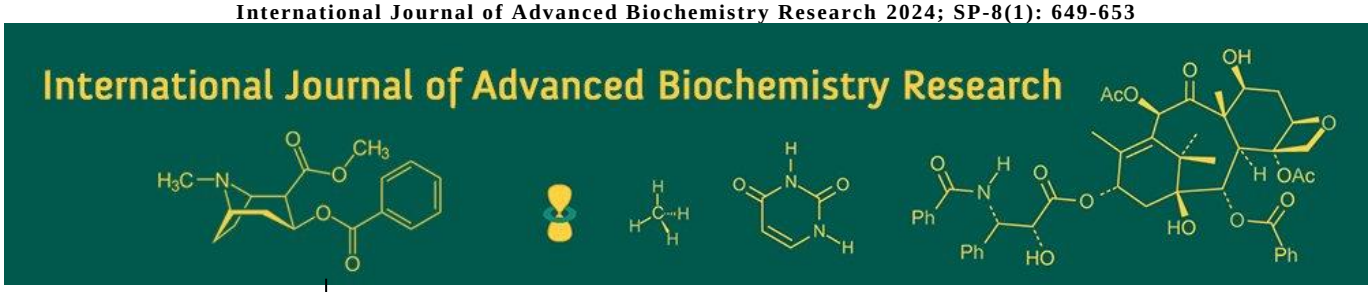

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Patho-morphological changes in hydropericardium-hepatitis syndrome in broiler chickens in Jammu region

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Abstract
Hydropericardium Hepatitis Syndrome (HHS) is an economically important poultry disease infecting wide range of poultry host including parrots, falcons and ostrich and has a significant negative economic impact on the global poultry industry. A study was conducted to investigate the pathology and molecular diagnosis of HHS in poultry birds from March 2022 to May 2023 in Jammu region. Outbreak of HHS was recorded in 2 different farms with sudden onset of high mortality in broiler birds of 4-6 weeks of age. Samples collected from broiler farms were examined for gross and histopathological changes in Fowl Adenovirus (FAdV) infection in the field samples. Gross examination showed characteristic straw coloured fluid in pericardial sac; pale, friable and enlarged livers with minute, petechial or ecchymotic haemorrhages and enlarged kidneys. Histologically, the liver of affected birds revealed presence of basophilic intranuclear inclusion bodies in hepatocytes, vacoular degeneration and focal hepatocytic necrosis. The kidneys displayed severe degeneration and necrosis of tubular epithelium, hypercellularity of glomerular tuft and interstitial haemorrhages. Spleen showed lymphocytolysis in splenic follicles in infected birds. Tissue samples (liver) of infected birds were used for DNA isolation and detection of FAdV using PCR to amplify hexon gene. The amplified PCR was analyzed by agarose gel electrophoresis indicated DNA fragments of approximately 890 bp as expected revealed presence of HPS virus. Keywords: Hydropericardium Hepatitis Syndrome (HHS), Fowl Adenovirus (FAdV), inclusion bodies, hexon.

Keywords: Patho-morphological, hydropericardium-hepatitis syndrome, broiler chickens

Introduction
Poultry industry is one of the fastest growing sector in India. Even though it is well established and organized, this sector of the economy nonetheless deals with a lot of acute and severe diseases. One of the economically significant poultry illness is hydropericardium syndrome (HPS), also known as inclusion body hepatitis-hydropericardium syndrome (IBH-HPS), hydropericardium-hepatitis syndrome (HHS), Angara disease (in Pakistan), and litchi heart disease (in India) (McFerran *et al.*, 2000) [11]. In India, HPS was first reported in the poultry belt of Jammu and Kashmir and Punjab in 1993 (Gowda *et al.*, 1994) [7]. The causative agent Fowl adenovirus is a member of the family adenoviridae and consists of a non-enveloped capsid icosahedral in shape, measuring 70-90 nm in size and containing a linear dsDNA of approximately 45 kb in size as its genome. The genome encodes for 10 primary structural proteins (hexon, penton base, fibre, terminal protein, protein, protein IIIa, protein V, protein VI, protein VII, and protein VIII) and 11 non-structural proteins [E1A, E1B, E2A (DBP), E3 (ADP), E4, EP, 33 K, 52/55 K, pol, pIVaII, and 100 K] (Zhao *et al.*, 2015) [23]. Hexon, one of the structural proteins, is a significant capsid protein that is visible on the surface of the virus and contains the loop1 structure as subtype-specific antigenic determinants (Gowthaman *et al.*, 2012) [8]. This virus can spread both horizontally and vertically. HPS is characterized by sudden onset, reduced productivity, immunosuppression and heavy mortality ranging from 20 to 75 per cent in healthy broiler flocks of 2-6 weeks age (Hussain *et al.*, 2012) [10]. The striking postmortem findings of the disease are accumulation of clear or straw coloured watery or jellylike fluid in the pericardial sac and the characteristic histologic changes include intranuclear basophilic or eosinophilic inclusion bodies in the

hepatocytes (Asrani *et al.*, 1997) [1]. Since, there has been no report on the outbreaks of disease from broilers based on clinic-pathological and molecular detection of HHS, the present paper describes the information about pathology and molecular detection of Fowl Adenovirus in the poultry population of Jammu region.

Material and Methods

Sample Collection

Samples for the present study were collected from a private broiler farm located at Kathua district of Jammu Region during the year 2023. Age of the broiler flock was around 28 days when all of sudden mortality started. The detailed postmortem examination of dead birds and gross lesions were systematically recorded. The liver specimens were collected in phosphate-buffered saline (PBS) and subsequently transported to the laboratory in an icebox. Afterward, the samples were preserved at -20 ºC for subsequent processing and viral detection.

Gross pathology

The dead birds were subjected to gross examination and suitable samples were collected at post mortem examination. Pathological changes in different organs were recorded in detail.

Histopathological examination

Pieces of tissues such as liver, kidneys, and spleen of infected dead chickens were collected in 10% neutral buffer saline for histopathological examination. Formalin fixed tissues were processed and sectioned and stained with haematoxylin and eosin (H&E) stain for observing the microscopic changes (Culling *et al.*, 1974) [4].

Polymerase Chain Reaction

Viral DNA was extracted from liver sample with the help of Qiagen DNeasy Tissue Kit as per the manufacturer's protocol. Using the previously described primer combination, the PCR was performed to amplify the FAdV hexon gene sequence of the viral DNA, producing a PCR product of size 900 bp [13]. The primer sequence used was: Forward -5'CAARTTCAGRCAGACGGT3' and Reverse-5'TAGTGATGMCGSGACATCAT3'. The reaction was carried out in a thermal cycler (Bio rad T100 tm) using the following conditions: initial denaturation at 94 °C for 5 min followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 58 °C for 1 min and extension at 72 °C for 10 min. Amplified PCR product was visualized in agarose gel electrophoresis using 1% gel containing ethidium bromide under ultraviolet light in a Gel Documentation System.

Results

Gross lesions

The current study has revealed HHS outbreak in 2 (20%) out of 10 poultry farms covering around 2000 poultry population. Out of 2000 birds, 240 birds were died due to HHS. The overall mortality rate was recorded 12% in broiler birds of 4-6 weeks of age. The dead birds were examined for gross pathological changes during post mortem examination. The pericardial sac showed the examination of straw coloured fluid with misshapen and flabby heart (Fig 1). In most of the cases, liver was pale, friable, and enlarged with presence of focal or diffuse area of necrosis (Fig 2). The kidneys were enlarged and congested with necrotic foci (Fig 3). Spleen was enlarged and friable and showed moderate congestion (Fig 4).

Histopathology

The liver showed severe hepatocytic vacuolar degeneration (Fig 5), focal area of necrosis with the presence of neutrophils (Fig 6). Pericentral infiltration of neutrophils with clumping of degenerated hepatocytes (Fig 7) and presence of characteristic basophilic intranuclear inclusion bodies in the hepatocytes (Fig 8) was also observed in infected birds. Liver parenchyma showing basophilic intranuclear inclusion bodies in the degenerating hepatocytes are considered as typical evidence of adenoviral infection. In kidneys, renal tubular epithelium showed swelling and severe degeneration causing complete occlusion of lumen and interstitial haemorrhages (Fig 9). Hypercellularity of glomerular tuft with occlusion of tubular lumen with degenerated and necrosed epithelial cells was also noticed (Fig 10). In spleen, vascular congestion and depletion of lymphocytes in the germinal center of follicle was prominent finding (Fig 11).

Molecular detection

All HHS outbreaks were further validated after histopathological evaluation by PCR identification of the FAdV hexon gene in liver tissue lesions. Out of 10 farms included in the study, 2(20%) farms were also tested positive for FAdV infection by PCR based detection of hexon gene region of 900 bp. The presentation of these gene bands was accomplished using gel electrophoresis for visualization (Fig 12).

Discussion

Viral infections in birds pose a significant danger to the poultry industry, resulting in high mortality and significant economic losses in terms of output and productivity (Chen *et al.*, 2015) [2]. These losses might be caused by either initial infections with potentially harmful viruses or subsequent infections with opportunistic pathogens. Hydropericardium hepatitis syndrome (HHS) has emerged as one of the crucial vertically transmitted illnesses affecting broiler chickens that address the expanding global poultry industry (Sawale *et al.*, 2012) [17].

The current investigation has verified the occurrence of HHS outbreaks in 2 out of 10 poultry farms, affecting a total chicken population of 2000. These outbreaks exclusively occurred in the broiler birds aged between 4 to 6 weeks. Similar findings were earlier observed by (Das *et al.*, 2015) [5]. These gross pathological lesions were comparable to the investigation by (Suohu and Rajkhowa 2021) [20]. The histopathological lesions observed in liver were in accordance with changes described by (Toro *et al.*, 1999 Thakor *et al.*, (2012), Palanivelu *et al.*, 2014 and Suohu and Rajkhowa 2021) [15, 20, 21, 22]. Lesions in the kidneys and spleen were in agreement with (Shivchandra *et al.*, 2003, Palanivelu *et al.*, 2014 and Dutta *et al.*, 2017) [6, 19, 20]. Lymphoid depletion in spleen indicates immunosuppression (Roy *et al.*, 2004 and Hafeez *et al.*, 2009) [9, 16]. Earlier workers (Meulemans *et al.*, 2004, Mittal *et al.*, 2014, Chitradevi *et al.*, 2018 and Shiyamala *et al.*, 2020) [3, 12, 13, 18] also earlier confirmed the presence of FAdV infection by detecting hexon gene by PCR testing. Precision in diagnosing the illness holds paramount significance. While gross and histopathological observations are primarily employed in diagnosing the disease, the molecular technique of PCR can be utilized to identify the specific gene associated with fowl adenovirus, serving as a confirmatory diagnostic tool.

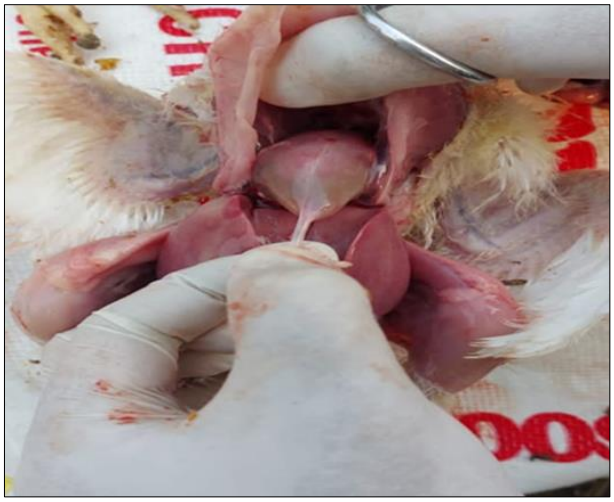

Fig 1: Characteristic hydropericardium in the pericardial sac (arrow) of the heart

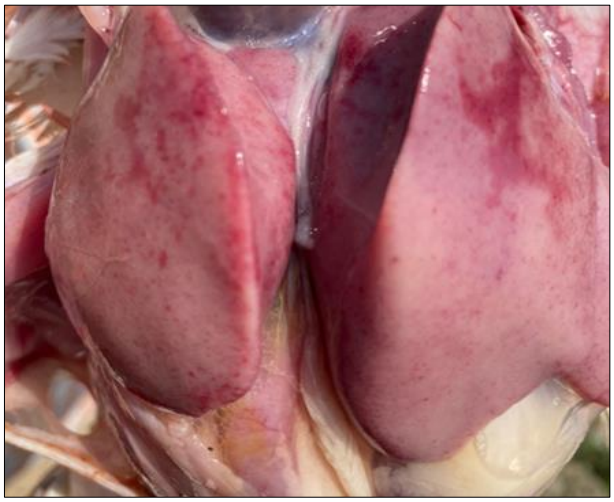

Fig 2: Pale enlarged liver with focal area of necrosis and haemorrhages

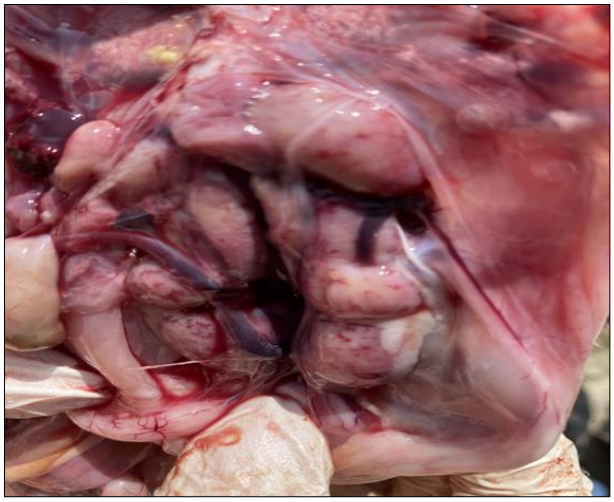

Fig 3: Kidneys were pale, enlarged and congested with necrotic foci in HHS affected birds

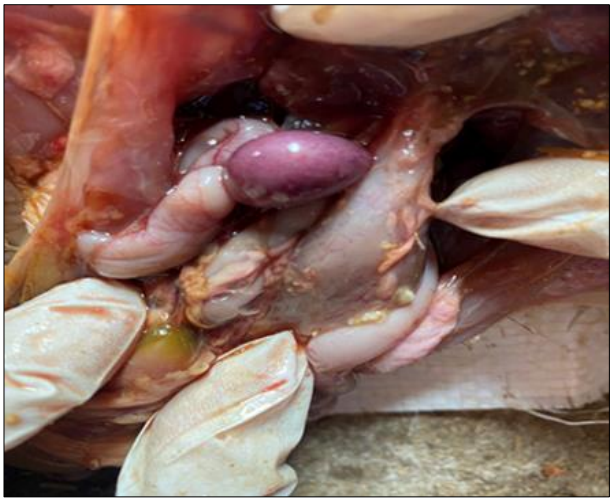

Fig 4: Spleen was enlarged with moderate congestion.

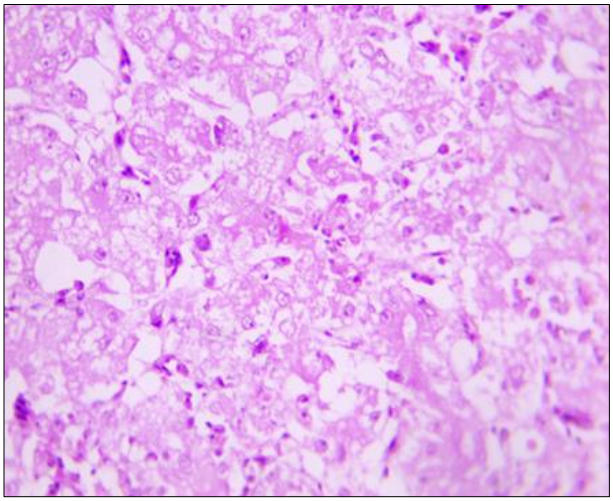

Fig 5: Liver showing severe vacuolar degeneration of hepatocytes in infected birds. H&E X 400

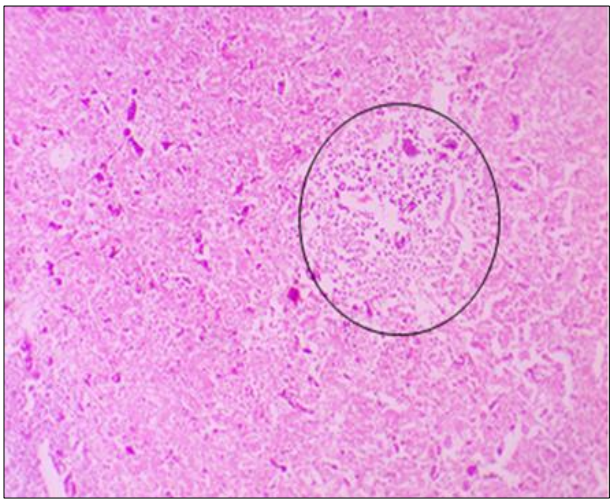

Fig 6: Liver showing focal area of necrosis of hepatocytes (circle) encroached by inflammatory cells in infected birds. H&E X 100

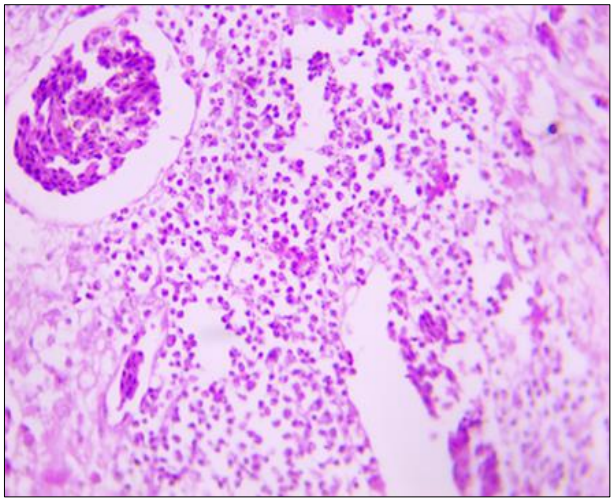

Fig 7: Liver showing pericentral infiltration of neutrophils with clumping of degenerated hepatocytes in infected birds. H&E X 400

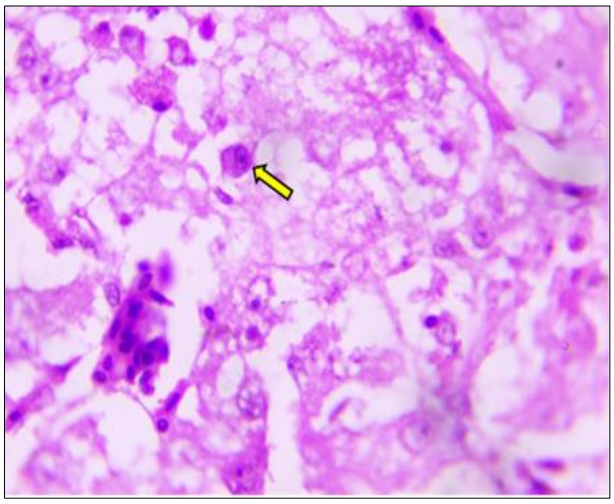

Fig 8: Liver showing characteristic basophilic intranuclear inclusion bodies in the hepatocytes in infected birds. H&E X 1000

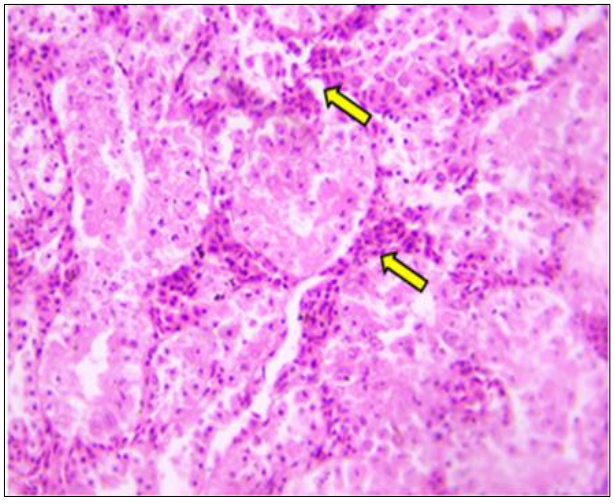

Fig 9: Renal tubular epithelium showing swelling and severe degeneration causing complete occlusion of lumen and interstitial haemorrhages (arrow) in infected birds. H&E X 400

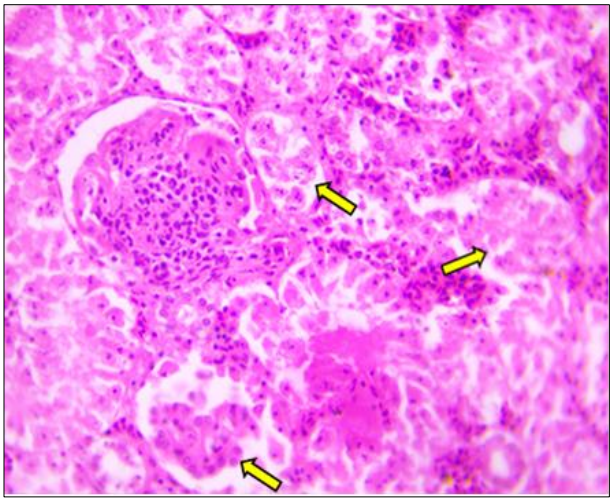

Fig 10: Kidney showing hypercellularity of glomerular tuft with occlusion of tubular lumen with degenerated and necrosed epithelial cells (arrows). H&E X 400

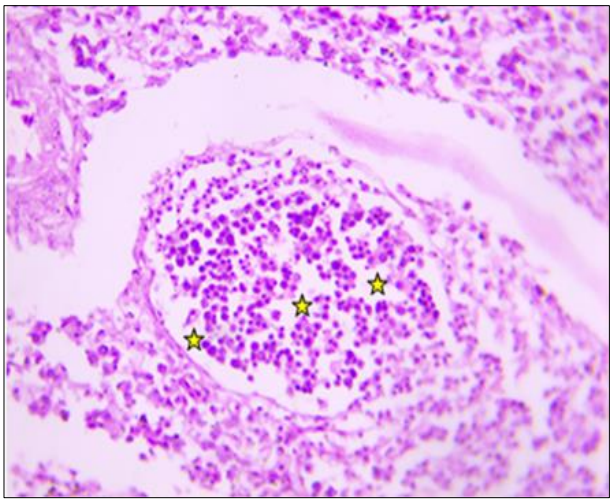

Fig 11: Spleen showing depletion of lymphocytes in the germinal centre of follicle. H&E X 400

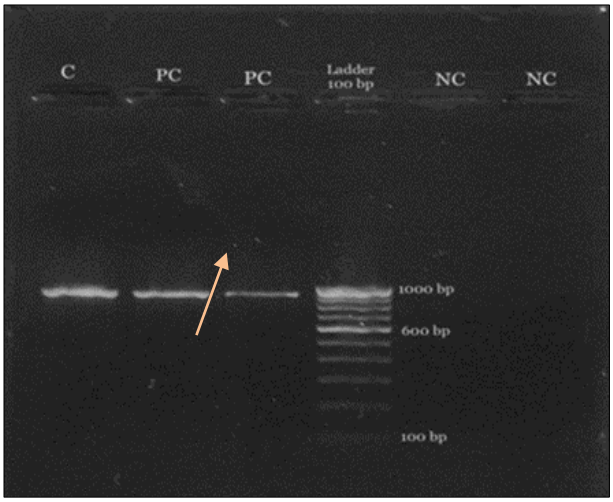

Fig 12: Agarose gel electrophoresis stained with Ethidium bromide showing the 900 bp hexon gene fragment in tissue sample. C = Positive control, PC = Positive sample, NC= Negative control

Conclusion

Present study was conducted to investigate the pathology and molecular diagnosis of HHS in poultry birds in Jammu region. Samples such as liver, kidneys and spleen collected from infected birds were examined for gross and histopathological changes. Gross examination showed characteristic straw coloured fluid in pericardial sac and presence of basophilic intranuclear inclusion bodies in hepatocytes microscopically. Tissue samples (liver) of infected birds showed positive for FAdV using PCR.

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