

# **Original Article**

# **Pathology**

pISSN 2466-1384 · eISSN 2466-1392 Korean J Vet Res 2025;65(3):e17 https://doi.org/10.14405/kjvr.20250016

## \*Corresponding author:

#### Harith Abdulla Najem

Department of Pathology and Poultry Diseases, College of Veterinary Medicine, University of Basrah, Basrah 61001, Iraq Tel: +964-7706756771

E-mail: harith.najem@uobasrah.edu.iq https://orcid.org/0000-0002-4203-5282

#### Conflict of interest:

The authors declare no conflict of interest.

Received: May 3, 2025 Revised: Sep 7, 2025 Accepted: Sep 26, 2025



- © 2025 The Korean Society of Veterinary Science.
- This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial license (https://creativecommons.org/licenses/by-nc/4.0/), which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

# Pathology and molecular comprehensive study of fowl adenovirus in broiler chicken in Basrah, Iraq

Budoor Muhammad Lateif<sup>1</sup>, Rana Kadhim Abdaljaleel<sup>1</sup>, Isam Azeez Khaleefah<sup>1</sup>, Sara Salim Mohammad<sup>2</sup>, Najlaa Hameed Megdad<sup>1</sup>, Harith Abdulla Najem<sup>1,\*</sup>

<sup>1</sup>Department of Pathology and Poultry Diseases, College of Veterinary Medicine, University of Basrah, Basrah 61001, Iraq

## **Abstract**

Viral diseases are a significant challenge facing the poultry industry, negatively impacting productivity and the agricultural economy. Among these viruses is fowl adenovirus (FAdV). The study aims to identify adenovirus in broilers in the Basrah province, Iraq. The study concentrated on clinical symptoms, postmortem lesions, histological abnormalities, and polymerase chain reaction (PCR) to validate a laboratory diagnosis. From October 2023 to April 2024, the present investigation collected 100 sick 10-day-old chickens from an unusual field in the Abu Sakhir district of Basrah. The poultry laboratory at the College of Veterinary Medicine, University of Basrah, handled the samples. Euthanized were chicks showing clinical symptoms. Every case was reviewed for gross lesions related to viscera. The livers of infected birds showed microhemorrhages or bruises with localized or diffuse areas of necrosis. The hearts showed a straw-coloured, diffuse fluid accumulation in the pericardial sac. The surface of the kidneys was enlarged and hemorrhagic. Microscopically, the liver exhibited basophilic intranuclear bodies, a necrotic area, and mononuclear cell infiltration. The heart showed vascular congestion in the pericardium, accompanied by inflammatory cell infiltration between the muscle fibres. Renal showed hemorrhage and degeneration of the epithelium, mononuclear cell infiltration and basophilic intranuclear bodies. PCR confirmed the presence of the adenovirus genome from infected birds. The study emphasizes the importance of early identification and accurate diagnosis to minimize the impact of FAdV on poultry health and productivity.

**Keywords:** fowl adenovirus; broilers; aviadenovirus; hexon gene; intranuclear inclusion bodies pathology

## Introduction

Viral diseases are one of the significant challenges facing the poultry industry, negatively impacting productivity and the agricultural economy [1]. Among these viruses, fowl adenovirus (FAdV) stands out as one of the most significant pathogens causing substantial economic losses in the poultry sector, particularly in broiler chickens [2]. This virus is characterized by its ability to cause a wide range of diseases, including inclusion body hepatitis (IBH) hydropericardium hepatitis syndrome (HHS), which lead to increased mortality rates and decreased feed con-

www.kjvr.org

<sup>&</sup>lt;sup>2</sup>Department of Veterinary Public Health, College of Veterinary Medicine, University of Basrah, Basrah 61001, Iraq

version efficiency [3–5]. FAdV belongs to the Adenoviridae family and is a non-enveloped virus containing double-stranded DNA (dsDNA). It is classified into 5 types (A–E), comprising more than 12 serotypes [6,7]. These viruses are characterized by their ability to infect a wide range of tissues, including the liver, kidney, respiratory tract, and gastrointestinal tract, leading to diverse diseases [8,9]. Infectious hepatitis (IBH) is characterized by the appearance of intranuclear bodies in hepatocytes, leading to acute hepatitis and sudden death in infected birds, especially chicks at 3 to 6 weeks of age [10–13]. Avian wasting syndrome (HHS) is characterized by fluid accumulation in the pericardium and hepatomegaly, leading to death in birds at 4 to 6 weeks of age [14].

In addition, the virus can cause other diseases, such as gastritis (gizzard erosion) and respiratory tract infection. In recent years, there has been an increasing interest in studying the pathological and molecular aspects of chicken adenovirus to understand the mechanisms of infection and disease progression, as well as to develop effective strategies for prevention and control [15]. Recent studies have included sequencing the virus genome, identifying genetic factors associated with increased virulence, and understanding the interactions between the virus and the immune system [16]. The virus genome contains approximately 43 to 45 kilobases and includes genes that control virus replication and viral capsid assembly, such as the Hexon, Penton, and Fibre genes [17]. Frequent genetic mutations in the virus produce new strains with different virulence, helping it evade the host's immunological response and making control more challenging [18,19]. Traditional laboratory diagnostic methods include virus isolation from liver or spleen tissue using cell cultures and histological examination to detect intranuclear bodies in liver cells [20]. Molecular diagnosis relies on techniques such as polymerase chain reaction (PCR) to amplify viral genes and identify strains, as well as genetic sequencing to analyze the complete genome sequence [21]. Control and prevention strategies include the use of live attenuated or inactivated vaccines, as well as recombinant vaccines developed through genetic engineering techniques [22]. Health management also includes cleaning and disinfecting farms periodically and immunizing mothers to provide transferred immunity to their chicks [23]. In addition, genes associated with virus resistance in chickens are currently being studied to develop more resistant strains [24]. The geographical distribution of viral strains varies from one region to another. In Asia, FAdV-4 is prevalent, causing avian wasting syndrome, whereas in Europe, FAdV-8 is prevalent and associated with systemic inflammation. In North America, there are diverse strains, including FAdV-2 and FAdV-

11 [25,26]. This study aims to identify FAdV in infected broilers in the Basrah province, Iraq.

## **Materials and Methods**

#### **Ethical consideration**

The current study was conducted with the permission of the Ethics Committee at the College of Veterinary Medicine, University of Basrah (reference number: 86/2024).

#### **Case history**

An outbreak with typical symptoms of adenovirus occurred in a flock of more than 100 infected 10-day-old chickens from an atypical field in the Abu Sakhir area of Basrah, Iraq. The study focused on postmortem changes, histopathological changes, and PCR to confirm diagnosis. Our study was continued from October 2023 to April 2024. The samples were processed in the poultry laboratory at the College of Veterinary Medicine, University of Basrah.

## Pathogenicity study

Every case was evaluated for gross lesions of the viscera. Postmortem lesions were prepared using the following guidelines [27,28]. A 10% formalin solution was used to preserve tissue samples obtained from internal organs, including the kidney, liver, and heart. The tissues were then routinely treated and rinsed overnight under running tap water and subsequently dehydrated for one hour using different ethanol concentrations—50%, 60%, 70%, 80%, 90%, and 100% alcohol in that sequence. After the tissues were cleansed with xylene, they were embedded in paraffin wax. Luna said the slices were histologically examined using hematoxylin and eosin staining [29].

## RNA extraction and PCR

Following the manufacturer's advised procedure, genomic DNA was isolated from visceral tissue lesions using a commercial purification kit (Wizard Genomic DNA Purification Kit; Promega, USA). Until further molecular study, the eluted DNA was stored at  $-20^{\circ}\text{C}$ . Twenty-five microliters of GoTaq G2 Colourless PCR Master Mix (Promega), 5  $\mu\text{L}$  of the extracted DNA template, 1.5  $\mu\text{L}$  of each primer (forward and reverse), and nuclease-free water to reach the final volume in a 50  $\mu\text{L}$  reaction vessel. Designed to target a conserved region of the adenoviral genome, the primers generated a 900-bp amplicon linked to the viral core protein. As before mentioned [30], the forward primer sequence was (forward, GAYRGYHGGRTNBTGGAYATG-GG and Reverse TACTTATCNACRGCYTGRTTCCA) An ini-

tial denaturation step at 94°C for 5 minutes, followed by 35 amplification cycles of denaturation at 94°C for 60 seconds, annealing at 61°C for 60-second extension, and so on comprised the thermal cycling schedule. To complete the amplification, a final extension step at 72°C for 10 minutes was performed [31,32].

## Results

#### **Gross lesions**

The postmortem examination of birds revealed gross lesions in the heart, liver, and kidneys in almost all infected birds. The liver was enlarged, pale, and friable, with localized or diffuse regions of necrosis. Occasionally, pinpoint or ecchymosis hemorrhages were visible in the hepatic parenchyma. The liver was icteric in a few isolated cases, either with or without hemorrhages (Fig. 1A). The pericardial sac is enlarged and accumulates straw-coloured fluid with a misshapen and flabby heart (Fig. 1B). Kidney swelling was noted during the necropsy, and a

localized region of necrosis. Hemorrhagic patches were visible on the surface of the congested kidneys (Fig. 1C).

## Histopathology result

Table 1 summarizes the histopathological analysis of the birds under examination revealed that renal tubular degeneration (75.0%) was the main renal lesion and hepatocellular necrosis (83.3%) was the most common hepatic lesion. Intranuclear inclusion bodies, vascular congestion, and mononuclear inflammatory infiltrations in the liver, heart, and kidneys were among the most often noted alterations.

#### Liver

In hepatocytes, the histopathology reveals basophilic intranuclear inclusion bodies, a necrotic region, and mononuclear cell infiltration (Fig. 2).

#### Heart

Severe diffuse pericarditis and congestion of blood vessels in



Fig. 1. (A) The liver of young broilers shows enlarged and yellowish discolouration (black arrows) and pinpoint hemorrhages (yellow arrows) and contains multiple hemorrhagic foci (blue arrow). (B) The heart of young broilers shows an accumulation of straw-coloured fluid (black arrow). (C) The kidneys of young broilers show swelling, congestion, and a localized region of necrosis (black arrows).

Table 1. Samples positive and the percentage of various histopathological lesions observed in the livers, hearts, and kidneys of 12 examined birds

Organ	Lesion	Positive samples (n, %)
Liver	Hepatocellular necrosis	10 (83.3)
	Intranuclear inclusion bodies	9 (75.0)
	Sinusoidal congestion	8 (66.7)
	Mononuclear inflammatory infiltration	7 (58.3)
Heart	Pericarditis	4 (33.3)
	Myocardial degeneration	5 (41.7)
	Congestion of cardiac vessels	6 (50.0)
	Focal mononuclear infiltrate	4 (33.3)
Kidney	Renal tubular degeneration	9 (75.0)
	Mononuclear cell infiltration and intranuclear inclusion bodies	8 (66.7)
	Necrosis	7 (58.3)
	Glomerular congestion	6 (50.0)

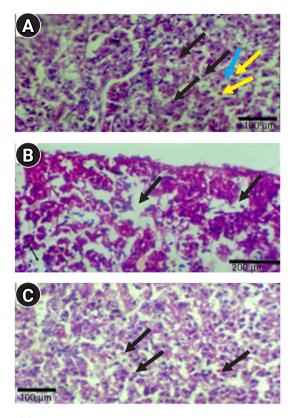


Fig. 2. (A) The liver histopathology shows a basophilic intranuclear inclusion body in hepatocytes (yellow arrows) with mononuclear cell infiltration (black arrows) and hepatocellular necrosis (blue arrow) (hematoxylin and eosin [H&E] stain, scale bar = 100  $\mu$ m). (B) The histopathology of the liver showing dilation of sinusoidal space (black arrows) (H&E stain, scale bar = 200  $\mu$ m). (C) The liver histopathology showing basophilic intranuclear inclusion bodies in the hepatocytes (black arrows) (H&E stain, scale bar = 100  $\mu$ m).

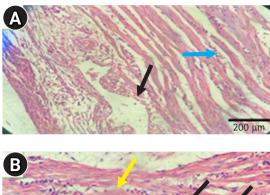
the epicardium with inflammatory cell infiltration between myofibers (Fig. 3). Degeneration of the cardiac muscle fibre and mild edema.

## Kidney

Kidneys observed hemorrhages and degeneration of renal epithelium. Infiltration of mononuclear cells and basophilic intranuclear inclusion bodies (Fig. 4).

## Detection of DNA virus using PCR

PCR amplification for adenovirus was performed using extracted viral DNA from samples, employing both forward and reverse primers. Twelve samples were analyzed using the PCR technique (Fig. 5). DNA bands measuring approximately 900 base pairs were observed following electrophoresis on an agarose gel. Highly conserved adenovirus core protein (adenovirus



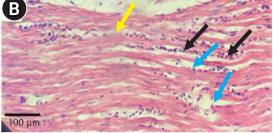
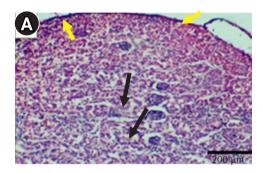


Fig. 3. (A) Histopathology of the heart showing severe diffuse pericarditis and congestion blood vessels in the epicardium (black arrow), infiltration inflammatory cell in myofibers (blue arrow) (hematoxylin and eosin [H&E] stain, scale bar = 200  $\mu m$ ). (B) The histopathology of the heart shows accumulation of mononuclear cells in the myocardium (black arrows) with degenerated cardiac muscle fibres (blue arrows) and mild edema (yellow arrow) (H&E stain, scale bar = 100  $\mu m$ ).



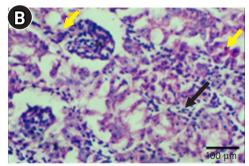
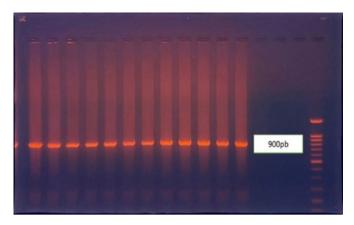


Fig. 4. (A) Histopathology of the kidney showing subcapsular hemorrhages (yellow arrows), necrosis, and degeneration of tubular epithelium (black arrows) (hematoxylin and eosin [H&E] stain, scale bar = 200  $\mu$ m). (B) The kidney's hstopathology shows necrosis and tubular epithelium degeneration accompanied by mononuclear cell infiltration (black arrow) and intranuclear inclusion bodies (yellow arrows) (H&E stain, scale bar = 100  $\mu$ m).



**Fig. 5.** Agarose gel electrophoresis of amplification products from field fowl adenovirus strains using serotype-specific primers. Field isolates included 12 samples that exhibited appropriately sized DNA bands.

nucleoprotein) was detected in 12 out of 100 clinically suspected samples. Twelve samples were extracted from random positive PCR samples.

## Discussion

FAdVs are non-enveloped, dsDNA viruses classified within the genus Aviadenovirus. FAdVs are categorized based on their serological affiliations; 12 fowl serotypes, including 8a and 8b, have been identified [33]. The present study was conducted under field conditions on farms experiencing clear and common clinical outbreaks. It was not practical for a group of healthy birds to remain completely free of exposure due to the widespread prevalence of the disease and the difficulty of ensuring that any flock remains free of infection under current epidemiological conditions. Furthermore, there was no access to unvaccinated birds or birds free of the same environmental disease background to serve as a reliable comparator. In the present study, the liver was enlarged, pale, and friable with widespread or isolated necrotic regions. Pointy or ecchymotic hemorrhages signal vascular impairment, most likely resulting from coagulopathy or injury to endothelial cells. Observed in a subset of birds, icteric colouration disturbed bilirubin metabolism and decreased liver function. The decrease in osmotic pressure of the blood can lead to the accumulation of fluid in body cavities, such as the pericardium, which appears as a straw-coloured fluid in this condition called "ascites" [34–36].

Additionally, our results regarding the cardiovascular system displayed evidence of severe involvement, most notably the accumulation of straw-coloured fluid within the pericardial sac and a flaccid, misshapen heart. These results suggest both car-

diac deterioration and increased vascular permeability [37,38].

In addition, renal examination findings of surface hemorrhages, localized necrosis, and increased vascular permeability due to the inflammatory reaction can lead to kidney swelling (renal oedema) and kidney dysfunction. In adenovirus (FAdV) infection, damage to the inner lining of blood vessels (endothelial dysfunction) may occur, leading to kidney bleeding and circulatory stress, which are connected to viremia and systemic inflammatory reactions. These gross lesions, taken together, confirm a severe, multi-organ impact of adenoviral infection, thereby highlighting the extensive tissue tropism of the virus [39–41].

Our results, observed under a microscope in the kidneys, included necrosis and degeneration in renal tubular cells, which occur when virus replication interferes with normal cellular functions. Where FAdV produces inclusion bodies in the nucleus of renal epithelial cells. Additionally, these inclusions comprise viral DNA, proteins, and cellular detritus. Inclusions cause necrosis by displacing chromatin and interfering with regular nuclear functions [42,43]. The present results, observed microscope changes in the liver's alterations, reveal basophilic intranuclear inclusion bodies with a necrotic region and mononuclear cell infiltration. The disease is caused by viral inclusion accumulation and replication-related damage, leading to hepatocytes becoming structurally and functionally disrupted and ultimately resulting in necrosis [36,44], as reported by researchers who found necrosis in the hepatocytes of the liver. The high concentration of viral DNA and related proteins causes basophilic staining because they firmly attach to hematoxylin during histological staining.

Present study, serotype-specific primers were employed to detect the presence of chicken adenovirus (FAdV) DNA in field samples using agarose gel electrophoresis. Twelve samples, taken from clinically afflicted birds, underwent PCR amplification. Indicating a 100% positive rate among the field isolates, the adequate amplification of a desired area of the FAdV genome is shown by the identification of bands of suitable molecular weight. Strong evidence that the amplified fragments match the expected virus serotype is the specificity of the primers used. These results are consistent with earlier reports indicating the widespread circulation of FAdV among poultry populations during outbreaks of IBH [9,45-49]. The confirmed presence of FAdV DNA in every examined sample indicates the circulation of the virus in the poultry population investigated. Additionally, molecular discoveries support the general diagnosis of FAdV-induced disease based on gross pathology and histological data. Moreover, the efficacy of serotype-specific primers

highlights the potential use of PCR as a standard diagnostic method for rapid and accurate FAdV diagnosis during an epidemic. The present work concludes that the FAdV infection aligns with gross and histological results, including necrosis, cellular degeneration, and intranuclear inclusion bodies. Sensitive molecular identification of the virus was made possible by serotype-specific primers and PCR, therefore verifying its presence in all examined samples. Pathology and molecular tools, used in conjunctions, provide a comprehensive diagnostic system. The results advance knowledge of FAdV and help guide future efforts in vaccination development and surveillance. Also, PCR used in this study was employed to support a primary diagnosis based on clinical signs, postmortem lesions, and histopathology. It was not designed to distinguish directly between different virus serotypes. The primers are used to target a conserved region in the hexon gene, enabling the detection of pan-adenoviruses; however, they alone do not allow for precise strain or serotype identification without sequencing. The primers were designed to target the Hexon gene, one of the main structural proteins in adenovirus and a popular target in molecular studies because it contains both conserved and variable regions that facilitate detection and classification.

## ORCID

Budoor Muhammad Lateif, https://orcid.org/0009-0001-0084-6175

Rana Kadhim Abdaljaleel, https://orcid.org/0009-0006-9455-0653

Isam Azeez Khaleefah, https://orcid.org/0000-0001-8050-7896 Sara Salim Mohammad, https://orcid.org/0009-0008-1301-2138 Najlaa Hameed Megdad, https://orcid.org/0009-0008-1650-6192

Harith Abdulla Najem, https://orcid.org/0000-0002-4203-5282

## **Author's Contributions**

Conceptualization: Lateif BM; Data curation: Megdad NH; Formal analysis: Mohammad SS; Investigation: Khaleefah IA; Methodology: Abdaljaleel RK; Project administration: Najem HA; Resources: Mohammad SS; Software: Lateif BM; Supervision: Najem HA; Validation: Khaleefah IA; Visualization: Lateif BM; Writing–original draft: Megdad NH; Writing–review & editing: Abdaljaleel RK.

# **Acknowledgments**

The authors thank the staff of the University of Basra's College of Veterinary Medicine for providing the necessary assistance and resources for the study project.

# **Data Availability Statement**

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

## References

- 1. Schachner A, Matos M, Grafl B, Hess M. Fowl adenovirus-induced diseases and strategies for their control: a review on the current global situation. *Avian Pathol.* 2018;47: 111–126.
- 2. Niczyporuk JS. Phylogenetic and geographic analysis of fowl adenovirus field strains isolated from poultry in Poland. *Arch Virol.* 2016;161:33–42.
- **3.** Steer PA, O'Rourke D, Ghorashi SA, Noormohammadi AH. Application of high-resolution melting curve analysis for typing of fowl adenoviruses in field cases of inclusion body hepatitis. *Aust Vet J.* 2011;89:184–192.
- **4.** Mase M, Nakamura K. Phylogenetic analysis of fowl adenoviruses isolated from chickens with gizzard erosion in Japan. *J Vet Med Sci.* 2014;76:1535–1538.
- 5. Niu Y, Sun Q, Zhang G, Sun W, Liu X, Xiao Y, et al. Pathogenicity and immunosuppressive potential of fowl adenovirus in specific pathogen free chickens. *Poult Sci.* 2017;96: 3885–3892.
- **6.** Ganesh K, Suryanarayana VV, Raghavan R. Detection of fowl adenovirus associated with hydropericardium hepatitis syndrome by a polymerase chain reaction. *Vet Res Commun.* 2002;26:73–80.
- 7. Kaján GL, Kecskeméti S, Harrach B, Benkő M. Molecular typing of fowl adenoviruses, isolated in Hungary recently, reveals high diversity. *Vet Microbiol*. 2013;167:357–363.
- **8.** Ojkic D, Martin E, Swinton J, Vaillancourt JP, Boulianne M, Gomis S. Genotyping of Canadian isolates of fowl adenoviruses. *Avian Pathol.* 2008;37:95–100.
- 9. Huang Q, Ma X, Huang X, Huang Y, Yang S, Zhang L, et al. Pathogenicity and complete genome sequence of a fowl adenovirus serotype 8b isolate from China. *Poult Sci.* 2019;98: 573–580.
- 10. Lim TH, Lee HJ, Lee DH, Lee YN, Park JK, Youn HN, et al.

- Identification and virulence characterization of fowl adenoviruses in Korea. *Avian Dis.* 2011;55:554–560.
- 11. Malik YS, Verma AK, Kumar N, Touil N, Karthik K, Tiwari R, et al. Advances in diagnostic approaches for viral etiologies of diarrhea: from the lab to the field. *Front Microbiol*. 2019;10:1957.
- 12. Tian F, Wang H, Zhang Z, Jin L, Zhan Z. Establishment of a continuous kidney cell line and its application in molecular characterization of a novel adenovirus from Chinese soft-shelled turtle, Pelodiscus sinensis. *Aquaculture*. 2025;606: 742574.
- **13.** Grafl B, Aigner F, Liebhart D, Marek A, Prokofieva I, Bachmeier J, et al. Vertical transmission and clinical signs in broiler breeders and broilers experiencing adenoviral gizzard erosion. *Avian Pathol*. 2012;41:599–604.
- 14. Li PH, Zheng PP, Zhang TF, Wen GY, Shao HB, Luo QP. Fowl adenovirus serotype 4: Epidemiology, pathogenesis, diagnostic detection, and vaccine strategies. *Poult Sci.* 2017; 96:2630–2640.
- Erny KM, Barr DA, Fahey KJ. Molecular characterization of highly virulent fowl adenoviruses associated with outbreaks of inclusion body hepatitis. *Avian Pathol.* 1991;20:597–606.
- 16. Matos M, Grafl B, Liebhart D, Hess M. The outcome of experimentally induced inclusion body hepatitis (IBH) by fowl aviadenoviruses (FAdVs) is crucially influenced by the genetic background of the host. *Vet Res.* 2016;47:69.
- 17. Pouladi I, Najafi H, Jaydari A. Research Note: Overview of fowl adenovirus serotype 4: structure, pathogenicity, and progress in vaccine development. *Poult Sci.* 2024;103: 103479.
- 18. Gaweł A, Nowak M, Ciaputa R, Bobrek K. Prevalence of inclusion body hepatitis (IBH) in Poland from 2010-2014. *Pol I Vet Sci.* 2016;19:889–891.
- 19. Noormohammadi AH. Inclusion body hepatitis and hepatitis hydropericardium syndrome in poultry. *Merck Vet Man.* 2023;1.
- 20. McFerran JB, Adair BM. Avian adenoviruses: a review. *Avian Pathol.* 1977;6:189–217.
- 21. Ghorani M, Ghalyanchi Langeroudi A, Khordadmehr M, Hojabr Rajeoni A. Molecular and histopathological characterization of inclusion body hepatitis (IBH) in broiler chickens in Isfahan province. *Iran J Virol.* 2021;15:28–33.
- 22. Grimes TM, King DJ, Kleven SH, Fletcher OJ. Involvement of a type-8 avian adenovirus in the etiology of inclusion body hepatitis. *Avian Dis.* 1977;21:26–38.
- **23.** Mariappan AK, Munusamy P, Latheef SK, Singh SD, Dhama K. Hepato nephropathology associated with inclusion body

- hepatitis complicated with citrinin mycotoxicosis in a broiler farm. *Vet World*. 2018;11:112–117.
- 24. Mirzazadeh A, Asasi K, Mosleh N, Abbasnia M, Abdi Hachesoo B. A primary occurrence of inclusion body hepatitis in absence of predisposing agents in commercial broilers in Iran: a case report. *Iran J Vet Res.* 2020;21:314–318.
- 25. Gayathri L, Karthikeyan BS, Rajalakshmi M, Dhanasekaran D, Li AP, Akbarsha MA. Metabolism-dependent cytotoxicity of citrinin and ochratoxin A alone and in combination as assessed adopting integrated discrete multiple organ co-culture (IdMOC). *Toxicol In Vitro*. 2018;46:166–177.
- 26. Mase M, Mitake H, Inoue T, Imada T. Identification of group I-III avian adenovirus by PCR coupled with direct sequencing of the hexon gene. *J Vet Med Sci.* 2009;71:1239–1242.
- 27. De Herdt P, Timmerman T, Defoort P, Lycke K, Jaspers R. Fowl adenovirus infections in Belgian broilers: a ten-year survey. *Vlaams Diergeneeskd Tijdschr.* 2013;82:125–133.
- 28. Khaleefah IA, Najem HA. Clinical, pathological and molecular study of Marek's disease in the Gallus gllus domesticus and brahma chickens of Basra province, Iraq. *Egypt J Vet Sci.* 2021;52:63–71.
- 29. Luna LG. *Manual of histologic staining methods of the Armed Forces Institute of Pathology*. 3rd ed. McGraw-Hill Book Company; 1968.
- 30. Mase M, Hiramatsu K, Nishijima N, Iseki H, Watanabe S. Identification of specific serotypes of fowl adenoviruses isolated from diseased chickens by PCR. *J Vet Med Sci.* 2021;83: 130–133.
- 31. Khaleefah IA, Al-Tameemi HM, Kraidi QA, Najem HA, Ahmed JA, Alrafas HR. Clinical and molecular detection of fowl pox in domestic pigeons in Basrah Southern of Iraq. *Korean J Vet Res.* 2024;64:e7.
- **32.** Meulemans G, Boschmans M, Berg TP, Decaesstecker M. Polymerase chain reaction combined with restriction enzyme analysis for detection and differentiation of fowl adenoviruses. *Avian Pathol.* 2001;30:655–660.
- **33.** Safwat MM, Sayed AS, Ali Elsayed MF, Ibrahim AA. Genotyping and pathogenicity of fowl adenovirus isolated from broiler chickens in Egypt. *BMC Vet Res.* 2022;18:325.
- 34. Oliver-Ferrando S, Dolz R, Calderón C, Valle R, Rivas R, Pérez M, et al. Epidemiological and pathological investigation of fowl aviadenovirus serotypes 8b and 11 isolated from chickens with inclusion body hepatitis in Spain (2011-2013). *Avian Pathol.* 2017;46:157–165.
- 35. Gomis S, Goodhope AR, Ojkic AD, Willson P. Inclusion body hepatitis as a primary disease in broilers in Saskatche-

- wan, Canada. Avian Dis. 2006;50:550–555.
- **36.** Lateif BM, Ahmed JA, Najem HA. Genotypic characterization of Escherichia coli isolated from infected chicken in Basrah, Iraq. *Online J Anim Feed Res.* 2024;14:29–39.
- 37. Ahamad DB, Selvaraj J, Sasikala M, Prasath NB. Pathological study of an outbreak of hydropericardium syndrome in Giriraja chicken. *Indian J Vet Pathol.* 2017;41:53–56.
- **38.** Chen L, Deng H, Cui H, Fang J, Zuo Z, Deng J, et al. Inflammatory responses and inflammation-associated diseases in organs. *Oncotarget*. 2017;9:7204–7218.
- **39.** Sohaimi NM, Clifford UC. Fowl adenovirus in chickens: Diseases, epidemiology, impact, and control strategies to the Malaysian poultry industry: a review. *J Worlds Poult Res.* 2021;11:387–396.
- **40.** Kombe Kombe AJ, Fotoohabadi L, Gerasimova Y, Nanduri R, Lama Tamang P, Kandala M, et al. The role of inflammation in the pathogenesis of viral respiratory infections. *Microorganisms*. 2024;12:2526.
- 41. Wang R, Lan C, Benlagha K, Camara NO, Miller H, Kubo M, et al. The interaction of innate immune and adaptive immune system. *MedComm* (2020). 2024;5:e714.
- **42.** Steer PA, Sandy JR, O'Rourke D, Scott PC, Browning GF, Noormohammadi AH. Chronological analysis of gross and histological lesions induced by field strains of fowl adenovirus serotypes 1, 8b and 11 in one-day-old chickens. *Avian Pathol.* 2015;44:106–113.
- 43. Nakamura K, Mase M, Yamaguchi S, Shibahara T, Yuasa N.

- Pathologic study of specific-pathogen-free chicks and hens inoculated with adenovirus isolated from hydropericardium syndrome. *Avian Dis.* 1999;43:414–423.
- 44. Hafez HM. Avian adenoviruses infections with special attention to inclusion body hepatitis/hydropericardium. *Pak J Zool.* 2009;41:269–276.
- 45. Benkő M, Harrach B, Both GW, Russell WC, Adair BM, Adam E, et al. Adenoviridae. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, Ball LA, eds. *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses.* Elsevier/Academic Press; 2005: p213–228.
- **46.** El-Tholoth M, Abou El-Azm KI. Molecular detection and characterization of fowl adenovirus associated with inclusion body hepatitis from broiler chickens in Egypt. *Trop Anim Health Prod.* 2019;51:1065–1071.
- 47. Nakamura K, Mase M, Yamamoto Y, Takizawa K, Kabeya M, Wakuda T, et al. Inclusion body hepatitis caused by fowl adenovirus in broiler chickens in Japan, 2009-2010. *Avian Dis.* 2011;55:719–723.
- **48.** Choi KS, Kye SJ, Kim JY, Jeon WJ, Lee EK, Park KY, et al. Epidemiological investigation of outbreaks of fowl adenovirus infection in commercial chickens in Korea. *Poult Sci.* 2012;91:2502–2506.
- **49.** Li S, Zhao R, Yang Q, Wu M, Ma J, Wei Y, et al. Phylogenetic and pathogenic characterization of current fowl adenoviruses in China. *Infect Genet Evol.* 2022;105:105366.