P-ISSN: 2410-8863
E-ISSN:2958-6178
https://djvs.uodiyala.edu.iq
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Diyala Journal for Veterinary Sciences
Vol. 3 No.4 February (2026)

https://doi.org/10.71375/djvs.2025.03403

Exploring Hydropericardium Hepatitis Syndrome (HHS) in Broiler Chickens: A Comprehensive Clinical and Histopathological Study in Diyala Governorate

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Important dates: **Received:** 08-August-2025; **Accepted:** 11-September-2025; **Published**: 15-**February** -2026

Abstract

Hydropericardium Hepatitis Syndrome (HHS), caused by Fowl Adenovirus serotype 4 (FAdV-4), is a significant viral disease in broiler chickens, leading to high mortality and substantial economic losses in the poultry industry. It primarily affects chickens aged 3 to 6 weeks, characterized by the accumulation of amber-colored fluid in the pericardial sac, distinguishing it from other adenoviral diseases like Inclusion Body Hepatitis (IBH).This study, conducted at the College of Veterinary Medicine, University of Diyala, aimed to identify FAdV-4 strains associated with (HHS) and confirm the presence of HHS in Diyala Governorate. To achieve this, tissue samples from 50 post-mortem cases across five regions Baqubah, Al-Galibia, Al-Khalis, Kanaan, and Baladrose were collected from clinically infected chickens, including liver, spleen, kidney, heart, and intestine. All samples were preserved in 10% formalin and processed for histopathological analysis. Clinical signs observed in infected chickens in the current study, included depression, reduced feed intake, and discolored excreta. The excreta of affected chickens showed discoloration, ranging from deep yellow to greenish, indicating intestinal distress. Post-mortem lesions were characterized by pericardial effusion, with clear or amber-colored fluid in the pericardial cavity, which was the most prominent lesion. Additional findings included enlarged, friable liver with areas of necrosis, congested kidneys with urate deposits, and splenomegaly. Histopathologically, the liver exhibited dilated central veins and sinusoids, with infiltration of polymorphonuclear and mononuclear cells, along with necrotic hepatocytes. The kidneys displayed severely dilated tubules packed with proteinaceous debris and widespread interstitial edema. The heart showed signs of myofiber necrosis, lymphocytic

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https://doi.org/10.71375/djvs.2025.03403

infiltration around the veins, and inflammation. The spleen exhibited loss of lymphoid follicles, inflammatory cell infiltration, and hemorrhage. The intestines manifested multiple deep ulcers, patches of mucosal necrosis, crypt epithelial atrophy, and irregular villous hyperplasia. These overlapping pathological features offer valuable insights into understanding the connection between the progressive clinical signs and the complex histological changes caused by FAdV-4 infection, the viral agent responsible for HHS. Clarifying this relationship is essential for guiding future efforts in epidemiological surveillance, enhancing disease control strategies, and developing more effective diagnostic tests and preventive measures.

Keyword: Black pigment, melano-macrophages, carcasses, kupffer cells, hepatocytes

Introduction:

Infection with fowl adenoviruses (FAdVs) can cause a range of syndromes in chickens, with hydropericardium hepatitis syndrome (HHS) being one of the most significant diseases. These infections, along with other related syndromes (HHS), have led to substantial economic losses in poultry production over the past three decades (Schachner and Hess, 2021). FAdVs belong to the genus Aviadenovirus within the family Adenoviridae. These are linear double-stranded DNA viruses, characterized by a non-enveloped capsid and a pseudo-cubic (icosahedral) arrangement of capsomers. The virus is classified into three divisions, as determined by serum cross-neutralization tests and restriction enzyme digest patterns. Group 1 can be broken down into a total of twelve different serotypes (FAdV-1-8a and 8b-11) and 5 different species (A–E) (Kim et al., 2014). Due to the widespread distribution of FAdV strains, they are associated to a variety of avian syndromes, such as inclusion body hepatitis, hydropericardium hepatitis syndrome (HHS), and Egg Drop Syndrome EDS. Most broiler chickens are a common susceptible to infection with FadV-4 which is the etiological agent of HHS (Davison *et al.*, 2003; Liu *et al.*, 2016; Niu *et al.*, 2018). (HHS), a previously unknown emerging disease, has shares clinical similarities with classical avian adenoviral disease, inclusion-body hepatitis (IBH) but is characterized by severe hydropericardium resulting in sudden death among all infected broiler flocks in the age group 3-6 weeks, with heavy mortality in broiler chickens reaching up to 75% (Abdulaziz and AL-Attar,1991; Ganesh et al., 2002).

In the late 1980s, a new viral disease affecting broiler chickens with clinical signs similar to classical inclusion body hepatitis (IBH) was first identified in the Angara Goth area of Karachi, Pakistan. Initially named "Angara disease" (Akhtar, 1994), it was later recognized as

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Hydropericardium Hepatitis Syndrome (HHS) caused by fowl adenovirus serotype-4(FAdV-4) is primary diagnosed through the observation of macroscopic lesions in affected organs, particularly the liver and heart (Mazaheri *et al.,* 1998; Meulemans *et al.*, 2004). The significant organ affected with the FAdV is the liver, performing pale yellow in colour, with a friable texture and swelling. It may also show enlargement accompanied with tiny white foci, and in rare instances, petechial or ecchymotic hemorrhages (Julian, 2005; Hussein *et al.,* 2023; Naikoo, 2023). Two types of intranuclear inclusion bodies (INIB) are commonly observed in degenerated hepatocytes. These inclusions bodies may appear as large, round, or irregularly shaped structures, typically surrounded by a pale halo and containing eosinophilic or basophilic material within the nucleus (Safwat *et al.,* 2022). Regarding the heart, the accumulation of clear or amber-colored, watery, or jelly-like fluid in the pericardial sac, with a pH of 7.0, is known as hydropericardium. Which is the most common and persistent gross lesion associated with HHS infection. The heart, may also be observed floating within the pericardial sac, further indicating the severity of the infection (Kataria et al., 2013; Hussein *et al.,* 2023). The disease was first reported in Iraq in 1989, with increasing cases by 1990, affecting broiler flocks and causing a mortality rate of 10-40%. The deaths often occurred just before the birds reached market weight, when they had exhausted. FAdVs reduce lymphocyte populations in lymphoid organs, such as the thymus and spleen, compromising the immune response in infected birds (Wang *et al.,* 2023). While primarily affecting chickens, FAdVs can occasionally be detected in other avian species. The virus spreads both vertically and horizontally, contributing to its global prevalence (Pouladi *et al.,* 2024). It can remain latent in chicks with high maternal antibody titers until they are 2-4 weeks old, after which it is excreted in their feces (Pereira *et al.,* 2014). The infections caused by FAdV-4 (HHS) in Diyala Governorate remain poorly understood. The objective of this study is to conduct clinical and histopathological examinations on homogenates from positive samples and identify pathological changes in the liver, heart, spleen, intestines, and kidneys.

Materials and Methods:

Sampels collection :

From September 2024 to January 2025, this study was conducted at the Department of Microbiology, College of Veterinary Medicine, University of Diyala. The primary objective of this study was to identify Hydropericardium Hepatitis Syndrome (HHS) in commercial broiler chicken flocks in Diyala Governorate. Detection was carried out through clinical and histopathological examinations of infected flocks. None of the broiler flocks involved in the present study had been vaccinated against Adenovirus or received the commercial inactivated vaccines pointing Fowl Adenovirus serotype-4 (FAdV-4). Samples were collected from

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postmortem birds from flocks suspected of Fowl Adenovirus infection, which is linked to Hydropericardium Hepatitis Syndrome. These farms were located in five distinct regions of the Diyala Governorate: Baqubah, Kanaan, Baladrose, Al-Khalis, and Al-Galibia, all of which are known for their long-established intensive broiler farming practices. The collected specimens included the liver, spleen, kidneys, heart, and intestines. The affected birds exhibited notable clinical signs of HHS, including depression and lethargy within most of the flock. The birds were observed huddling together with ruffled feathers and producing mucoid, yellowish droppings. The mortality rate was significantly high, ranging from 10% - 40% at the age of (3-6 weeks), with sudden deaths occurring among the broiler flocks. Pathological lesions were characterized by an enlarged, pale liver; swollen kidneys with obstructed renal tubules; and the accumulation of 5 to 10 mL of clear fluid in the pericardial sac. These clinical and gross pathological features strongly indicated infection with Fowl Adenovirus serotype-4 (FAdV-4). A total of ten samples were taken from each region, with two tissue samples collected from each of the five organs: liver, kidney, heart, intestine, and spleen. These samples were obtained from birds that had been confirmed positive for HHS through PCR testing and were subsequently used for routine histopathological analysis.

Tissue preparation:

A total of fifty tissue samples were extracted from various organs, included the liver, spleen, kidney, intestine and heart. Following that, tissue samples 4-6 mm in thickness, were taken and submerged in a 10% neutral-buffered formalin solution at room temperature for 24 hours to ensure proper fixation. Following fixation, the samples tissues were dehydrated in graded ethanol series at room temperature for two hours (70% for two hours, 80% for two hours, 90% for two hours, and 100% for two hours), then the samples were submerged in xylene for two hours, and then infiltrated with molten paraffin wax for three hours (Spencer *et al.*, 2012). The samples were then carefully oriented and embedded in fresh paraffin to form paraffin blocks, which were sectioned at a thickness of five µm using a microtome and fixed on glass slides. Following standard protocols (Spencer *et al.*, 2012), the sections were routinely stained with hematoxylin and eosin (H&E). The stained sections were then examined under a light microscope equipped with a digital camera (Omax, USA), and photomicrographs were captured to aid in histopathological analysis.

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Results:

Fowl adenovirus serotype 4 (FAdV-4) is a major pathogen causing significant financial losses in the global poultry industry. In recent years, native flocks have faced epidemics from mixed infections, including FAdV linked to inclusion body hepatitis and hydropericardium hepatitis syndrome (HHS), along with virulent strains of Newcastle disease, H9N2 avian influenza, and other infections. These mixed infections compromise immune resistance and spread new viral strains. FAdV-4 is the primary cause of HHS in broiler chickens aged 3 to 6 weeks. Clinical signs of HHS include depression, loss of appetite, reduced activity, and discolored droppings ranging from yellowish to greenish. The primary clinical signs observed in broiler chickens essentially infected with HHS included depression, loss of appetite, reduced activity, and a tendency to huddle together with minimal avoidance of movement, which is indicative of severe weakness and systemic distress. The droppings of the infected flocks were discolored, varying in color from deeply yellowish to greenish.

Result of clinical sings

The Postmortem results of broiler chickens naturally infected with FAdV-4 revealed characteristic lesions with hydropericardium-hepatitis syndrome (HHS). Key findings included marked pericardial effusion, with 5 to 15 mL of clear or amber-colored fluid in the pericardial sac, causing the heart to appear flabby and often float in the sac. The liver exhibited predominant changes, including enlargement, paleness, and friability, with small multifocal area of necrosis, petechial and ecchymosis hemorrhage in the heart musculature and other organ. Intranuclear inclusion bodies (INIBs) were also present in the liver, a hallmark of adenoviral infection. Other commonly observed lesions included swollen, pale kidneys with the deposition of urates in the kidney tubules and ureters. Clear pathological changes were observed in the intestines and spleen due to FAdV-4 infection. The spleen showed lesions characterized by mild to moderate splenomegaly and congestion. Whereas, the gross lesions in the intestine display non-specific pathological changes, characterized by congestion in the mucosa, dilution of the intestinal wall and catarrhal enteritis as shown in figure (1,2,3).

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Result of Histopathology

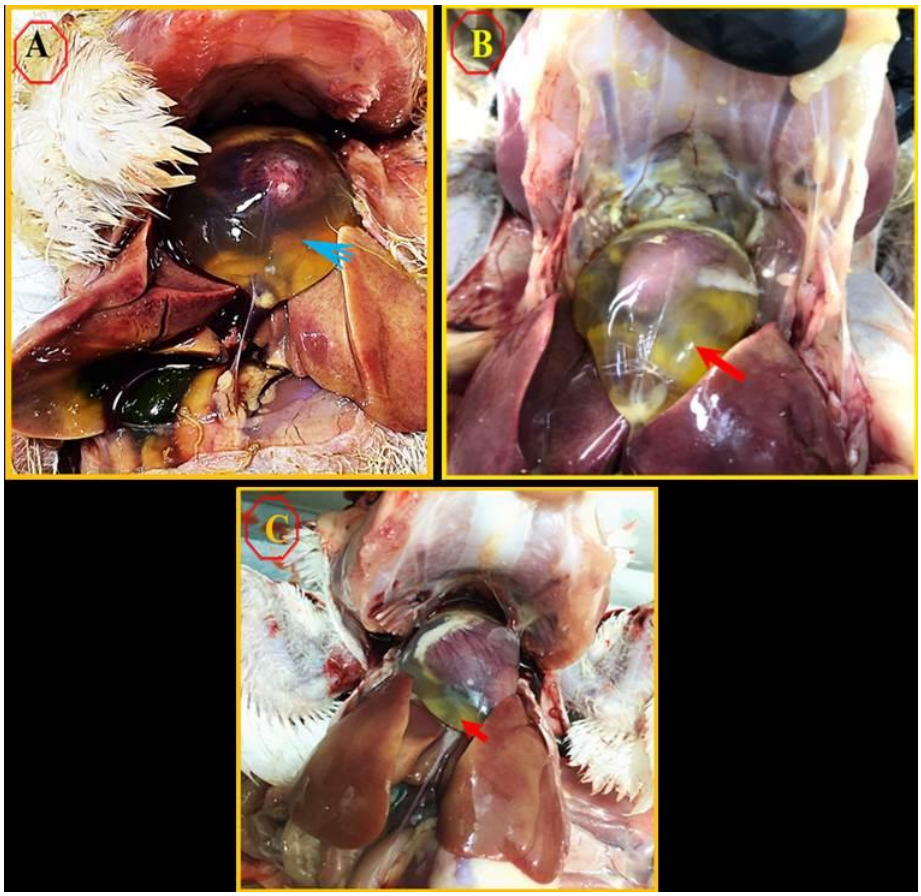

Fig. 1 Broiler chickens infected with HHS exhibit a marked buildup of yellowish fluid within the pericardial sac giving it floating form (arrow)

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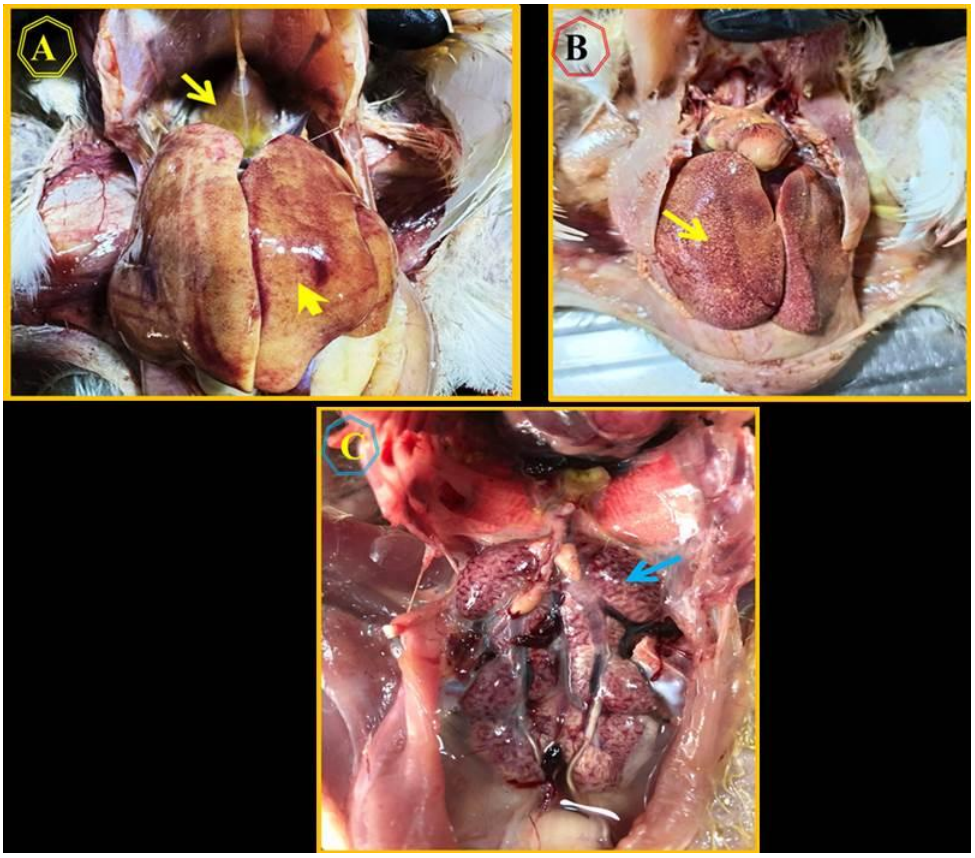

Fig 2.Broiler chickens (3-6 weeks age)infected with HHS exhibit a noticeably enlarged liver, characterized by multifocal small white foci, a friable and mottled appearance, indicative of hepatocellular necrosis and fatty degeneration (arrows A, B). Whereas C showed pale and swollen kidneys with distinct lobulation and deposition of urate proposing renal pathology associated with HHS (blue arrow).

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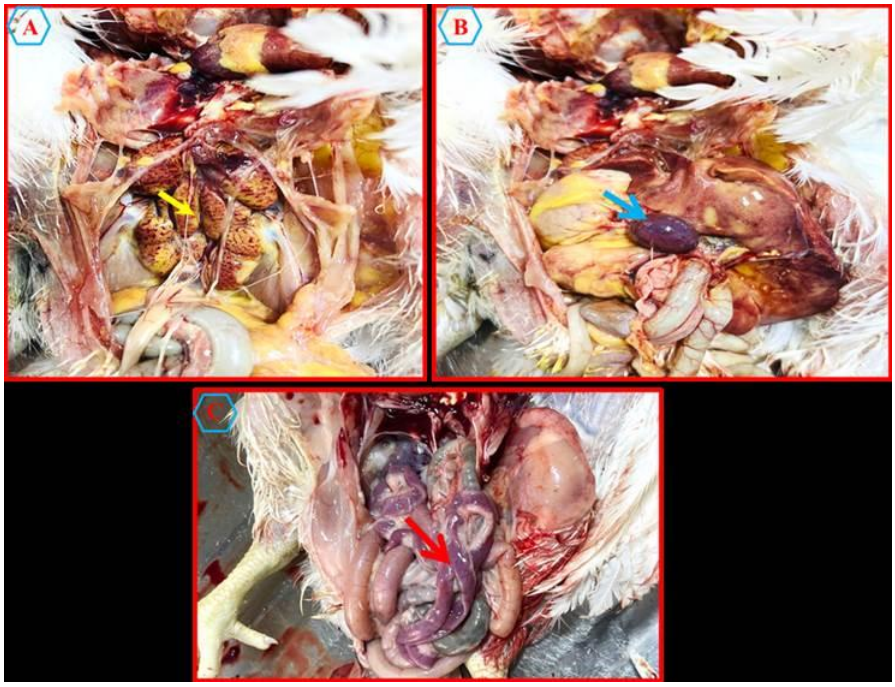

Fig 3. Broiler chickens naturally infected with (HHS). A. Kidneys appear pale, significantly swollen, with diver's lobulation and urate deposition (yellow arrow).B. The spleen shows mild to moderate splenomegaly with evident congestion and hemorrhages (blue arrow). C. Intestinal loops display non-specific pathological changes, including congestion in the mucosa, dilution of the intestinal wall and catarrhal enteritis (red arrow).

Result of Histopathology

Liver

Microscopic examination of the liver from (normal) chickens showed a typical structure, including a central vein, hepatocytes, and sinusoids. In contrast, liver samples from FAdV-4 (HHS)-infected birds exhibited significant histopathological changes, including dilated central veins and sinusoids, infiltration by polymorphonuclear and mononuclear cells, hemorrhagic interstitial areas, and necrotic hepatocytes around the portal area. Additionally, multiple basophilic intranuclear inclusion bodies were observed within the hepatocytes. The liver also appeared enlarged, friable, and hemorrhagic, with intranuclear inclusion bodies. Acute cellular swelling, vacuolation of hepatocytes, congestion, and dilated veins filled with RBCs and inflammatory cells were also present. Edema, necrosis, and mononuclear cell infiltration in the interstitial layer further contributed to the overall necrotic hepatitis (Fig, 4, 5)

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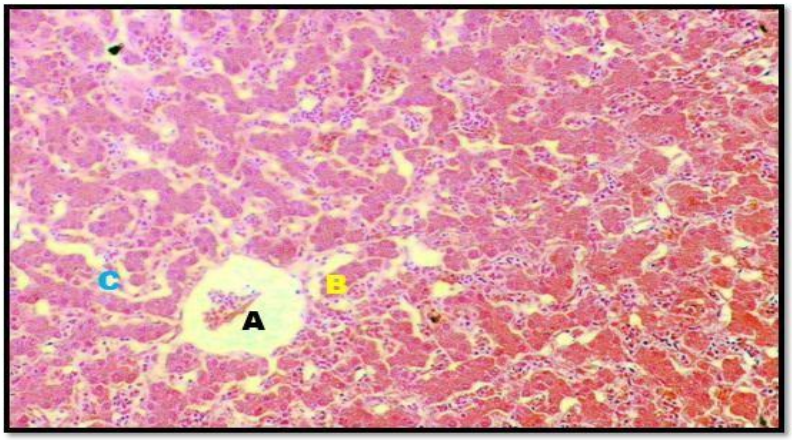

Fig 4. Microscopic section in normal liver showing A-central vein B-hepatocyte C-sinusoids.

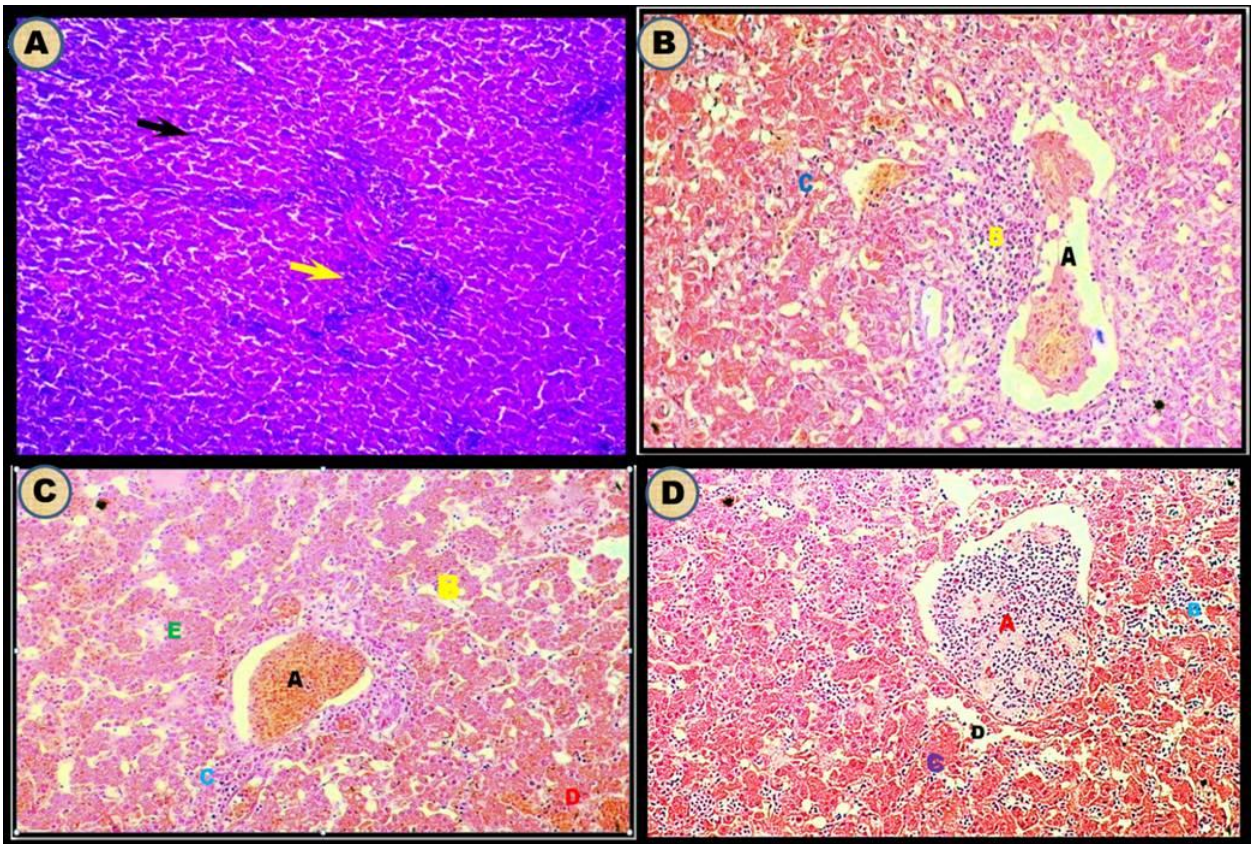

Fig 5, Microscopic section in liver infected with FAdV-4 (A) showing the sinusoids dilated, and there is infiltration of polymorphonuclear and mononuclear cells, with hemorrhagic interstitial areas and necrotic hepatocytes (blue arrow). Whereas the yellow arrow indicates the multiple basophilic intranuclear inclusion body. (B) A-sever dilated central vein B- mononuclear inflammatory cells cuffing the central vein C- acute

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cellular swelling (vacuolation) of hepatocytes.(C) dilated and congested central vein in portal area A, B- dilated sinusoids C- polymorphic and mononuclear cell infiltrated surrounded portal area D- interstitial hemorrhage E- necrotic hepatocyte.(D) sever dilated central vein with RBC& inflammatory cells (A), (B)- mononuclear cells infiltration in sinusoids (C)- necrotic hepatocytes (D)- edema. (X200).

Kidney

Microscopic examination of the kidney from (normal) chickens showed normal cortical architecture, with well-defined glomeruli and proximal convoluted tubules. In contrast, FAdV-4 (HHS)-infected kidneys exhibited significant changes, including dilated tubules filled with proteinaceous material, indicating acute cellular swelling. The glomerular capsule cells were thickened, with hyperplasia and mesangial cell proliferation. The interstitial tissue was edematous, with an increase in fibroblasts and infiltration of neutrophils. Additional sections showed interstitial edema, hemorrhagic dilated tubules, and mononuclear inflammatory cells in the interstitial space (Fig 6, 7).

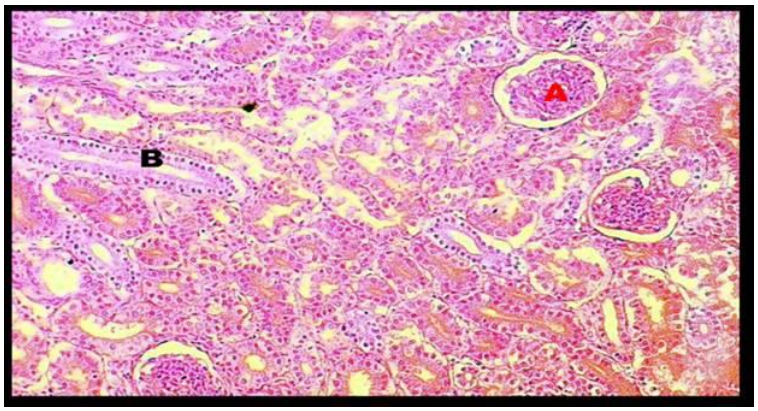

Fig 6, Microscopic section in kidney in normal, showing A- normal glomeruli (consist of glomerulituffts,bowman space ,capsule) B- proximal convoluted tubules (H and E stain; X200).

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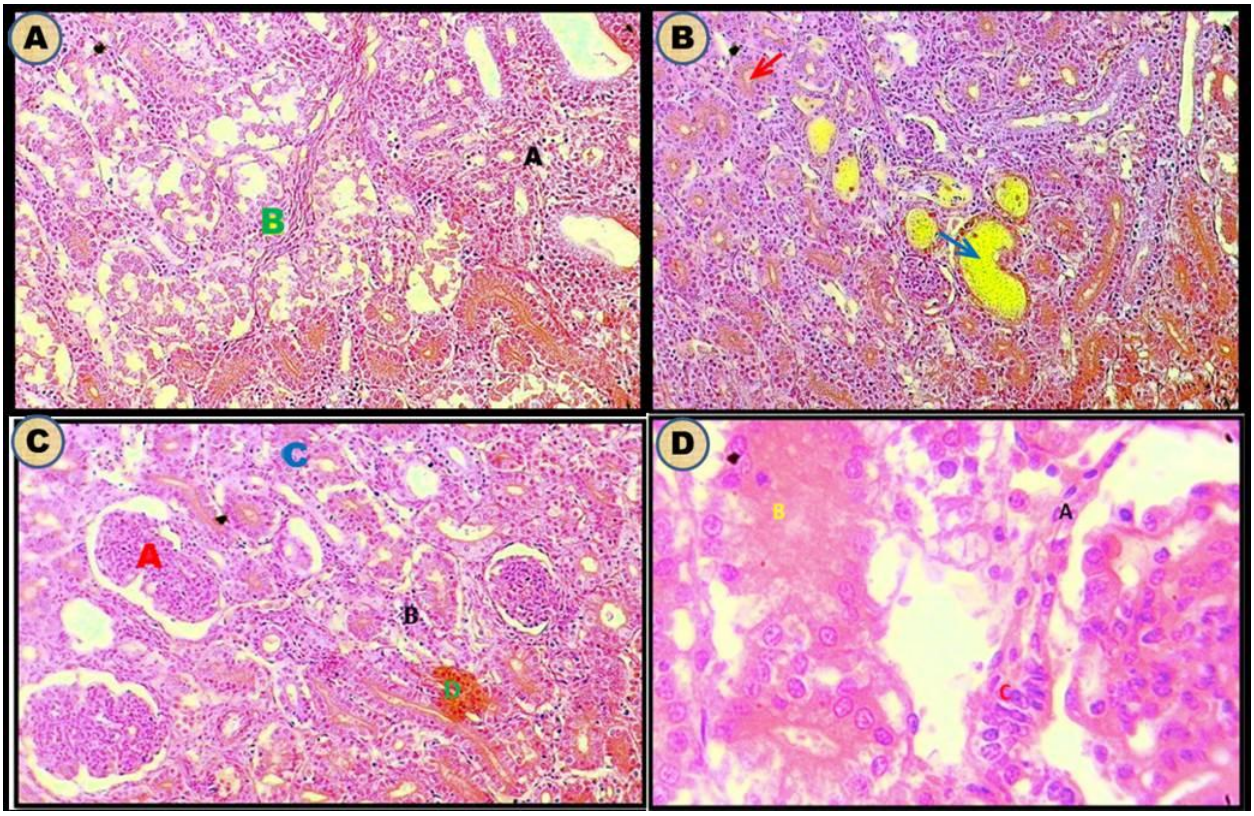

Fig 7, Microscopic section in kidney infected with FAdV-4 showing, (A) acute cellular swelling(A), (B) – increase in fibroblasts in interstitial tissue. (B) Dilated tubules with proteinous material (blue arrow), acute cellular swelling (Claudy swelling of tubules) (red arrow). (C) Large glomeruli with glomerular hyperplasia –(A), (B) – hyperplasia of mesangial cells (C)- acute cellular swelling (D) – interstitial hemorrhage. (D) Thinking of glomerular capsule with hyperplasia of mesangial cells –(A), (B) – interstitial edema (C)- polymorphic cells infiltration (neutrophiles) (H and E stain; X200).

Heart

Microscopic examination of the heart in control chickens showed normal muscle fibers. In contrast, FAdV-4-infected hearts displayed necrotic muscle fibers with infiltration of mononuclear and polymorphonuclear cells. Lymphocytic cuffing was observed around veins. Additional sections revealed edema, necrosis, inflammatory cell infiltration (lymphocytes, neutrophils, and macrophages), and interstitial hemorrhage (Fig 8, 9).

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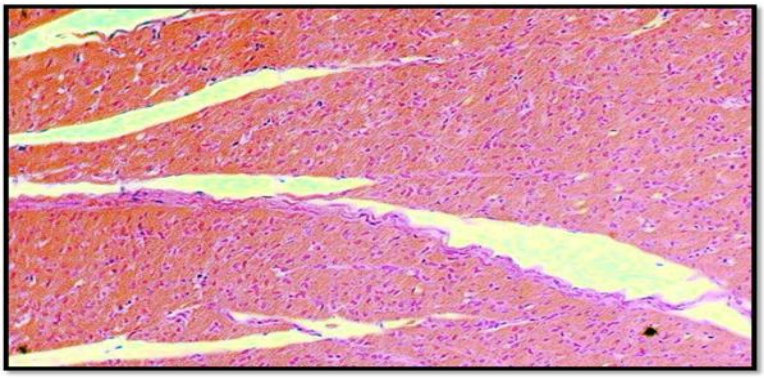

Fig 8, Microscopic section in normal heart showing normal muscle fibers (H and E stain; X200).

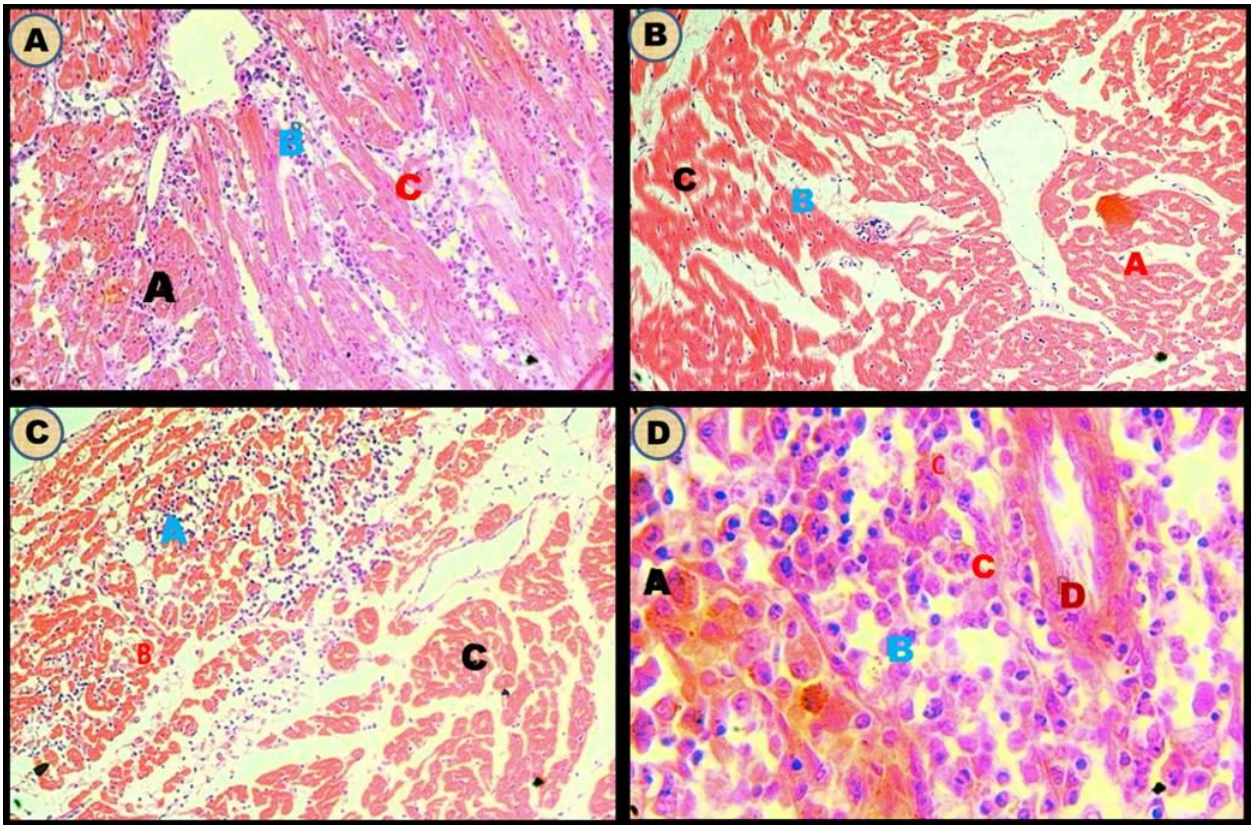

Fig 9, Microscopic section in heart infected with FAdV-4 showing (A), (A)-necrosis in all heart muscle fibers (B) – poly and mononuclear cells infiltration (C)-lymphocytic cuffing vein. (B) Showing (A)-edema, (B)– inflammatory cells infiltration (C)-necrosis.(C) (A)-heavy infiltration with lymphocytes in the heart muscle (B) – necrotic cells (C)- edema. (D) (A)-infiltration with inflammatory cells mostly neutrophils (B) - lymphocytes (C)- macrophages (D) – interstitial hemorrhage (H and E stain; X400).

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Spleen

Microscopic examination of the spleen in control chickens showed normal architecture with white and red pulp. In contrast, FAdV-4 (HHS)-infected spleens exhibited lymphoid follicle depletion, severe infiltration of mononuclear and polymorphonuclear cells in pulp types, hemorrhage, edema, and thickening of the capsule. Additional sections revealed further depletion of both red and white pulp, with dilated and congested blood vessels (Fig 10, 11).

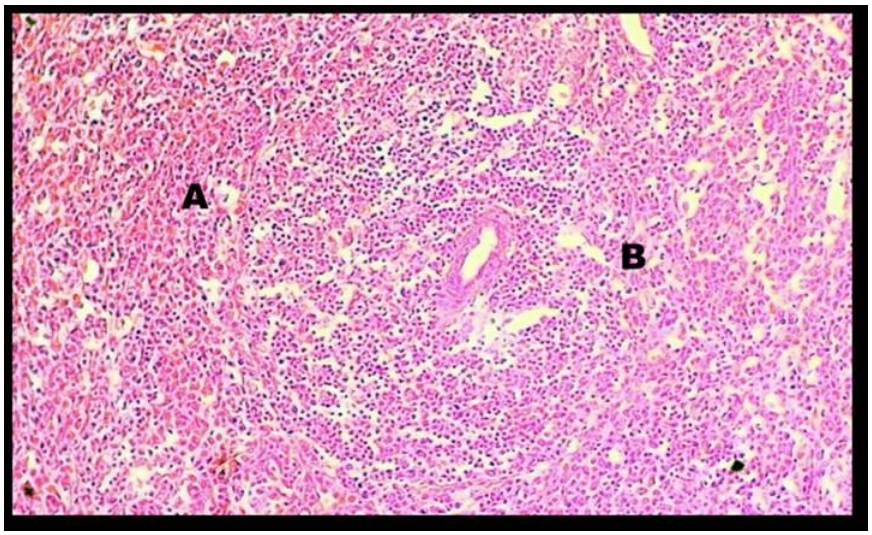

Fig 10, Microscopic section in normal spleen showing A-white pulp malpighian corpuscles (central artery with lymphocyte) B – red pulp (H and E stain; X200).

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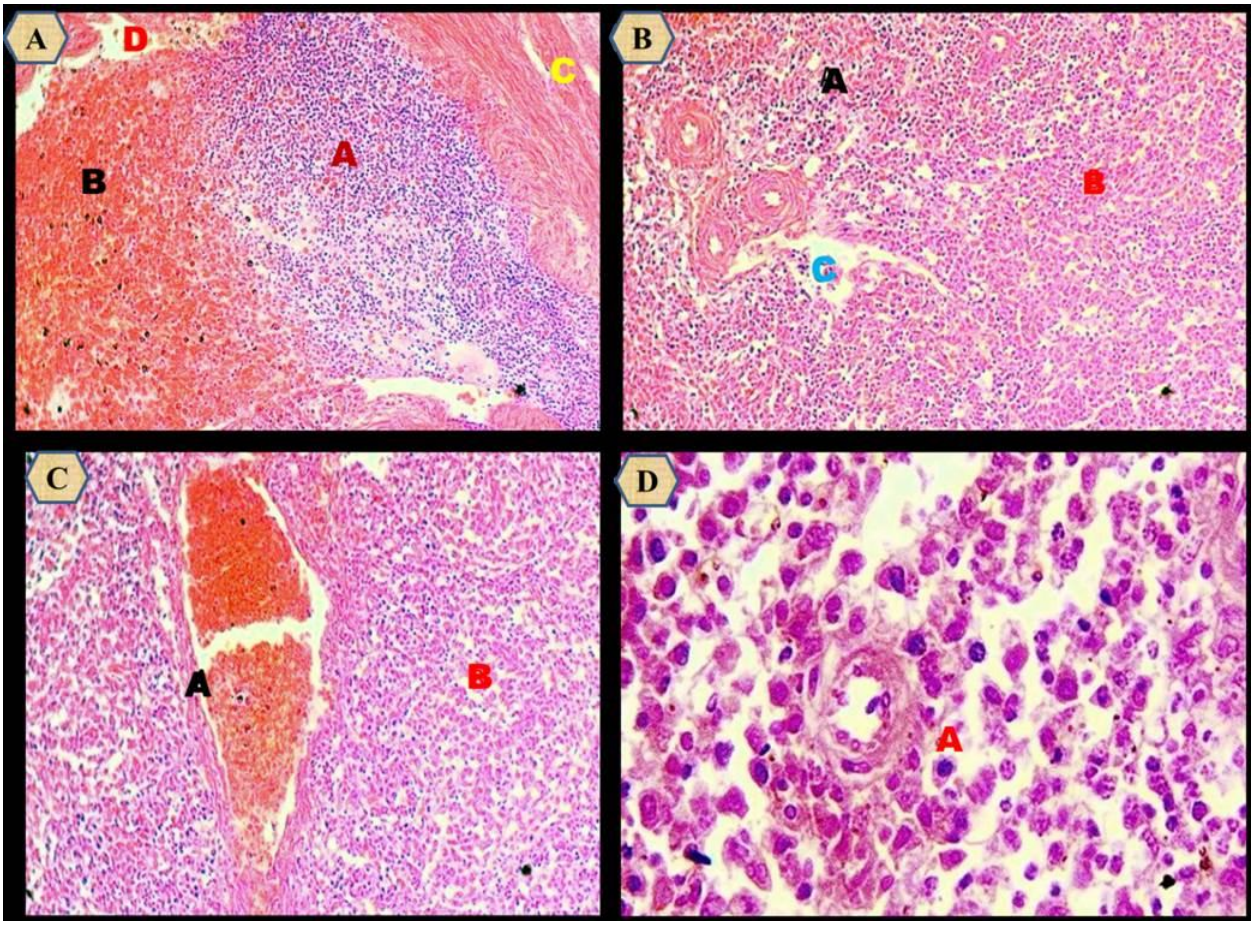

Fig 11, Microscopic section in spleen infected with FAdV-4 showing, (A) (A)- sever infiltration of mononuclear and polymorphic cells in white and red pulp (B) -sever hemorrhage (C)- thickened capsule layer (D) – edema.(B) (A)- depleted white pulp (B) - edema (C)- depleted red pulp. (C) – (A) sever dilated and congested blood vessel, (B) depletion of white and red pulp. (H and E stain; X200). (D) Showing (A)- central artery surrounded by macrophages cells (H and E stain; X400).

Intestine

Microscopic examination of the normal intestine showed a well-organized structure with villi, crypts, and distinct layers. In contrast, FAdV-4 (HHS)-infected intestines exhibited ulceration, necrosis, and atrophied crypts in the mucosal layer. Infiltration of mononuclear and polymorphonuclear cells was observed in the intestinal stroma. Additional sections revealed sloughing, desquamation, and hyperplasia of the mucosal layer, with extensive necrosis and mononuclear cell infiltration (Fig, 12).

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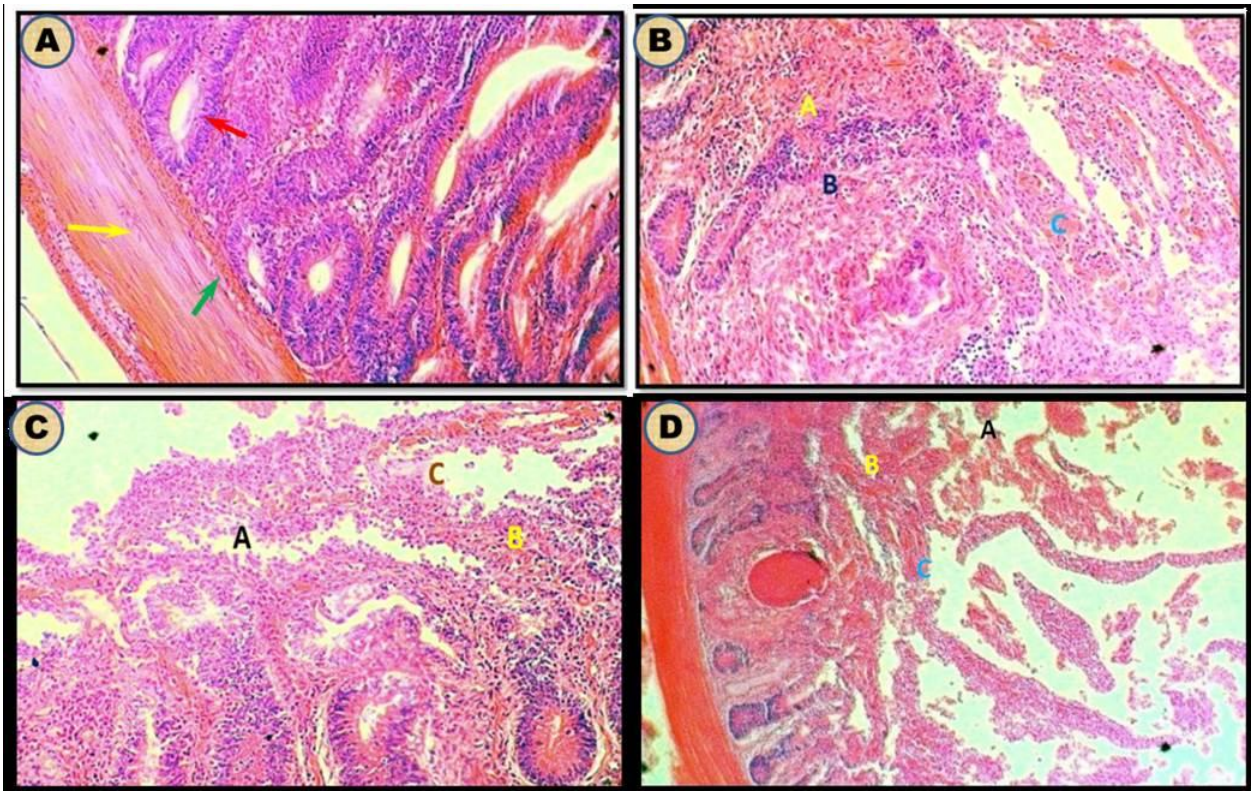

Fig 12, (A) Microscopic section in normal intestine showing mucosal layer with villi (green arrow), submucosa layer (yellow arrow) crypt (red arrow). (B) **(A)–**large area of necrosis in mucosal layer, **(B)** – mononuclear cells infiltration, **(C)-**atrophied crypt. (C) **(A)** -sloughing and desquamation of mucosal layer, **(B)** – necrotic area, **(C)-**atrophied crypt. (D) **(A)-**ulceration of mucosal layer, (B) – necrotic area, **(C)-**atrophied crypt (H and E stain; X100).

Discussion

Hydropericardium Hepatitis Syndrome (HHS) is a contagious viral infection caused by Fowl Adenovirus serotype 4 (FAdV-4), primarily affecting broiler chickens aged 3-6 weeks. In recent years, HHS has been reported globally, with FAdV-4 being the most common causative agent, resulting in significant financial losses for the poultry industry (Schachner and Hess, 2024). FAdV-4 infection causes various pathological changes, and the liver is the target and susceptible organ. Histopathological examinations of tissues revealed the most severe lesions in the liver, as well as in the kidney, heart, intestine, and spleen, in chickens from various infection groups compared to the controls. Multiple changes were observed in the liver tissue of the broilers, including inflammatory cell infiltration, congestion in central veins, necrosis, edema, and fibrosis. These

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findings align with those reported by Yu et al. (2018), Liu *et al.* (2021), and Sultan *et al.* (2021), who also observed similar pathological lesions in the livers of FAdV-4-infected birds, such as inflammatory and necrotic alterations. In the present study, these alterations were again observed, supporting the results of Wei *et al.* (2024), who documented comparable liver damage, including inflammatory and necrotic changes, in FAdV-4-infected birds. The presence of necrosis hinders liver function, suggesting that the condition has deteriorated significantly, leaving the broilers in poor health. Additionally, vacuolar degeneration and acute hepatic necrosis were evident in the current research in agreement with those found by Etbestway *et al.* (2020) and Abouzied et al. (2021) showing comparable liver lesions in FAdV-4 infected broiler chickens. These changes, particularly vacuolar degeneration, could be representing an early cellular reaction to viral infection, in which the infected hepatocytes appear to be damaged and distressed. The liver alterations during the present study align with those documented by Ishag *et al.* (2022). Who revealed degeneration, necrosis, lymphocytic infiltration, and inclusion bodies in the liver of broilers infected with FAdV-4. Sultan et al. in (2021) also portrayed multifocal necrotic foci, noted morphologic alteration of the vascular structures, and demonstrated the presence of basophilic intranuclear inclusion bodies (INIB) inside hepatocytes of birds naturally-infected with HHS. These results align with our data, suggesting that both HHS and FAdV-4 can induce comparable liver damage. The damage characterized by necrosis and the presence of inclusion bodies. The presence of edema and fibrosis in our study suggests a chronic response to (HHS) infection, with ongoing liver tissue degeneration potentially leading to dysfunction. These findings elucidate that HHS can induce both acute and chronic liver damage if remaining unchecked, as explored by (Schachner *et al.* in 2018). Moreover, our data suggest that hexon gene mutations may mold the virulence of FAdV-4, and genetic differences could explain variations in liver lesion severity. The genetic diversity of circulating FAdV-4 in different regions may account for differences in pathogenesis and liver damage severity (Schachner *et al.,* 2018; Liu *et al.,* 2021). Both eosinophilic and basophilic intranuclear inclusions are observed in FAdV-4-infected cells. Most of the inclusions were basophilic in some studies; they occupied the entire nucleus being dense to coarse. This finding agree with that of Goyal et al. (2009) who also reported central location and periphery this halo on eosinophils inclusions.

Granular degeneration, congestion, and myocytolysis with widened intercellular spaces in the myocardium as observed in our present study were consistent with those observed by Yu *et al.* (2018). In addition, Mariappan *et al.* (2018) also observed myocardial necrosis and proliferation of fibroblasts and inflammatory cells consistent to myocardial necrosis and inflammatory infiltrates of this study. However, significant histopathological changes were found in the FAdV-4 infected hearts. These involved the necrotic myocardial fibers, with both mononuclear and polymorphonuclear cell infiltration. Veins were enclosed by small lymphocytic cuffs.

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Microscopically, edema, necrosis, and inflammatory cell infiltration were observed. Infected hearts showed necrotic myocardial fibers, edema, venous dilation, and severe inflammatory infiltrates, confirming severe myocardial damage caused by FAdV-4 (Zhang *et al.*, 2021; Rashid *et al.*, 2024).

Microscopic examination of the spleen in birds infected with FAdV-4 revealed depletion of lymphoid follicles and severe infiltration of both mononuclear and polymorphonuclear cells in the white and red pulp areas, accompanied by hemorrhaging, edema, and thickening of the capsule. Further evaluation showed dilated and congested blood vessels alongside additional depletion of lymphoid follicles. These findings highlight the significant histopathological changes caused by FAdV-4 infection, which systemically suppresses the immune system and leads to widespread damage of tissues. Comparable alterations have also been documented by Yu and colleagues in 2018, as well as Venkatesha and others in 2005, Naguib and coauthors in 2021, and Mariappan et al. in 2018 - including hemorrhaging, depletion of lymphoid tissues, and inflammatory infiltration. These observations underscore the severity of FAdV-4 infection in broiler chickens, resulting in destruction of tissue integrity and systemic spread of the infection.

Kidney sections from unaffected chickens showed usual structure with assembled glomeruli and proximal convoluted tubules. In contrast, infected kidneys revealed severe tissue changes, such as swollen tubules filled with proteinaceous material, indicating acute cellular swelling. The glomerular capsule was thickened with mesangial cell proliferation,, and the among area was edematous, infiltrated by neutrophils and fibroblasts. Dilated tubules contained hemorrhaging and mononuclear inflammatory cells. These discoveries correspond with those of Wilson et al. (2010), Zadravec *et al.* (2013),Yu *et al.* (2018), Faisal and Al-Azzawi, 2023 and Thaeer and Al-Azzawi (2025), who reported tubular degeneration, epithelial detachment, necrosis, basophilic intranuclear inclusion bodies, and karyorrhexis of the tubular epithelial cells and glomerular abnormalities in infected kidneys. Additional studies by Kim et al. (2008), Wang et al. (2023), and others highlighted similar kidney pathology, including tubular degeneration, interstitial hemorrhages, and glomerulonephritis. Collectively, these outcomes verify that infection causes severe renal harm, impacting both tubules and glomeruli, and showing systemic effects of the virus on kidney function. Histopathological changes in the intestines of FAdV-4 (HHS)-infected chickens included ulceration of the mucosal surface, necrotic slough, and atrophic crypts. Infiltration of mononuclear and polymorphonuclear cells extended deeply into the intestinal stroma, accompanied by near-total sloughing and shedding of the epithelial surface. Furthermore, hyperplasia of the mucosal layer (villi), extensive necrosis, and atrophied crypts were noted. These findings align with the observations of Yu and colleagues in 2018 who reported hemorrhagic enteritis and necrotic foci in infected chickens. Bayraktar *et al.* (2024), described similar intestinal damage. While the liver,

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E-ISSN:2958-6178
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heart, and kidneys experienced more pronounced effects, the intestines also demonstrated meaningful involvement. One study portrayed mild intestinal thickening and yellow semisolid discharges, relating to necrosis and damaged crypts seen in our study. Hussein *et al.* (2023), documented exfoliation of the intestinal mucosa and lymphocytic infiltration. These histological changes highlight the pervasive damage caused by FAdV-4 across organs, emphasizing the severity of disease. As Schachner and colleagues asserted, updated diagnostic and preventive measures are needed. The histopathological alterations and clinical manifestations observed emphasize the extensive impact of FAdV-4 (HHS) infection on multiple tissues, including severe damage to the liver, heart, kidneys, and intestines, highlighting the serious and systemic nature of disease.

Acknowledgments:

The authors would like to express their sincere appreciation to the staff at the poultry farm and the experimental animal facility for their valuable assistance during the study. Special thanks are also extended to the Department of Microbiology, College of Veterinary Medicine, University of Diyala, for their essential logistical support.

Competing interests:

The authors declare no conflict of interest.

Authors' Contributions

Ahmed Thaban and Amer Al-Azzawi co-developed the hypothesis, designed the study, collected samples from various poultry farms, and conducted the histopathological analysis. Ahmed Thaban, Amer Al-Azzawi and AL-Bayati, A.J contributed to the manuscript preparation and approved the final version of the article.

Funding

this research was not funded by any institution or organization

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