, anorexia	CAV
15.	3,000	30	Dullness, anorexia	CAV + MDV
16.	42,000	17	Pale discoloration of skin, comb, wattles	CAV
17.	3,300	16	Emaciation, dullness, anorexia	CAV
18.	10,000	14	Sudden death	Negative
19.	8,000	11	Emaciation, dullness, anorexia	Negative
20.	12,000	12	Pale discoloration of skin, comb, wattles	CAV
21.	36,000	10	Emaciation, dullness, anorexia	CAV
22.	42,000	13	Pale discoloration of skin, emaciation	CAV
23.	5,000	10	Moist exudative, edematous lesions in wings, thigh	CAV + <i>S. aureus</i> + <i>E. coli</i>
24.	19,000	18	Emaciation, dullness	Negative
25.	12,000	9	Pododermatitis	CAV + <i>S. aureus</i>
26.	7,000	11	Pale discoloration of skin	CAV
27.	20,000	10	Moist exudative, edematous lesions in wings	CAV + <i>Cl. perfringens</i> + <i>S. aureus</i>
28.	12,000	8	Dullness, anorexia	Negative
29.	18,000	17	Sudden death	Negative
30.	12,500	9	Emaciation, dullness, anorexia	CAV
31.	50,000	10	Serosanguinous exudates of wings	CAV + <i>S. aureus</i> + <i>E. coli</i>
32.	20,000	15	Emaciation, dullness	Negative
33.	40,000	10	Pale discoloration of skin, comb, wattles	CAV
34.	23,000	14	Pale discoloration of skin, emaciation	CAV
35.	15,000	11	Emaciation, dullness, anorexia	CAV
36.	41,000	9	Greenish-black discoloration with gangrenous changes of skin	CAV + <i>Cl. perfringens</i> + <i>S. aureus</i>
37.	50,000	8	Emaciation, dullness, anorexia	CAV
38.	3,000	13	Pale discoloration of skin, emaciation	CAV
39.	5,000	14	Emaciation, dullness, anorexia	Negative
40.	2,500	10	Pale discoloration of skin, emaciation	CAV
41.	5,000	12	Emaciation, dullness, anorexia	CAV
42.	2,000	24	Greenish-black discoloration with gangrenous changes of skin	CAV + <i>Cl. perfringens</i> + <i>S. aureus</i>
43.	10,000	10	Emaciation, dullness, anorexia	CAV
44.	13,000	12	Emaciation, dullness	Negative
45.	9,000	18	Emaciation, dullness, anorexia	Negative
46.	7,000	17	Greenish-black discoloration with gangrenous changes of skin	CAV + <i>Cl. perfringens</i> + <i>S. aureus</i>

Table 2 (Continued...)

Table 2. (Concluded)

Farm no.	Flock size	Age (wk)	Clinical signs	Etiology detected by PCR and culture
47.	6,000	18	Pale discoloration of skin, emaciation	CAV
48.	44,000	11	Greenish black discoloration with gangrenous changes of skin	CAV + <i>Cl. perfringens</i> + <i>S. aureus</i>
49	41,000	11	Greenish black discoloration with gangrenous changes of skin	CAV + <i>Cl. perfringens</i> + <i>S. aureus</i>
50	6,000	20	Sudden death	CAV + MDV
51	8,000	16	Emaciation, dullness, anorexia	Negative
52	2,500	25	Emaciation, dullness, anorexia	CAV
53	10,000	13	Pale discoloration of skin, emaciation	CAV
54	5,000	31	Unilateral paralysis of leg	CAV + MDV
55	25,000	13	Emaciation, dullness, anorexia	CAV
56	15,000	15	Dullness, anorexia	Negative
57	10,000	26	Sudden death	CAV + MDV
58	10,000	9	Pale discoloration of skin, emaciation	CAV
59	8,000	55	Dullness, anorexia	CAV + MDV
60	25,000	50	Unilateral paralysis of leg	CAV + MDV

Clinical signs: The CAV affected birds showed dullness, depression, emaciation, severe anaemia, pale skin, combs, wattle and shanks. In some cases, sudden mortality was also noticed (Hussein *et al.* 2016). The birds affected with gangrenous dermatitis due to concurrent infection of CAV with bacterial agents exhibited moist exudative lesions particularly in the wings, back, breast and thigh region. The paleness and anaemic signs noted in the present study were related with the pale bone marrow in necropsy and poor erythropoietic progenitor cells in histopathology (Arulmozhi *et al.* 2021). In a few cases in CAV + MDV, the affected birds revealed unilateral paralysis characterized by stretching of one leg forward and one leg backward (Reddy *et al.* 2022).

Diagnosis of concurrent infection of CAV with viral etiologies: The genome of CAV was detected in 46 flocks by amplification of product of 419 bp of VP2 gene (Fig. 1A). The CAV positive flocks were screened for MDV by targeting two genes namely *meq* gene with amplicon size of 1081 bp (Fig. 1B) and 132 bp gene (amplicon size 434 bp). Six flocks (13.04%) were found positive for MDV. The concurrent infection of CAV + MDV recorded in the present study was supported by Yao *et al.* (2019) who reported that 35 (48.6%) out of 72 samples had dual infection of CAV + MDV.

Though all the samples were subjected to FAdV by targeting Hexon gene, none of the flocks were found positive for FAdV. This finding is contrary to Chitradevi *et al.* (2020) who observed CAV + FAdV, where incidence were observed in 7 (7.4%) out of 94 flocks indicating lesser incidence of CAV + FAdV co-infection.

Diagnosis of concurrent infection of CAV with bacterial etiologies: The stormy clot formation in skim milk media; zone of opalescence on egg yolk agar medium (Nagler's reaction) and gram-positive rods with subterminal spores in Gram's stain confirmed the *Clostridium perfringens*

Table 3. Incidence of CAV and concurrent infections in poultry flocks

CAV and concurrent infections	Number of flocks	Incidence (%)
CAV	29	63.04
CAV + MDV	06	13.04
CAV + FAV	0	0
CAV + <i>Cl. perfringens</i> + <i>S. aureus</i>	06	13.04
CAV + <i>S. aureus</i> + <i>E. coli</i>	03	6.52
CAV + <i>S. aureus</i>	02	4.35
Total	46	100.00

infection. The presence of *Staphylococcus aureus* was detected by presence of round, minute yellow colored colonies in MSA agar; clusters or bunches of gram positive cocci in Gram's stain and clot formation in tube coagulase test. The *E. coli* was identified by lactose fermenting pink colonies on MacConkey's agar; green metallic sheen colonies on EMB agar; gram negative bacilli in Gram's stain and confirmed by biochemical test viz. positive for indole and methyl red; negative for Vogues Proskauer's test and citrate utilization test (Chandrashekaraiiah *et al.* 2020, Arulmozhi *et al.* 2021).

Gross pathology

Chicken infectious anaemia: The atrophy of the thymic lobes (Fig. 2A) and pale and anaemic bone marrow (Fig. 2B) were the consistent lesions recorded in CAV affected birds. The bone marrow of the affected birds revealed pale discoloration, yellowish pink and fatty appearance. The liver and kidneys of the affected birds exhibited pale discoloration and spleen revealed mild atrophy (Andrabi *et al.* 2021).

Chicken infectious anaemia + Marek's disease (MD): The affected birds exhibited enlargement and congestion of thymic lobes; mild nodularity in bone marrow; massive

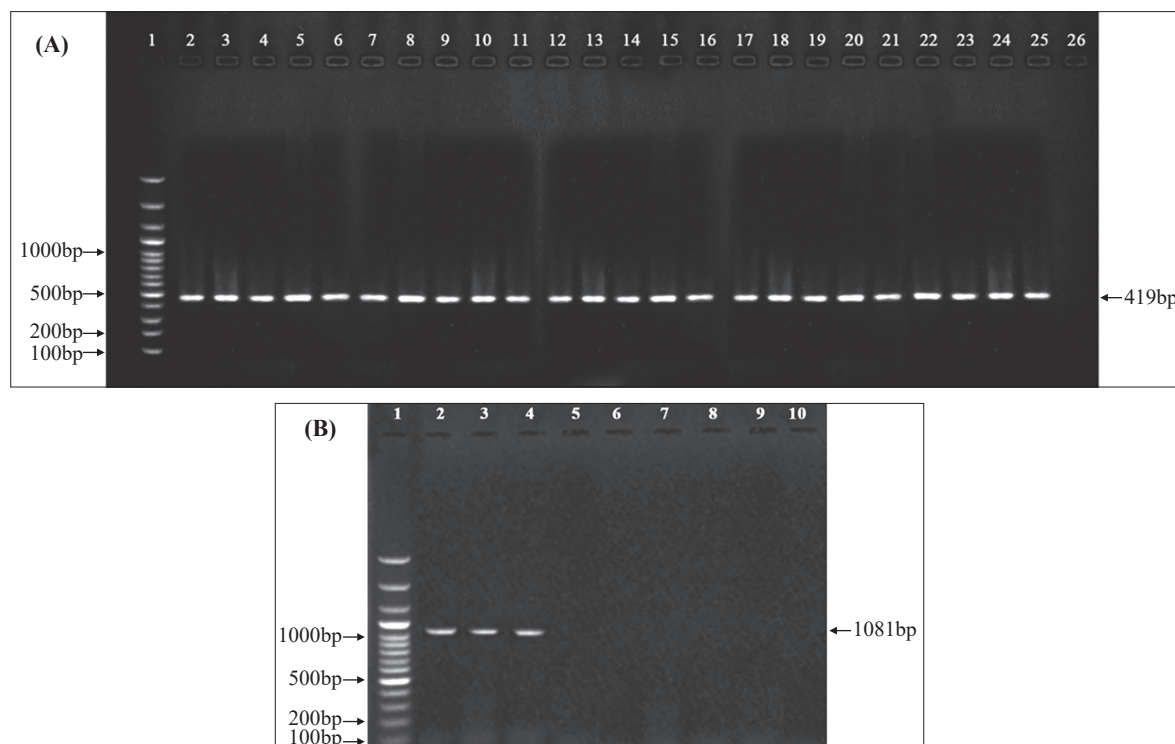


Fig. 1. (A) CAV: CAV specific amplicons visualized by agarose gel electrophoresis [Lane 1: 100 bp molecular weight marker; Lanes 2-24: Samples showing CAV specific 419 bp product of VP2 gene; Lanes 25: Positive control; Lanes 26: Negative control]; (B) MDV: MDV specific amplicons visualized by agarose gel electrophoresis [Lane 1: 100 bp molecular weight marker; Lanes 2,3: samples showing MDV specific 1081 bp product of meq gene; Lanes 4: Positive control; Lanes 5: Negative control; Lanes 6-10: samples showing negative for MDV gene].

enlargement with multifocal whitish nodules in spleen and severe enlargement with varying sizes of multiple nodules in liver (Fig. 2C). These observations are in accordance with the findings of Das *et al.* (2018).

Chicken infectious anaemia + Gangrenous dermatitis (GD): In CAV + GD infections, the vascular changes like congestion, haemorrhage and edema were more pronounced in liver, spleen and kidney than the individual infection of CAV alone. There was atrophy of thymus and mild congestion of the bone marrow were the major lesions in most of the cases. The haemorrhagic and gangrenous dermatitis of the wings (Fig. 2D), blood tinged and serosanguinous exudates in the skin, wing region and breast muscles were noticed (Chandrashekaraiah *et al.* 2020).

Cytology: The CAV affected thymus showed lymphoid depletion and bone marrow exhibited poor cellularity of erythroid and myeloid series as compared to normal

control bone marrow which had densely packed cells (Chandrashekaraiah *et al.* 2020) (Supplementary Fig. 1). Intranuclear inclusion bodies in the haemocytoblast could also be detected in the bone marrow. The cytology of bone marrow revealed pleomorphic lymphocytes in CAV and MD infection and is in correlation with the histopathological lesions. In CAV with GD affected cases, there was presence of bacteria in bone marrow which indicated septicemia and entry of bacteria via haematogenous route due to immunosuppression by CAV (Das *et al.* 2018).

Histopathology

CAV with concurrent infections, viz. MD and GD caused more intense pathological changes as compared to CAV alone.

Chicken infectious anaemia: The thymus of the affected birds revealed varying degree of moderate to severe

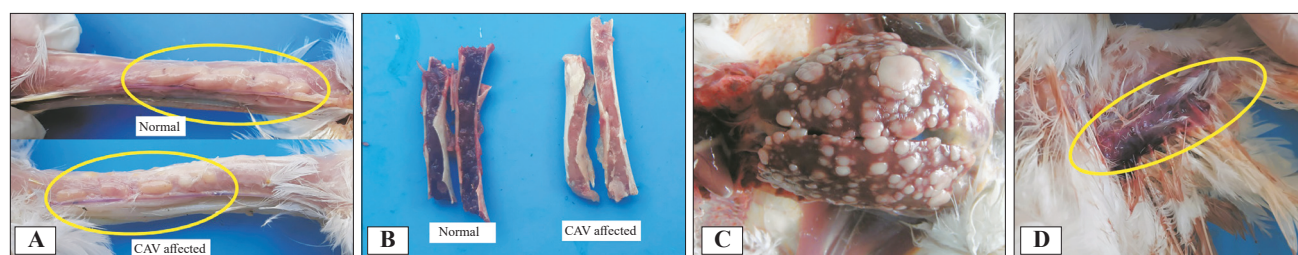


Fig. 2. (A) CAV: Affected bird showing atrophy of thymic lobes; (B) CAV: Pale discoloration of bone marrow as compared to normal dark reddish bone marrow; (C) CAV + MDV: Liver showing multiple nodules of varying sizes on the surface; (D) CAV + *Cl.perfringens* + *S.aureus*: Affected birds showing haemorrhagic and gangrenous dermatitis of wings.

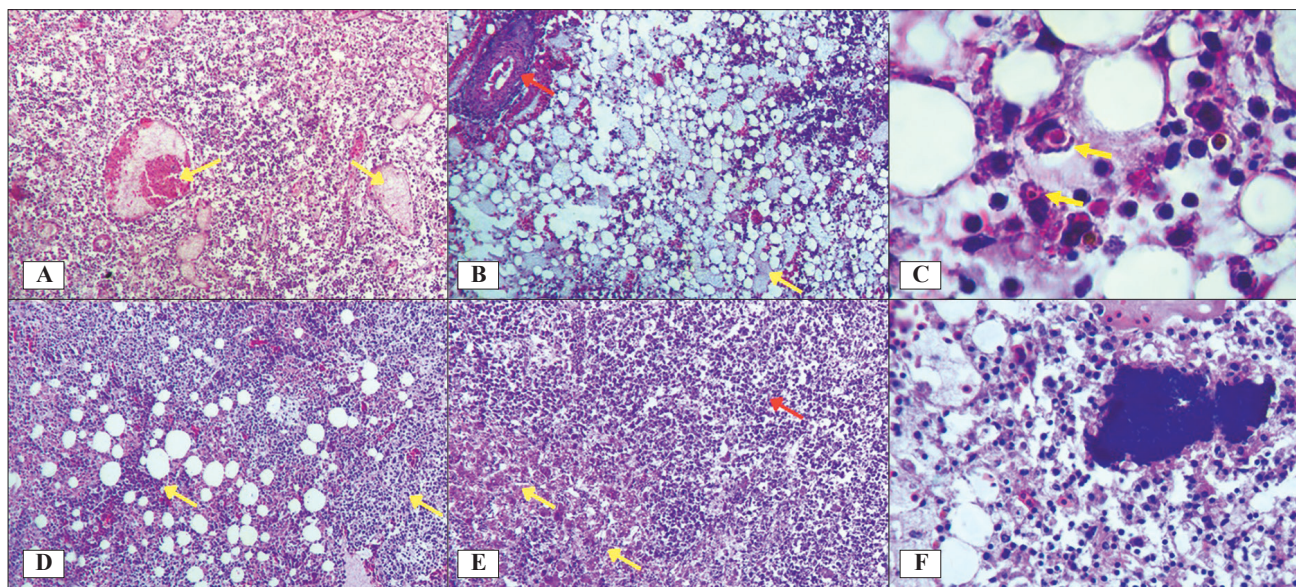


Fig. 3. (A) CAV: Thymus showing moderate lymphoid depletion, fibrinous and cellular thrombi (arrows) (H&E $\times 100$); (B) CAV: Bone marrow showing fibrinous exudate (yellow arrow), vasculoclerosis and haemorrhage (red arrow) (H&E $\times 100$); (C) CAV: Bone marrow showing intracytoplasmic inclusion bodies (arrows) in haemocytoblasts (Phloxine tartrazine $\times 1000$); (D) CAV + MDV: Bone marrow showing haematopoietic tissue being replaced with neoplastic lymphoid cell infiltration (arrows) (H&E $\times 100$); (E) CAV + MDV: Liver showing neoplastic lymphoid cell infiltration (red arrow), vacuolar degeneration and fibrinous exudate of hepatocytes (yellow arrows) (H&E $\times 100$); (F) GD: CAV + *Cl. perfringens* + *S. aureus*: Bone marrow showing large cluster of bacterial colony with hypoplastic changes (H&E $\times 400$).

lymphoid depletion with fibrinous and cellular thrombi (Fig. 3A) and severe depletion of erythropoietic and leukopoietic tissue along with degenerative and necrotic changes in bone marrow (Fig. 3B). The viral intracytoplasmic (Fig. 3C) as well as intranuclear inclusions in thymus and bone marrow could be demonstrated by special staining method Phloxine tartrazine. The vascular changes like congestion, fibrinous exudates and cellular thrombi were observed in liver, kidney and spleen (Elsamadony *et al.* 2019).

Chicken infectious anaemia and Marek's disease: In combined infection of CAV and MDV, there were pleomorphic lymphocyte infiltration replacing the normal architecture of bone marrow (Fig. 3D), thymus, liver and spleen. The neoplastic lymphocytes exhibited anisocytosis, anisokaryosis, pleomorphism and prominent nucleoli. In the liver, the characteristic lesions of lymphoma with vacuolar degeneration and fibrinous exudate in hepatic parenchyma (Fig. 3E) clearly indicated the concurrent infection of CAV and MDV. Similar histopathological lesions were also noted by Das *et al.* (2018) and Reddy *et al.* (2022).

Chicken infectious anaemia and gangrenous dermatitis: In the concurrent infection of CIA with GD, vascular changes were more predominant in bone marrow, liver and spleen than individual infection of CIA alone. The bacterial cluster was also observed in bone marrow (Fig. 3F) and other organs which indicate septicemic condition and entry of bacteria into the visceral organs via haematogenous route (Arulmozhi *et al.* 2021). The cutaneous lesions/gangrenous lesions were characterized by moderate to

severe dermal necrosis with numerous bacterial clumps and serosanguinous to haemorrhagic exudate in subcutaneous tissue. The thymus and bone marrow did not show specific microscopic lesions except mild to moderate lymphoid depletion of thymocytes and severe hypoplasia of bone marrow. These microscopic changes observed are in line with the findings of Chandrashekaraiah *et al.* (2020) who also reported that chicken infectious anaemia, being an immunosuppressive disease, causes dysfunction of humoral as well as cellular immune response on lymphoid organs of bone marrow and thymus, therefore increasing susceptibility to secondary infection and vaccine failure.

The present study documents the disease pattern of chicken infectious anaemia with other concurrent viral and bacterial infections. The immunosuppression by chicken anaemia virus (CAV) leads to susceptibility of the birds to other viral and secondary bacterial infections. In addition, immunosuppression caused by CAV results in vaccination failure, recurrent outbreak of various diseases, higher mortality and lower production performance in field conditions. Hence, proper CAV vaccination at breeder stock level and strict biosecurity measures at farm level are warranted to curtail the CAV and concurrent infection.

ACKNOWLEDGEMENT

The authors are thankful to the Tamil Nadu Veterinary and Animal Sciences University for providing the necessary facilities to carry out the research.

REFERENCES

Andrabi S A, Kimi L, Deka D, Gupta K and Singh A. 2021.

- Pathology and molecular characterization of field isolates of chicken anaemia virus circulating in commercial poultry in and around Punjab. *Indian Journal of Veterinary Pathology* **45**(3): 195–201.
- Arulmozhi A, Balasubramaniam G A, Gopalakrishnamurthy T R and Sivaseelan S. 2021. Assessment of predisposing effect of Chicken Anaemia Virus (CAV) in Gangrenous Dermatitis (GD) outbreaks in commercial layer chicken. *Indian Journal of Veterinary Pathology* **45**(4): 284–89.
- Bancroft J D and Gamble M. 2008. *Theory and Practice of Histological Techniques*. 6th edn. Churchill Livingstone, London.
- Becker Y, Asher Y, Tabor E, Davidson I, Malkinson M and Weisman Y. 1992. Polymerase chain reaction for differentiation between pathogenic and non-pathogenic serotype 1 Marek's disease viruses (MDV) and vaccine viruses of MDV-serotypes 2 and 3. *Journal of Virological Methods* **40**: 307–22.
- Bhatt P, Shukla S K, Mahendran M, Dhama K, Chawak M M and Kataria J M. 2011. Prevalence of chicken infectious anaemia virus (CIAV) in commercial poultry flocks of northern India: a serological survey. *Transboundary and Emerging Diseases* **58**(5): 458–60.
- Chandrashekaraiyah G B, Karamala S, Doddappaiah N H, Karumuri N K and Kumar V. 2020. Pathology of chicken infectious anemia (CIA) with concurrent infections. *Journal of Entomology and Zoology Studies* **8**(2): 519–24.
- Chitradevi S, Sukumar K, Suresh P, Balasubramaniam G A and Kannan D. 2020. Molecular typing of fowl adenovirus associated with gizzard erosion in commercial layer grower chicken in Tamil Nadu. *The Indian Journal of Animal Sciences* **90**(7): 977–81.
- Das S, Das D, Panda S, Sagarika S and Jena B. 2018. Clinico-pathological studies of Marek's disease in chickens. *International Journal of Livestock Research* **8**(1): 207–17.
- Elsamadony H A, Tantawy L A, Omar S E and Alah H A A. 2019. Molecular and pathological study on Chicken Anemia Virus. *Journal of Virological Sciences* **5**: 22–34.
- Francesco A D, Quaglia G, Salvatore D, Sakhría S, Catelli E, Bessoussa G, Kaboudi K, Ben Chehida N and Lupini C. 2021. Occurrence of chicken infectious anemia virus in industrial and backyard Tunisian broilers: Preliminary results. *Animals* **12**(1): 62.
- Gong Z, L Zhang, J Wang, L Chen, H Shan, Z Wang and H Ma. 2013. Isolation and analysis of a very virulent Marek's disease virus strain in China. *Virology Journal* **10**: 155.
- Hussein E, Arafa A E, Anwar N and Khafaga A. 2016. Molecular and pathological analysis of chicken anemia virus isolated from field infection in three Egyptian Provinces. *Advances in Animal and Veterinary Sciences* **4**(5): 218–29.
- Kamdi B P, Kolhe R P, Dhaygude V S and Mote C S. 2020. Chicken infectious anemia: An emerging immunosuppressive viral threat to the poultry industry. *Journal of Poultry Science and Technology* **8**: 16–22.
- Markey B, Leonard F, Archambault M, Cullinane A and Maguire D. 2013. *Clinical Veterinary Microbiology*. 2nd Ed. Mosby Elsevier, Edinburgh.
- Ottiger H P. 2010. Development, standardization and assessment of PCR systems for purity testing of avian viral vaccines. *Biologicals* **38**(3): 381–88.
- Reddy Y, Kumar A A and Amaravathi P. 2022. Diagnosis of Marek's disease through histological, immunohistochemical and molecular applications: A comparative study. *Indian Journal of Veterinary Pathology* **46**(3): 201–07.
- Umar S, Ullah S, Yaqoob M, Shah M and Ducatez M. 2014. Chicken infectious anaemia, an immunosuppressive disease of poultry birds. *World's Poultry Science Journal* **70**(4): 759–66.
- Yao S, Tuo T, Gao X, Han C, Yan N, Liu A, Gao H, Gao Y, Cui H, Liu C and Zhang Y. 2019. Molecular epidemiology of chicken anaemia virus in sick chickens in China from 2014 to 2015. *PLoS One* **14**(1): e0210696.