Diyala Journal for Veterinary Sciences
Open Access Journal Published by College of Veterinary Medicine
University of Diyala, Iraq
P-ISSN: 2410-8863
E-ISSN:2958-6178
Vol. 3, NO. 1, March 2025

Journal Homepage: https://djvs.uodiyala.edu.iq

Histopathological Investigation of Fowl Adenovirus (FAdV) Infections in Broiler Chickens: A Study in Diyala Governorate

Walaa Thaeer and Amer Al-Azzawi

Department of Microbiology, College of Veterinary Medicine, Diyala University, Iraq

Corresponding Author: E. mail: Amer_alazawy@yahoo.com

Abstract:

Inclusion Body Hepatitis (IBH), is the main serotype caused by Fowl Adenovirus (FAdV), that affecting chickens leading to substantial economic losses in poultry industry worldwide. The virus mostly affects broiler chickens aged 3-6 weeks, resulting in mortality rates ranging from 10-30%. IBH is characterized by friable, enlarged livers with hepatic necrosis and ecchymotic hemorrhages, often accompanied by lesions in the kidney and spleen. This study was performed at the College of Veterinary Medicine, University of Diyala, aim to identifying the strains of this virus and detect the disease to confirm its presence in Diyala Governorate. This was achieved by conducting histopathological examinations on samples collected from post-mortem cases. Twenty-five tissue samples were collected from several organs, including the liver, spleen, and kidney, from five location farms in Baqubah, Al-Khalis, Al-Muqdadiyah, Al-Wajhiyah, and Kanaan. These sections were stained using routine hematoxylin and eosin (H&E) staining protocols. Histopathological examination of tissues infected broiler chickens with IBH- exposed significant pathological changes. Liver sections displayed coagulative necrosis, basophilic intranuclear inclusion bodies within hepatocytes, fatty infiltration, and subacute periportal hepatitis. In the spleen, vascular congestion, hemorrhage, and lymphocyte depletion were detected. Kidney segments exhibited sign of nephritis, tubular degeneration, and mild peritubular mononuclear infiltration. Additionally, the glomeruli displayed mesangial hyperplasia, proliferative glomerulonephritis, and thickening of Bowman's capsule, while intranuclear inclusion bodies were rarely detected in renal tissues. These findings highlight the extensive tissue damage caused by IBH in affected organs. The results of the current study highlight the pathological effects of FAdV in broiler chickens emphasize the importance of timely detection and characterization of the virus to alleviate its economic effect on poultry rearing in Diyala Governorate

Keywords: IBH, fowl adenoviruses (FAdVs), histopathological changes, Diyala Governorate

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E-ISSN:2958-6178
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Introduction:

Fowl adenovirus (FAdV) infection poses an increasing threat to commercial broiler flocks, leading to extensive economic losses in the global poultry industry (Schachner *et al.*, 2021). Fowl adenovirus is classified into three groups, group I consist of fowl adenovirus (FAdV), group II included (Hemorrhagic Enteritis (HE) in turkey, Marble Spleen disease (MSD) in pheasants, and Avian Adenoviral Splenomegaly (AAS), while group III contains only one disease called Egg Drop Syndrome (EDS76) which has emerged as an inclusive concern, causing disease in layer chickens (Hess, 2013). Fowl Adenoviruses (FAdVs), which are mainly responsible for naturally outbreaks of two important viral infection namely (inclusion body hepatitis (IBH), hydropericardium hepatitis syndrome (HHS) and and gizzard erosion (GE) categorized into five different species (FAdV-A to FAdV-E) based on their genetic structure and phylogenetic relationships. These species are further divided into 12 serotypes (FAdV-1 to -8a, -8b, and -11), recognized through cross-neutralization tests (Benko *et al.*, 2005; Adair and Fitzgerald, 2008; Benkő *et al.*, 2022).

IBH is caused by FAdV serotype 8a and 8b within species D and E respectively (Schachner et al., 2018). It has a major economic impact since it causes a sharp increase in mortality, lower production , growth retardation and significant economic losses to the poultry industry especially the last two decades globally (Alvarado *et al.*, 2007; Zahid *et al.*, 2024) The virus mostly affects broiler chickens aged 3-6 weeks, with duration continues 2-3 weeks resulting in mortality rates ranging from 10-30% or up to 80% in more complex outbreaks that happed in India and Canada (McFerran and Smyth, 2000; Gomis *et al.*, 2006). Fowl adenovirus can be transmitted from hens to offspring vertically and horizontally from one bird to another through contact with respiratory discharges, feces, and contaminated tools (Pereira *et al.*, 2014; Arazi *et al.*, 2020). IBH was recognized to manifest as a secondary or concurrent pathogen, particularly in the presence of immunosu ppressive factors like IBD, CIAV avian reticuloendotheliosis virus (ARV), avian leukosis virus (ALV), and also due to the others factors like mycotoxins (Meng et al., 2018). Affected birds with IBH, exhibited depression, Ruffled feathers, watery droppings, with severe anemia (Zadravec *et al.*, 2011).

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The principal organ afflicted in these birds is the liver, which appears pale, enlarged, necrosis and ecchymotic haemorrhage in most cases (Steer *et al.*, 2015; Mohamed et al., 2018). Histopathological examination of affected birds exhibited necrotic hepatitis accompanied by basophilic or eosinophilic inclusion bodies in hepatocytes (Kim *et al.*, 2008; Dar *et al.*, 2012).

In Iraq Eighteen outbreaks of inclusion body hepatitis (IBH) were recognized in broiler chickens in Baghdad documented since 1979. Infected birds exhibited clear clinical signs such as flock lethargy, distinct yellow-tinged droppings with occasionally appeared as mucus-like consistency. Typical gross lesion of the infected broiler chickens included, enlarged, hemorrhagic and friability liver, Icteric skin, particularly in the non-feathering regions, and swollen enlarged kidneys and enlarged and mottled spleen (Al-Sheikhly and Mutalib, 1979). Due to the limited evidence and details regarding the recent FAdV (IBH)outbreak in Diyala Governorate, this study focuses on histological identification of FAdV strains from the recent IBH outbreaks in the Diyala Governorate.

Materials and Methods:

1.Sampel collection:

Between August 2023 and January 2024, the current study was done at the Department of Microbiology, College of Veterinary Medicine/ University of Diyala. The main aim of this study is to identify inclusion body hepatitis virus (IBH)among a commercial broiler flock farm of Diyala Province. Detection was accomplished using histopathological examination of infected tissues sample. All broiler flocks in this investigation were not vaccinated against Adenovirus by using the commercial inactivated vaccines against FAdV serotype 8b. For histopathological examination, samples collected from liver, kidney, and spleen of broiler chicken farms infected with FAdV, the birds exhibited a wide range of virulence and distinct clinical symptoms of the (IBH) characterized by lethargic of the most birds in the flocks, huddling with ruffled feathers with mucoid yellowish droppings.

The farms, which housed broilers aged 2-3 weeks, these farms were placed in 5 regions included, Baqubah, Al-Khalis, Al-Muqdadiyah, Al-Wajhiyah, and Kanaan, with five farms from each region were selected for histological investigation.

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2.Tissue preparation:

Tissue samples (twenty-five) were extracted from various organs included (liver, spleen, and kidney).

The histological investigation procedures followed in the current study were based on the procedures documented by Faisal and Al-Azzawi (2023) for viral analysis and collecting the tissues sample for this examination, with some minor modifications as follow, tissue samples (4-6 mm thick) were submerged in a 10% fixative formalin solution at room temperature for 24 hours before being processed. The samples were dehydrated in a series of graded ethanol at room temperature for two hours (70% for two hours, 80% for two hours, 90% for two hours, and 100% for two hours),to removal the water from the tissue samples then soaked in xylene for two hours, followed by three hours in molten paraffin wax. The samples were then orientated and embedded in new paraffin (paraffin blocks). The tissue blocks were sectioned with of rotary microtome (Letitz 1512, Germany) at a thickness of 5 µm and mounted on microscope slides. Routine stain hematoxylin and eosin (H&E) was used to staining the sections of the tissues of the samples according to the procedure down by (Spencer *et al.,* 2012). Photomicrographs of each section were examined under light microscope (Omax, USA) using a digital camera (Omax, USA) attached to a digital camera to facilitate detailed examination of the tissue sections.

Results

Recently, IBH have been considered the most important among adenovirus infections in chickens and their prevalence has increased widely in most of broiler flocks in Diyala Governorate. The main clinical signs of the (IBH) characterized by depression lethargic of the most birds in the flocks, huddling with tousled feathers with mucoid yellowish droppings. The duration of the disease will continue 2-3 weeks with mortality rate reach from 5-10%. The gross lesions observed in the broiler chickens infected with IBH involved, increased the size of the liver with mottled appearance, pale to icteric (yellowish) of the skin, swollen and pale kidneys, whereas the skeletal muscle showed moderate to severe hemorrhages (Fig, 1, 2 and 3).

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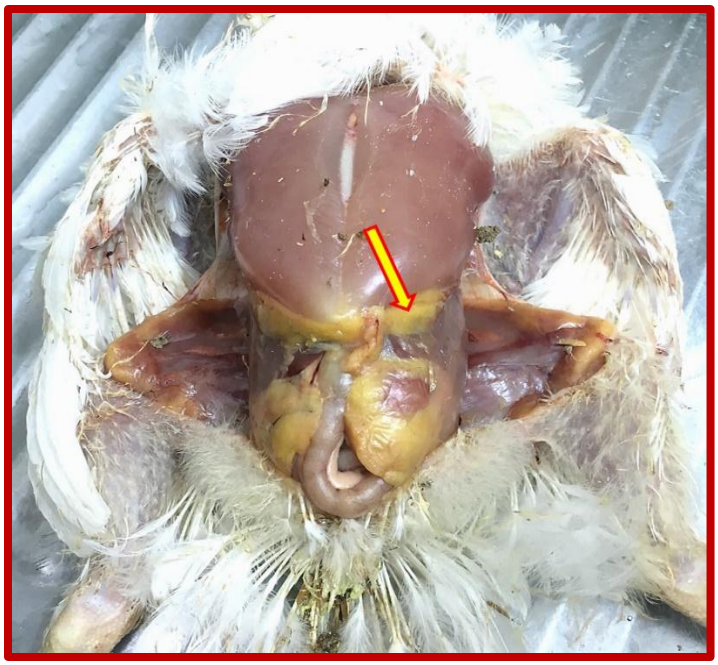

Figure 1: Broiler chicken infected by inclusion body hepatitis (IBH), displaying pale to yellow (icteric) skin as shown by the yellow arrow.

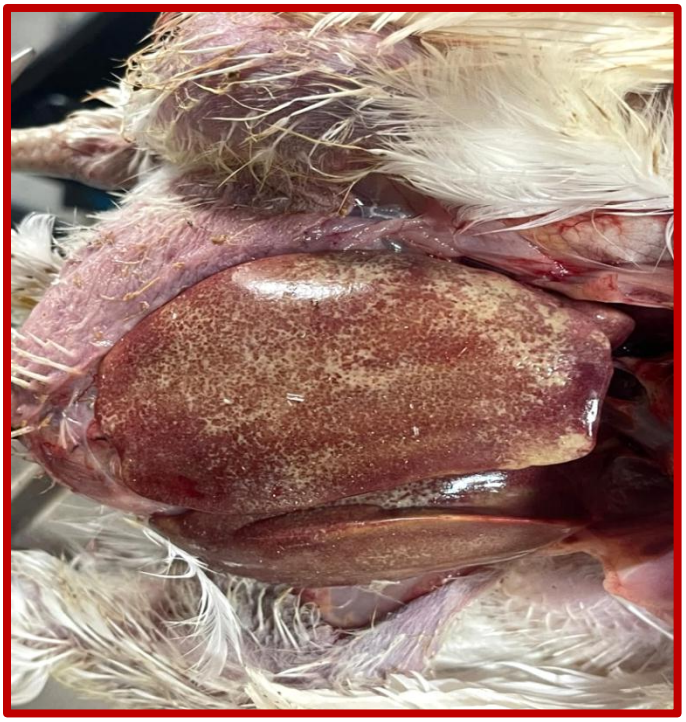

Figure 2: Gross lesions in broiler chickens infected by IBH characteristically include an enlarged, mottled liver.

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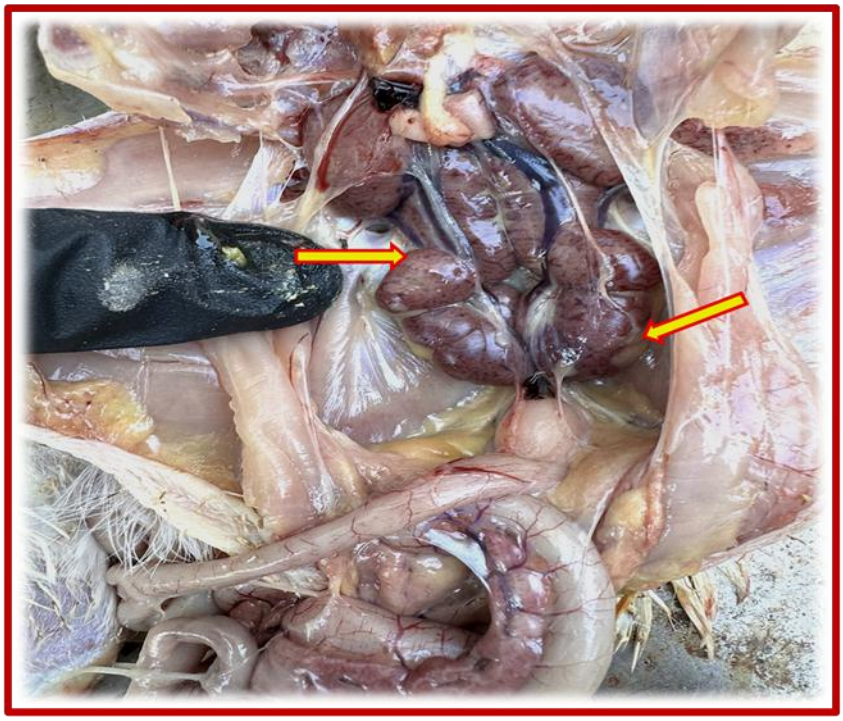

Figure 3: A broiler chicken infected with adenovirus displays swollen and pale kidneys as shown by the yellow arrow.

Histopathological results in the infected birds from the current study focused on the three important organs (liver, kidney and spleen) for studying the tissue changes due to infection with FAdV. Microscopic examination of the liver in the control group at all-time intervals revealed normal histological structure (Fig 4). Three days post infection with FAdV, the liver exhibited coagulative necrosis, basophilic intranuclear inclusion bodies, fatty changes with dilation of sinusoidal space, and signs of subacute periportal hepatitis. Severely affected birds display large basophilic intranuclear inclusion bodies within hepatocyte cells, accompanied by degeneration and necrosis. Degenerative hepatocytes can be identified by one or more large cytoplasmic vacuoles, accompanied by different degrees of pyknosis, karyorrhexis, and karyolitic.

In addition, some hepatocytes in sever liver damage have lost their nuclei, giving the appearance of ghost cells. Known as big Cowdry Type A intranuclear inclusion bodies, basophilic or eosinophilic intranuclear inclusions can occasionally be seen particularly during degeneration of the hepatocytes.

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Hepatic multifocal lymphocyte infiltration has also been discovered. One can see modest mononuclear cell infiltration, degeneration, and necrosis. The presence of fatty infiltration, basophilic intranuclear inclusion bodies, coagulative necrosis, and subacute periportal hepatitis were indicative of severe adenoviral infection as shown in (Fig, 5, 6 and 7).

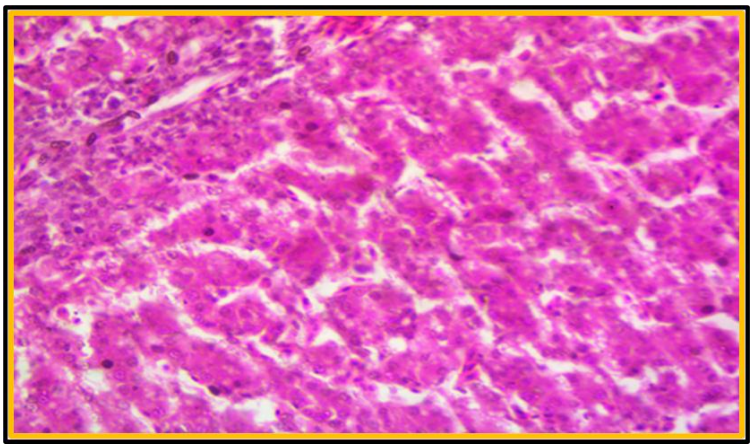

Figure 4: Histological image of a normal chicken liver stained with H&E, displays proper histological structure, with well-defined hepatic lobules and hepatocyte beams radiating towards the central vein. Small cytoplasmic vacuoles are existent in a few hepatocytes. (Magnification: X20).

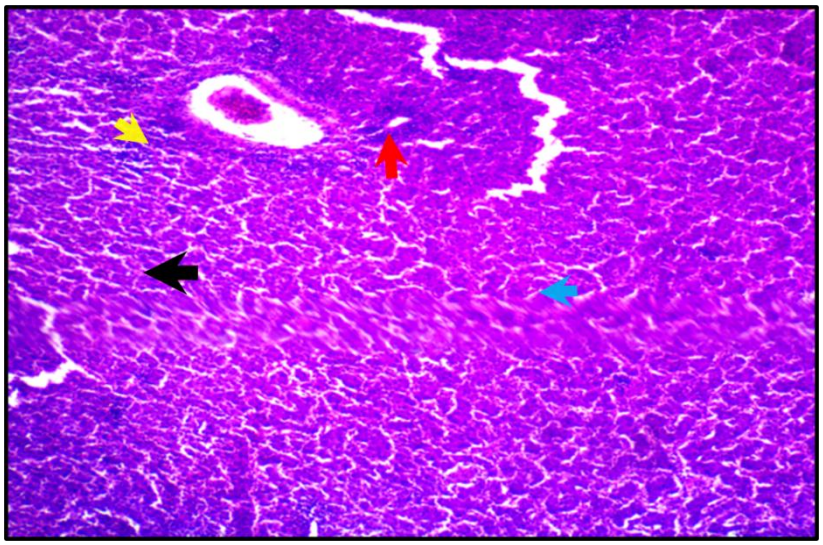

Figure 5: Liver Histopathological section. (Red arrow) multiple basophilic intranuclear inclusion body. (Blue arrow) hematogenous protein material in periportal ears. (Yellow arrow) infiltration of inflammatory cell. (Black arrow) congestion and hemorrhage in periporteal ears (H&E X20)

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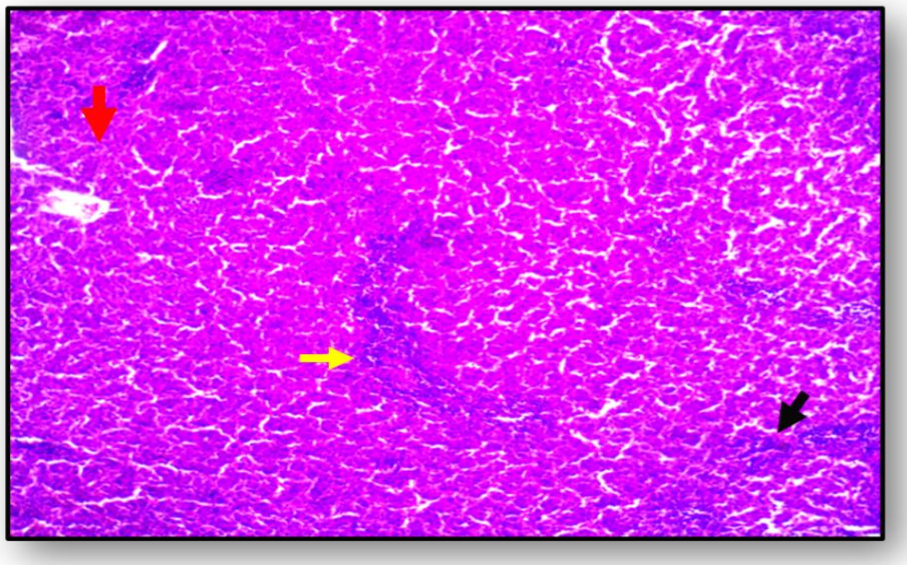

Figure 6: Histopathological section of the liver showing key features of FAdV infection. (Black arrow) indicates an intranuclear inclusion body, which is characteristic of focal necrosis associated with adenovirus. (Yellow arrow) highlights the infiltration of mononuclear cells, (red arrow0 points to congestion in the periportal areas, indicative of inflammation. (H & X20).

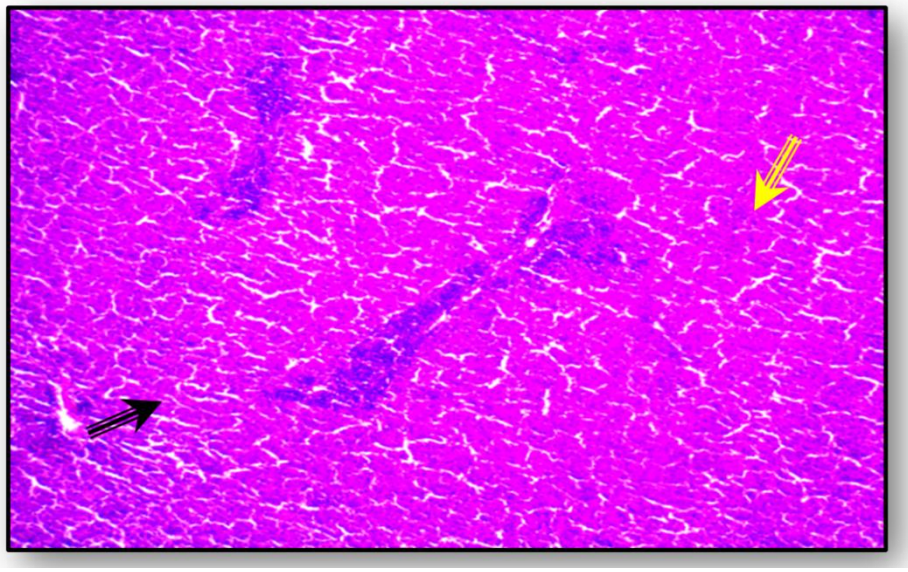

Figure 7: Liver histopathological section showed :(black arrow) congestion in periportal ears. (Yellow arrow) infiltration of inflammatory cells. (H&E X20).

Histopathological examination of the spleen from broiler chickens infected with Inclusion Body Hepatitis (IBH) caused by avian adenovirus (FAdV) revealed significant changes due to the virus's affinity for lymphoid organs, infiltration of

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E-ISSN:2958-6178
Vol. 3, NO. 1, March 2025

inflammatory cell resulting in immunosuppression. Common findings included vascular congestion, focal areas of hemorrhage, and lymphocyte depletion as observed in comparison to the control group. (Fig 8, 9, 10, and 11).

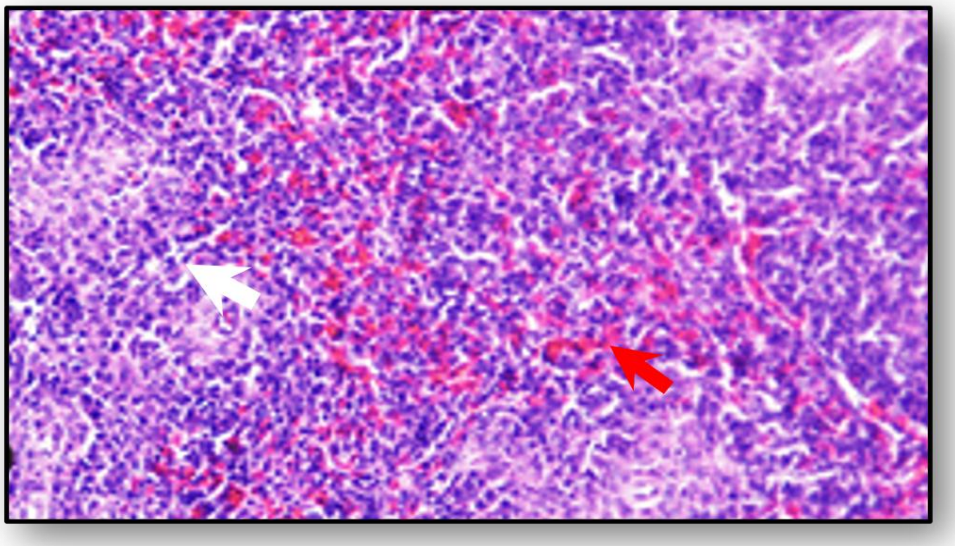

Figure 8: Histological structure of the control chicken spleen with HE staining. a. The compartments of the chicken spleen are red pulp (red arrow) and white pulp (white arrow).

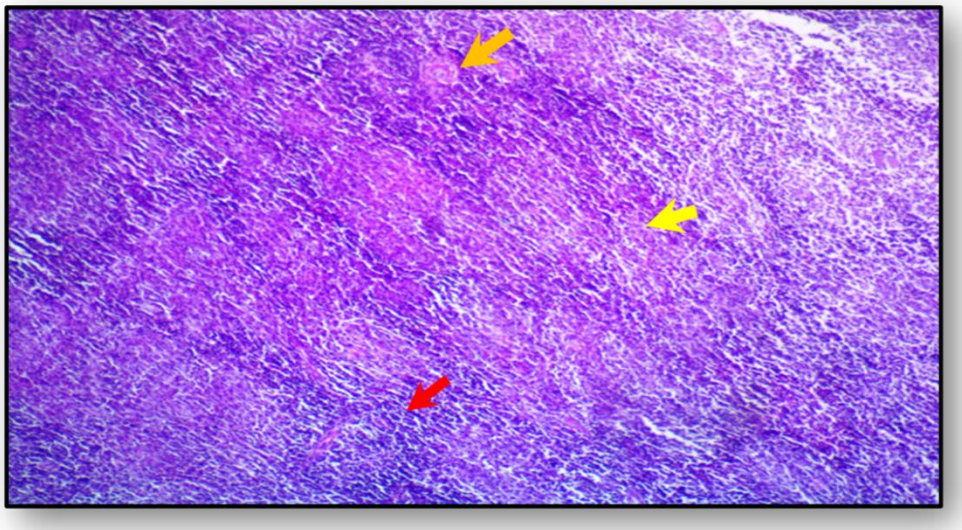

Figure 9: Histopathological section of the spleen. The orange arrow indicates focal areas of necrosis with inclusion bodies. yellow arrow shows infiltration of hematogenous protein material, and the dark red arrow highlights infiltration of mononuclear cells. (H&E staining, X10 magnification). (H&E X20).

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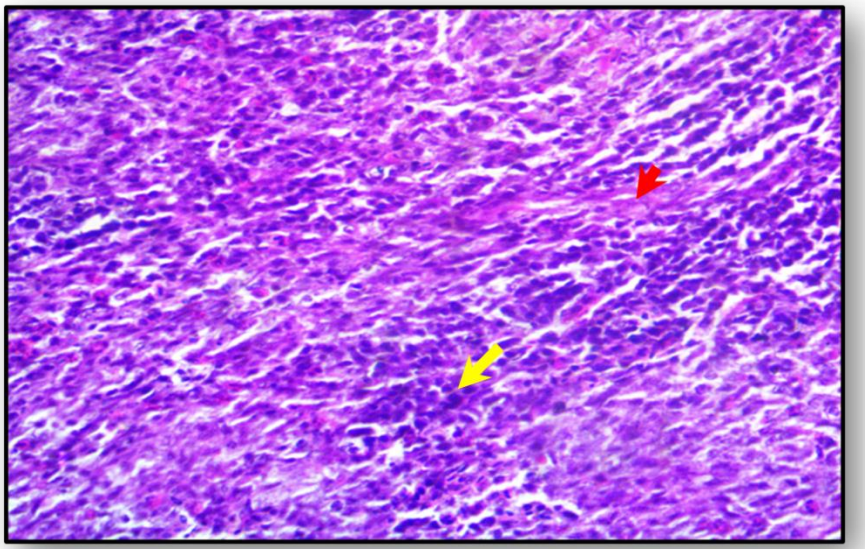

Figure 10: Histopathological section of the spleen. (Yellow arrow) infiltration of mononuclear. (Red arrow) mild infiltration of hematogenous protein material. (H&E X20).

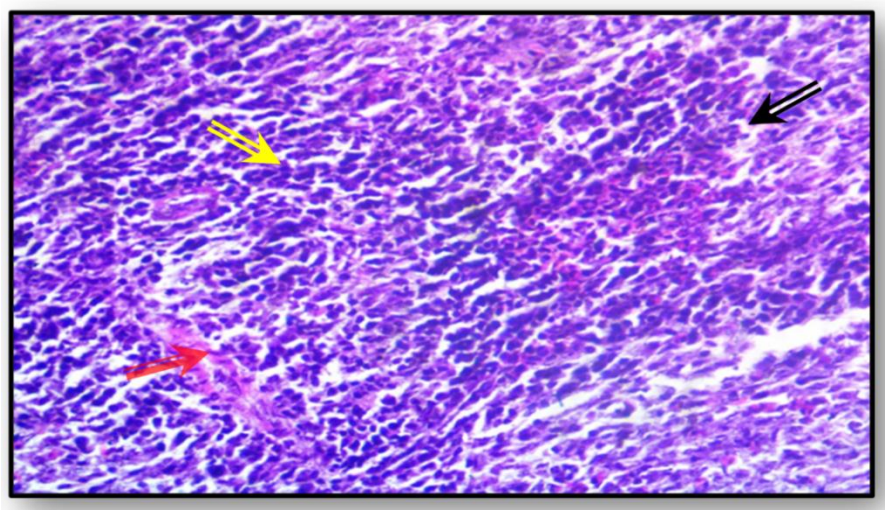

Figure 11: Spleen Histopathological section (yellow arrow) infiltration of inflammatory cell mononuclear cell. (red arrow) mild congestion. (Black arrow) focal ears of necrosis (inclusion body). (H&E X20).

Histological examination of the kidney of chicken in the control group throughout the study revealed normal histological structures (Fig 12). Renal pathology associated with IBH includes nephritis and tubular degeneration in the renal parenchyma. The kidneys exhibit inflammatory lesions, severe degeneration, necrosis and interstitial nephritis affecting the overall health and growth performance of broiler chickens. Large intranuclear basophilic inclusion body and karyorrhexis in the tubular cells of the

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University of Diyala, Iraq
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E-ISSN:2958-6178
Vol. 3, NO. 1, March 2025

kidney. At 3 DPI, the kidney of chicken revealed mild multifocal areas of peritubular mononuclear cells infiltration and mild degeneration of tubular epithelium. The glomeruli showed mesangial hyperplasia with thickening of Bowman's capsule and narrowing of Bowman's space which was consistent with proliferative glomerulonephritis. Whereas the inclusion bodies were poorly observed in the kidneys according to the infection with IBH as shown in Fig 13, 14, 1, 16 and 17.

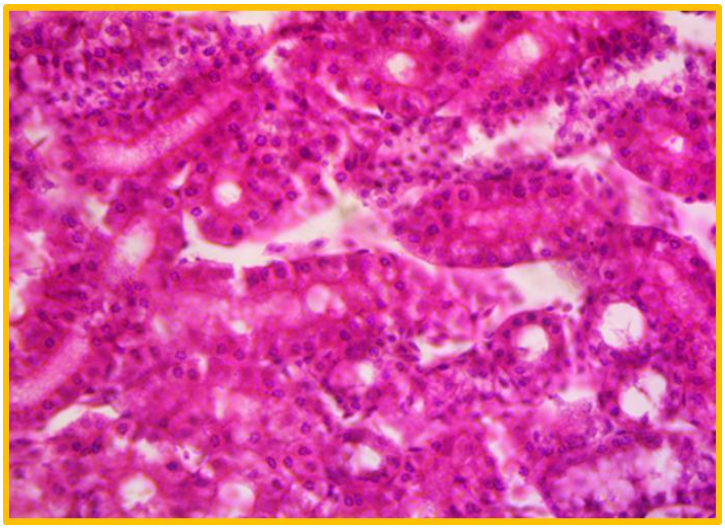

Figure 12: Normal histology of the renal cortex showing the glomerulus, capillary networks, and renal tubule lumens.

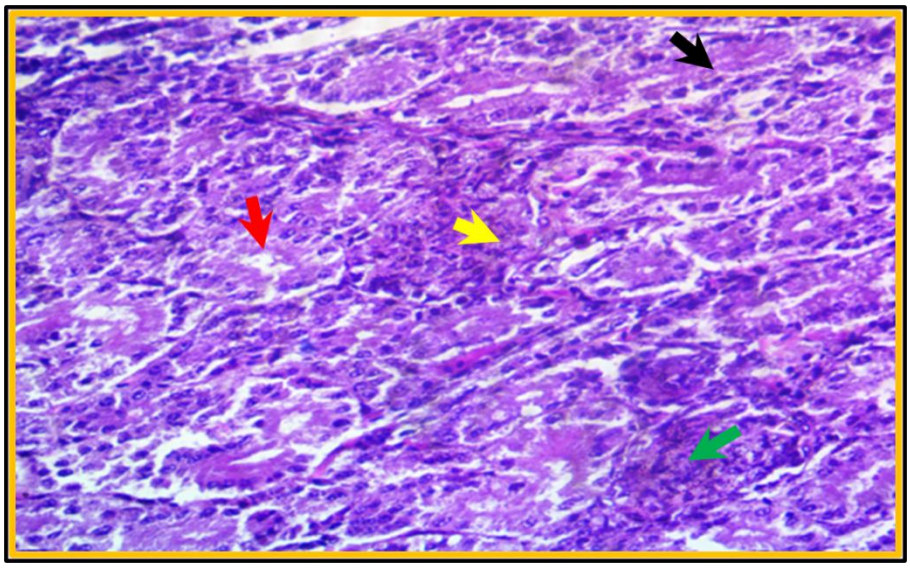

Figure 13: Kidney histological changes after infection with IBH, showed congestion (red arrow), severe degeneration and necrosis (green arrow), and interstitial nephritis (yellow arrow), Massive

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University of Diyala, Iraq
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Vol. 3, NO. 1, March 2025

infiltration of leucocytes, blood vessel congestion, severe degenerative changes and hemorrhages (black arrow).

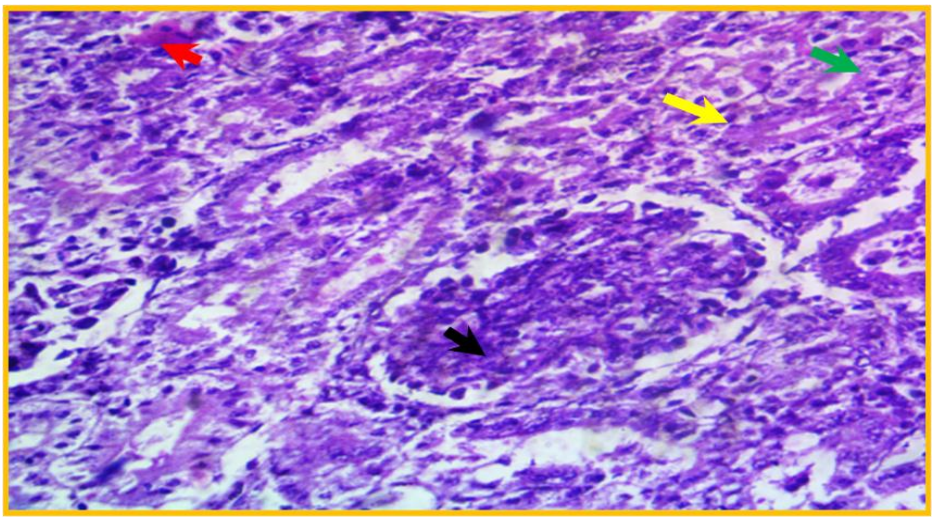

Figure 14: Kidney infected with IBHV, showed local area of granuloma (black arrow). The glomeruli showed mesangial hyperplasia with thickening of Bowman's capsule (yellow arrow) Bowman's space which was consistent with proliferative glomerulonephritis (green arrow), and congestion of the blood vessel (red arrow).

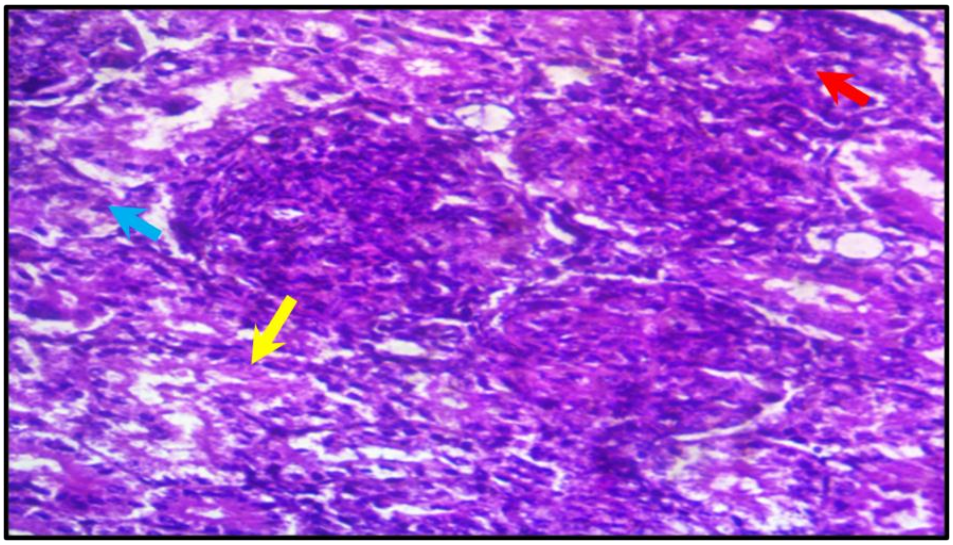

Figure 15: kidney Histopathological section. (red arrow)focal ears of necrosis (inclusion body)(yellow arrow)infiltration of hematogenous material .(blue arrow)infiltration of inflammatory cell.(H&E X20).

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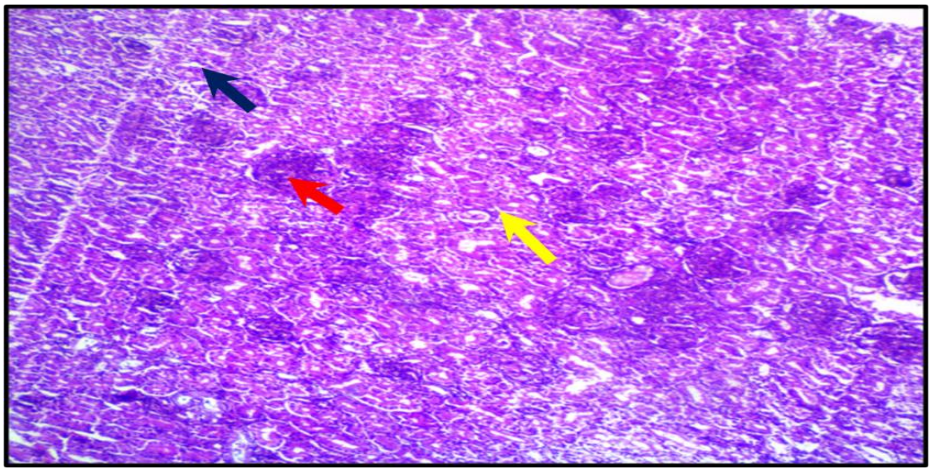

Figure 16: Histopathological section of the kidney. (red arrow) focal ears of necrosis (inclusion body). (Yellow arrow) mild congestion. (Black arrow) infiltration of mononuclear cell. (H&E X20)

Discussion

Inclusion Body Hepatitis (IBH) is an acute viral disease affecting young broiler chickens aged 3 to 6 weeks. It is caused by various serotypes of fowl adenovirus (FAdVs) and can occur regardless of the breeders' immunization status (Hoerr, 1996; Eregae et al., 2014). IBH primarily affects commercial broiler flocks, leading to significant economic losses due to increased mortality and reduced performance (Hess, 2013). In the past two decades, outbreaks of Inclusion Body Hepatitis have been documented in multiple geographical sites, highlighting FAdVs' global spread this virus. Similarly to FAdVs, most of the virus such as (AIV and IBDLV have also contributed to caused immunosuppression effect to the poultry industry (Gomis *et al.*, 2006; Schachner *et al.*, 2016; Al-Talabani and Al-Azzawi, 2023). Clinical isolates of diseased birds have shaped much of our understanding of fowl adenovirus (FAdV). In this study, broiler chickens infected with Inclusion Body Hepatitis (IBH) exhibited lethargy, huddling, ruffled feathers, and yellowish mucoid droppings, primarily in birds two weeks or older. These findings align with previous reports by Matos et al. (2016), Norina et al. (2016), and Schachner et al. (2018), which noted similar symptoms, including poor growth, depression, diarrhea, and inappetence shortly before death. Inclusion Body Hepatitis (IBH) primarily affects chickens aged 3–6 weeks but has also been reported in chicks under one week old (Philippe et al., 2005).

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In such cases, a virulent adenovirus is vertically transmitted from an immunologically naïve hen (Hoerr, 1996). In this study, increased mortality was observed in broiler farms with chickens aged 14–30 days, along with macroscopic liver lesions such as pinpoint hemorrhages, pale and friable livers, and occasional hydropericardium, consistent with findings by Grimes et al. (1978) and Mariappan et al. (2018). Histopathological analysis of the liver, spleen, and kidney offered valuable insights into the tissue-specific impacts of IBH infection in broiler chickens (Dutta et al., 2017). The observed changes in these organs indicate that IBH may have systemic effects, affecting multiple organ systems (El-Shall et al., 2022).

Histopathological analysis revealed intranuclear inclusion bodies in hepatocytes, confirming IBH infection. Two types were noted: eosinophilic inclusions with a surrounding halo and dense basophilic inclusions occupying most of the nucleus. These findings are consistent with Farag et al. (2024), who reported hepatic degeneration, widespread necrosis infiltrated by mononuclear inflammatory cells, bile duct hyperplasia, and scattered intranuclear inclusion bodies (INIBs). In contrast, vaccinated and uninfected controls showed normal liver tissue. The current study revealed that severely affected birds exhibited large basophilic intranuclear inclusion bodies in hepatocytes, accompanied by degeneration, necrosis, cytoplasmic vacuolation, and varying degrees of pyknosis, karyorrhexis, and karyolysis. These findings are consistent with reports by Farag et al. (2024) and Hair-Bejo (2005), who documented similar necrotic changes, extensive inflammatory cell infiltration, and intranuclear inclusions in adenovirus-infected broilers. Notably, varying levels of nuclear damage were observed across most hepatic cells. These results also align with studies by Wilson et al. (2010) and Matos et al. (2016), which described multifocal hepatocellular necrosis and vacuolar degeneration associated with large basophilic intranuclear inclusions. The severe necrotic changes observed in liver tissues further corroborate the gross pathological findings.

Our findings reveal that some hepatocytes in the affected birds lose their nuclei, giving rise to a ghost cell appearance. Additionally, large Cowdry Type A intranuclear inclusion bodies, either basophilic or eosinophilic, were occasionally observed in the degenerating hepatocytes. These inclusion bodies were irregular in shape and

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surrounded by a clear halo. Our observations align with those of Padhy et al. (2015), who reported that in many hepatic cells, the nuclei completely disappear, resulting in ghost cells containing one or more large vacuoles. In some areas of the liver, inclusion bodies were very numerous. Multifocal lymphocyte infiltration in the liver was also noted, along with mild mononuclear cell infiltration, degeneration, and necrosis. These pathological observations are consistent with previous studies that reported similar findings as hallmarks of IBH infection. For example, Ishag *et al.* (2022) observed degeneration, necrosis, lymphocytic infiltration, and intranuclear inclusion bodies in hepatocytes, aligning closely with the present findings. Likewise, Wilson *et al.* (2010) and Schachner *et al.* (2018) reported histopathological examinations in IBH-affected livers of the broiler chickens, showing variable areas of multifocal hepatocellular necrosis, vacuolar degeneration, and lymphoid infiltration—further supporting our observations. The current study revealed significant kidney pathology, including tubular degeneration, basophilic intranuclear inclusion bodies, and karyorrhexis in tubular cells, consistent with previous findings. Zadravec et al. (2013) noted similar karyorrhexis in kidney tubular cells, while Itakura et al. (1974) documented nephrotic changes, such as vacuolation in proximal tubules and regressive changes like karyopyknosis, karyorrhexis, and karyolysis in severe lesions. Dutta et al. (2017) and Wilson et al. (2010) reported renal damage, including glomerulonephritis, tubular necrosis, and vasculitis, highlighting IBH's impact on kidney function. Kim et al. (2008) and Wang et al. (2022) observed renal swelling, tubular degeneration, and interstitial hemorrhages, aligning with the current findings.This study's observations of mild multifocal peritubular infiltration and glomerular changes, such as mesangial hyperplasia and Bowman’s capsule thickening, suggest proliferative glomerulonephritis. These results align with Wilson et al. (2010), Mariappan et al. (2018), Şahindokuyucu et al. (2020), and Naguib et al. (2021), who reported similar histopathological features in IBH-affected kidneys. Collectively, these findings confirm that IBH consistently induces severe renal pathology, impairing kidney function. Upon infecting the host, the virus primarily replicates in lymphoid organs, including the spleen, thymus, bursa of Fabricius, and cecal tonsils, causing significant immunosuppression due to reduced growth

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E-ISSN:2958-6178
Vol. 3, NO. 1, March 2025

in these tissues (McFerran and Adair, 2003). In this study, broiler chickens with Inclusion Body Hepatitis (IBH) caused by Fowl Adenovirus (FAdV) exhibited marked histopathological changes in the spleen, including vascular congestion, focal hemorrhages, and lymphocyte depletion, reflecting the virus's tropism for lymphoid organs. These findings align with Itakura et al. (1974), who reported lymphocyte depletion, reticular cell proliferation, and macrophage infiltration, sometimes accompanied by necrosis. Similarly, Naguib et al. (2021) observed splenic necrosis, lymphocyte depletion, and nuclear changes like pyknosis and karyorrhexis. Mariappan et al. (2018) documented splenitis with lymphocyte depopulation, congestion, and reticular endothelial cell hyperplasia. These results support Steer et al. (2015), who demonstrated that FAdV strains, such as FAdV-8b and FAdV-11, significantly impair lymphoid organs, contributing to immunosuppression.

Conclusion

The study confirmed the presence of Fowl Adenovirus (FAdV) associated with inclusion body hepatitis (IBH) among broiler chickens farm in Diyala Governorate. The disease exhibited distinct clinical symptoms, including enlarged and pale of the liver, kidney and spleen along with mortality rate of up to 30% in addition to pathological changes that provided definitive confirmation of the viral infection. Histopathological examination of IBH-infected broiler chickens revealed basophilic intranuclear inclusion bodies, cellular degeneration, and necrosis in hepatocytes, with periportal inflammatory infiltration, congestion, and hemorrhage. The spleen showed focal necrosis with inclusion bodies and mononuclear cell infiltration. Kidney histology displayed severe degeneration, necrosis, interstitial nephritis, leukocyte infiltration, vascular congestion, and hemorrhages.

Diyala Journal for Veterinary Sciences
Open Access Journal Published by College of Veterinary Medicine
University of Diyala, Iraq
P-ISSN: 2410-8863
E-ISSN:2958-6178
Vol. 3, NO. 1, March 2025

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