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Incidence of Hepatitis Hydropericardium Syndrome in Broiler Chickens Caused by a New Fowl Adenovirus Strain in Iraq

Melad Ibrahim Oraibi*[1], Majid Haqi Khaleel[2], and Amer Abdulameer A. Al-Baldawi[3]

Al-Manara College for Medical Sciences, Misan[1], Uruk Laboratory for Molecular, Bacterial, and Serological Tests[2], Iraq

*Corresponding author's Email: Meladibrahim1975@gmail.com

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ABSTRACT

Hepatitis Hydropericardium syndrome (HHS) is an acute infectious disease affecting broiler chickens. It is caused by a fowl adenovirus (FAdV) of Group I, serotype 4. This disease is characterized by sudden deaths in broilers as young as three weeks, with mortality rates reaching up to 65%. The current study aimed to evaluate the outbreak of HHS in three broiler farms in southern Iraq. It also sought to identify the specific serotypes of fowl adenovirus (FAdV) responsible for this outbreak, primarily focusing on its genetic characteristics and diversity. Ten liver and heart tissue samples were collected from broiler chickens (Ross 308) that had displayed clinical signs of depression, ruffled feathers, and a tendency to huddle in corners before death. Viral DNA was extracted from liver tissues for further virus detection using PCR and RT-PCR. A post-mortem examination showed a turmeric-yellow discoloration in the dividing lines between the pectoral muscles and the abdominal cavity. The livers of infected chickens were markedly enlarged, and clear, yellow-colored fluid was observed in the pericardial sac. Histopathological analysis of stained liver and heart tissues revealed small multifocal areas of necrosis and mononuclear cell infiltration, including basophilic intranuclear inclusion bodies in hepatocytes and lymphocytic infiltrates. Conventional PCR analysis of liver tissues confirmed the presence of FAdV serotype 4, identifying all samples as the Melad strain, a novel strain responsible for the ongoing epidemic in Iraq. This study confirmed the presence of FAdV serotype 4 and identified all samples as the Melad strain. This research also addresses the need to investigate FAdV with molecular techniques for a better understanding of the epidemiology of the disease.

Keywords: Fowl adenovirus, Hepatitis Hydropericardium Syndrome, Melad strain serotype 4.

INTRODUCTION

Hepatitis Hydropericardium Syndrome (HHS) has emerged as a critical challenge in the global poultry industry, leading to substantial economic losses and high mortality rates, particularly in Iraq since its recognition in the early 1990s (Abdul-Aziz and Al-Attar, 1991; Abdul-Aziz and Hassan, 1995). HHS primarily affects broiler chickens aged 3 to 6 weeks and is characterized by abrupt outbreaks that severely impact poultry production (Liu et al., 2022; Oraibi and Abdalmaged, 2022). Key clinical manifestations of HHS include hepatomegaly, hepatic discoloration, and the accumulation of gelatinous fluid within the pericardium, highlighting the severe pathophysiological effects on infected flocks (Li et al., 2016). The etiological agent responsible for HHS is a non-enveloped double-stranded DNA virus, classified within the Adenovirus family and specifically under the fowl adenovirus (FAdV) group (Meulemans et al., 2004; Fitzgerald et al., 2020). Fowl adenoviruses (FAdVs) are classified into twelve serotypes, grouped into five species including FAdV-A to FAdV-E (Mirzazadeh et al., 2020; El-Shall et al., 2022). Among these, FAdV-C, particularly serotype 4, is most frequently linked to HHS outbreaks worldwide and is recognized as the principal causative agent of the disease (Pallister et al., 1996; Liu et al., 2016; Niczyporuk, 2016; Mirzazadeh et al., 2020; El-Shall et al., 2022). The *hexon* protein, located on the virion capsid, plays a crucial role in classifying FAdVs into specific groups and types, while the fiber protein at the distal C-terminal head of the virion contains type-specific antigens (Hess, 2000; Mase et al., 2010; Zhao et al., 2016; Li et al., 2023). HHS is known by various names across different regions, reflecting its widespread impact and local nomenclature. In Pakistan, it is called "Angara disease," named after the region near Karachi, where it was first reported (Cheema et al., 1989; Kataria et al., 1997). In India, it is commonly called "Leachy" (Afzal et al., 1991; Gowda and Satyanarayana, 1994). To differentiate it from classical inclusion body hepatitis (IBH), some researchers call it "infectious hydropericardium" (IH) (Mirzazadeh et al., 2020). Additionally, it is recognized as "inclusion body hepatitis-hydropericardium syndrome" (IBH-HPS) and "hydropericardium hepatitis syndrome" (HHS) in various scientific literature.

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Traditionally, the disease is diagnosed by post-mortem examination, histological findings of the liver and other organs in the affected bird, and various serological tests (Zhao et al., 2016). However, more accurate and sensitive molecular techniques include polymerase chain reaction (PCR), Real-time PCR (RT-PCR), and restriction fragment length polymorphism (RFLP) (Meulemans et al., 2004; EL-Shall et al., 2022).

The current study aimed to evluate the outbreak of Hepatitis Hydropericardium Syndrome (HHS) in broiler farms located in the Shatra district of Thi-Qar Governorate, southern Iraq. It also sought to identify the specific serotypes of fowl adenovirus (FAdV) responsible for this outbreak, primarily focusing on its genetic characteristics and diversity.

MATERIALS AND METHODS

Ethical approval

The authors considered ethical concerns and farmers' consent before the surveys. This article was originally written without copying from other articles. All broiler chickens involved in this research were treated humanely, according to the guidelines outlined by the College of Veterinary Medicine, Baghdad University, adhering to the procedures outlined by international and national animal care, and using criteria of ethical standards defined in the 1964 Declaration of Helsinki.

Environmental conditions

This study began following a notification from a poultry farm owner in December 2023, who reported a significant loss of broilers at his farm in the Al-Shatra district of Thi-Qar Governorate, Iraq. Alarmed by the high mortality rate, the owner contacted the research team to investigate the sudden deaths of the broiler chickens. Initial observations were conducted during a comprehensive visit to the farm, spanning from December 2023 to May 2024. The farm owner reported that the mortality rate was about 65%.

During the visit, the research team meticulously assessed the farm environment. The farm consisted of three broiler houses (Breed Ross 308) situated in a rural area with a semi-arid climate, characterized by significant temperature fluctuations, reaching around 30°C between day and night, and a humidity of 60%. The farm operates under an open-house system with essential ventilation and temperature control measures. The broiler chickens are raised on deep litter bedding, and the farm follows a standard feeding regimen, utilizing commercially available poultry diet plate fodder that contains 22% starter protein and has a metabolizable energy content of about 3200 kcal. Routine management practices at the farm include vaccinations against common poultry diseases. At one day old, the chicks receive the Newcastle Disease Virus (NDV) vaccine, Clone, and Ma5 IB (Inervet, Netherlands). At five days old, they are injected with the Avian Influenza virus (AIV Hg) and Newcastle disease virus (NDV) Lasota killed vaccine (Vaxxon, Italy). The Newcastle disease (ND) Lasota vaccine is administered again at eight days of age through drinking water. Finally, on day 14, the chicks receive the Infectious Bronchitis Disease (IBD) vaccine D78 (Intervet, Netherlands) via drinking water. Periodic health check-ups and regular cleaning and disinfection of the poultry houses are also conducted.

Clinical signs

The clinical signs observed in the affected broilers included depression, ruffled feathers, and a tendency to huddle in corners before death. The droppings were discolored, varying from yellowish to greenish, and the chickens exhibited lethargy and elevated body temperatures. These observations, combined with a high mortality rate, necessitated further investigation through post-mortem examinations and molecular diagnostic techniques to identify the causative agent of the outbreak (Chen et al., 2019).

Post-Mortem examination

Post-mortem examinations adhered to the protocols described by Kalai (2024) with additional precision to ensure comprehensive analysis. The external examination involved a detailed inspection for signs of trauma, external parasites, or abnormalities in the plumage and skin. A systematic approach was followed, opening the body cavity to expose the thoracic and abdominal organs. Standard dissection instruments, including scalpels, forceps, and scissors, were used. The organs were inspected in situ for any gross lesions before being removed for further examination. Particular attention was given to the liver, heart, lungs, kidneys, and gastrointestinal tract.

Sample collection

Ten tissue samples from the liver and heart of affected broiler chickens were collected and divided into two parts. Part 1 was used for molecular techniques, and Part 2 was fixed in 10% neutral buffered formalin for histopathological analysis.

Histopathology

The liver and heart samples of the affected chickens were processed with care for the histological examination. The samples were fixed in 10% neutral buffered formalin for 24 hours. After fixation, the tissues were dehydrated through a graded series of ethanol, cleared in xylene, and then embedded in paraffin wax. Paraffin blocks were sectioned to a thickness of 5 µm using a rotary microtome. The sections were stained using the hematoxylin and eosin (H and E) protocol (Hess, 2000). Hematoxylin staining was performed for 5 minutes, followed by differentiation in acid alcohol, and then rinsed in alkaline tap water. Eosin staining was carried out for 2 minutes. After staining, the sections were dehydrated, cleared, and mounted with coverslips. Any deviations from the standard H and E protocol were carefully noted to ensure consistent staining quality. The prepared slides were examined under a light microscope (Olympus, Japan) at a specified magnification to identify and evaluate the presence and extent of lesions. The detailed histopathological analysis aimed to detect cellular and tissue-level abnormalities indicative of the pathological condition (Gallina et al., 1973).

Molecular technique

A polymerase chain reaction (PCR) assay was developed following the protocol outlined by Ganesh et al. (2000) to detect fowl adenovirus (FAdV) associated with Hepatitis Hydropericardium Syndrome (HHS). According to the manufacturer's instructions, viral DNA was purified from infected liver tissues using the KYLT RNA/DNA Purification Kit (Anicon, Germany). Primers targeting the *hexon gene* of FAdV were designed based on previously published sequences (Werner et al., 2024).

PCR protocol

The PCR was conducted using a thermocycler (Applied Biosystems™ Veriti™ 96-Well Thermal Cycler, USA) with the following cycling conditions, thirty cycles at 90°C for 30 seconds, 50°C for 30 seconds, and 72°C for 30 seconds. The target products were detected using specific *hexon* gene primers and subsequently subjected to DNA sequencing. The results of nucleotide sequencing confirmed the nature of the product (Liu et al., 2016; Werner et al., 2024).

Controls and replicates

To ensure reliability, each PCR assay included positive and negative controls. The positive control consisted of known FAdV DNA, while the negative control utilized nuclease-free water. all PCR reactions were performed in triplicate to ensure consistency and reproducibility of the results.

Detection of virus DNA

The PCR products were analyzed using agarose gel electrophoresis, which confirmed the specific *hexon* gene by comparing the band patterns with a molecular weight marker. The amplified products were then purified and subjected to DNA sequencing using the Sanger sequencing method on an ABI 3730xl DNA Analyzer (Applied Biosystems). The nucleotide sequencing results were analyzed to verify the specific nature of the amplified products, ensuring accurate identification of the fowl adenovirus associated with HHS (Liu et al., 2016).

RT-PCR for variant identification

Hexon gene-specific and variant-specific RT-PCR assays were conducted according to the protocols provided by AniCon Labor GmbH (AniCon Labor GmbH, Muehlenstr. Hoeltinghausen, Germany). Hybridization probes specific to HHS-FAdV, *Hexon* A, *Hexon* B, and *Hexon* C were utilized, following the instructions for the Kylt® HHS-FAdV assay (Liu et al., 2016; Werner et al., 2024).

Variant identification

To identify and detect variant mutations and single nucleotide polymorphisms (SNPs), DNA sequences were analyzed using bioinformatics tools and software recommended in the protocol (Yamaguchi et al., 2022; Werner et al., 2024). The sequencing data were aligned and compared with reference sequences to identify any mutations or variations. The sequences of the new strain were validated and deposited in the GenBank database, which is accessible through the National Center for Biotechnology Information (NCBI, Bethesda, MD20894, USA). This comprehensive approach ensured the accurate detection and characterization of FAdV variants, providing valuable insights into the genetic diversity and epidemiology of the virus (Liu et al., 2016).

RESULTS

Clinical signs

The research team identified several factors that may have contributed to the outbreak, including high broiler chicken density, potential breaches in biosecurity protocols, and the possible introduction of the virus through contaminated feed or equipment. The mortality rate reached about 60%; the affected broiler chickens exhibited a range of clinical signs indicative of severe illness, with symptoms progressing consistently across the observed population, though some variability in severity was noted. Elevated body temperatures were observed, suggesting a systemic inflammatory response or infection. The chicken's feathers appeared fluffy and unkempt, a common sign of poor health or systemic disease. The droppings were notably yellowish-green and phosphorescent, indicating possible liver dysfunction or biliary involvement. The comb and wattles of the affected chickens were pale, signifying anemia or circulatory compromise. Many chicks were found dead while lying on their stomachs, a posture suggesting respiratory distress or sudden cardiac failure. Difficulty breathing was noted, attributed to the fluid-filled sac around the heart (pericardial effusion), which likely compromised cardiac function and contributed to the respiratory difficulties observed (Choi et al., 2012; Chen et al., 2019).

Post-mortem

Post-mortem examinations revealed several distinctive pathological findings that provided significant insights into the disease process. Externally, a notable turmeric-yellow discoloration was observed in the dividing lines between the pectoral muscles and the abdominal cavity (Figure 1). The broiler duodenal loop showed multifocal necrosis in the pancreas. Additionally, the subcutaneous fat exhibited a dark yellow hue, indicating jaundice or extensive lipid deposition (Figure 1a). Internally, significant congestion and an increase in the thymus were observed, suggesting an immune response or inflammation. Sporadic hemorrhagic spots were present in the leg and chest areas, indicative of vascular damage or coagulopathy. The livers of the infected chickens were markedly enlarged, and clear, yellow-colored fluid was present in the pericardial sac (Figures 1b, c). A yellow, watery sac was found surrounding the heart within the endocardium, which initially appeared as a yellow liquid and later turned gelatinous in the advanced stages of infection, impairing cardiac function (Figure 1d).

Necrotic foci were evident in the pancreas, indicating localized cell death likely due to viral infection or secondary complications. The spleen was pale and enlarged, with signs of congestion in the surrounding intestines, suggesting splenomegaly and an active immune response (Figures 1-4). The kidneys were also enlarged and pale, indicative of renal compromise or infection. Blood spots were observed in the duodenal area, pointing towards gastrointestinal involvement and possible hemorrhagic enteritis. These detailed post-mortem findings provided crucial insights into the pathophysiological changes occurring in the affected chickens, highlighting the systemic nature of the infection and its impact on multiple organs.

Histopathological findings

Histopathological examination of liver samples from infected chickens revealed significant pathological changes, providing crucial insights into the disease's impact on cellular structures. Liver tissue sections were stained using the hematoxylin and eosin (H & E) technique, a standard method for highlighting cellular and tissue morphology. These sections were then examined under a light microscope at a magnification of X400 to allow detailed observation of cellular changes.

The findings showed numerous necrotic foci within the liver parenchyma, indicating areas where hepatocytes had died due to the infection (Figure 2). This necrosis disrupted normal liver function, suggesting an advanced stage of disease progression. Additionally, basophilic intranuclear inclusion bodies were prominently observed within the hepatocytes. These inclusions, stained dark blue to purple, indicate viral replication, confirming the presence of a viral pathogen actively infecting the liver cells. Furthermore, the examination revealed blue and red corpuscles within the nuclei of hepatocytes. These corpuscles represent abnormal nuclear structures and inclusions, signifying severe cellular distress and disruption of normal nuclear functions. The presence of these nuclear inclusions and necrotic areas are hallmark features of viral hepatitis, suggesting an aggressive and extensive viral infection. These histopathological findings underscore the severity of the liver damage caused by the infection, highlighting the aggressive nature of the disease and its profound impact on the affected broiler chickens. The extensive necrosis and the presence of intranuclear inclusion bodies are critical indicators of the pathological processes at play, providing essential information for understanding the disease mechanism and progression.

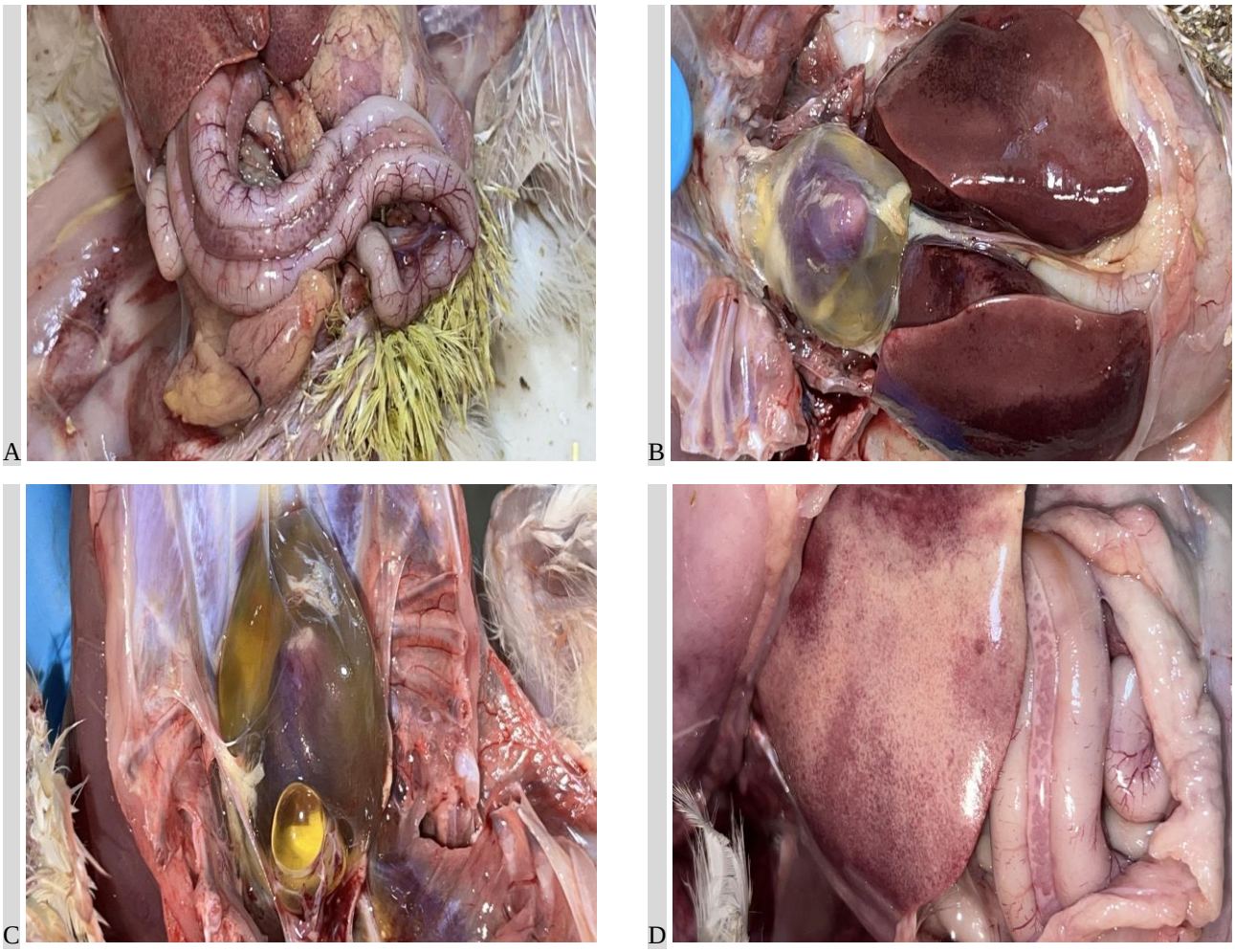

Figure 1. Gross lesions in 24-day-old broiler chickens affected by hepatitis hydropericardium syndrome. **A:** The broiler duodenal loop shows multifocal necrosis in the pancreas. **B:** Enlargement of the liver of infected chicken and the accumulation of clear, yellow-colored fluid in the pericardial sac. **C:** A yellow watery sac surrounding the heart within the endocardium in a bird affected by HSS. **D:** Enlarged spleen and congested intestines in the affected broiler chickens.

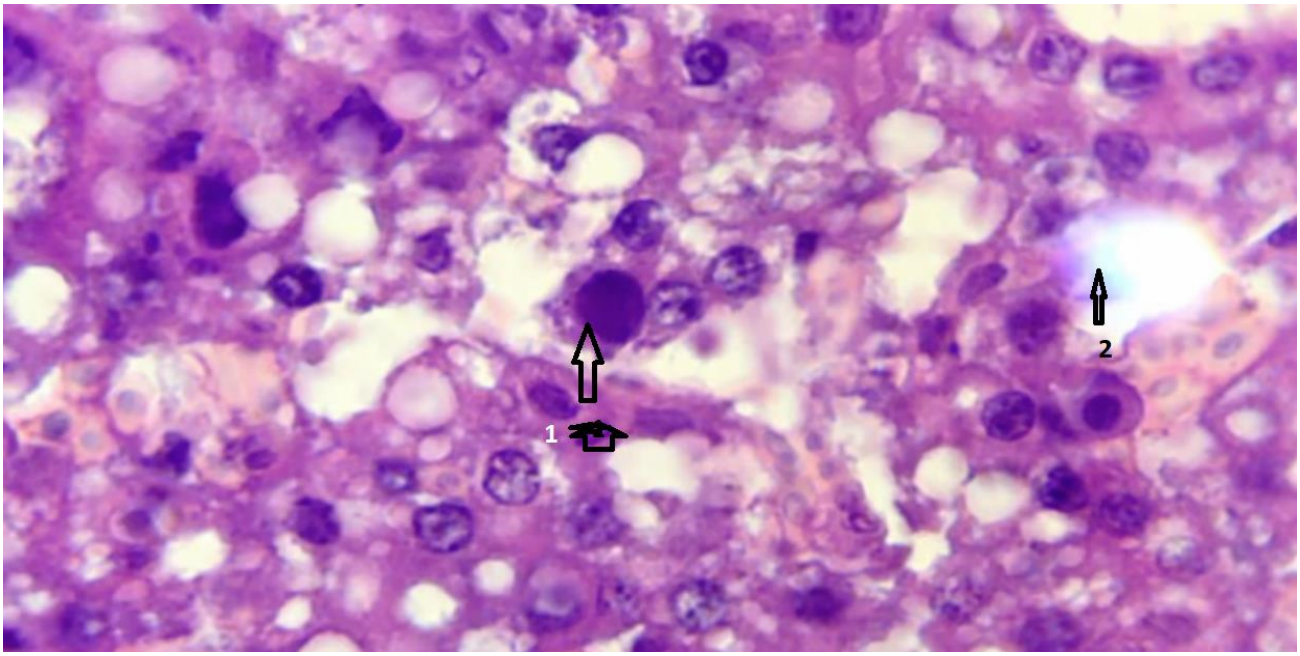

Figure 2. The liver of an affected broiler chicken with hepatitis hydropericardium syndrome at 24 days of age. The numerous basophilic intranuclear inclusion bodies in the hepatocytes. (**1**) Small multifocal areas of necrosis (**2**). Blue and red corpuscles within the nuclei of hepatocytes are also observed (H and E stain, magnification X400).

Molecular techniques

Liver samples from affected broilers collected from the farm located in the Al-Shatra district tested positive for HHS adenovirus using the polymerase chain reaction (PCR) technique.

PCR protocol

The PCR protocol followed a meticulous step-by-step process to ensure the accuracy and reliability of the results.

All ten samples taken from the affected liver were positive. Each PCR run included both positive and negative controls. While the positive control used known HHS adenovirus DNA, the negative control used nuclease-free water instead of DNA. Each sample and control were run in triplicate to ensure the reproducibility and reliability of the results. The PCR products were analyzed using agarose gel electrophoresis, which confirmed the presence of the specific *hexon* gene fragment at approximately 580 bp (Figure 3).

DNA sequences

The PCR products were then subjected to DNA sequencing to confirm the identity of the adenovirus. The sequencing was performed using the Sanger sequencing method on an ABI 3730xl DNA Analyzer (Applied Biosystems). The complete genomic sequences of FAdV-4 were analyzed and deposited in the GenBank database under accession number PP129627 (Figure 4).

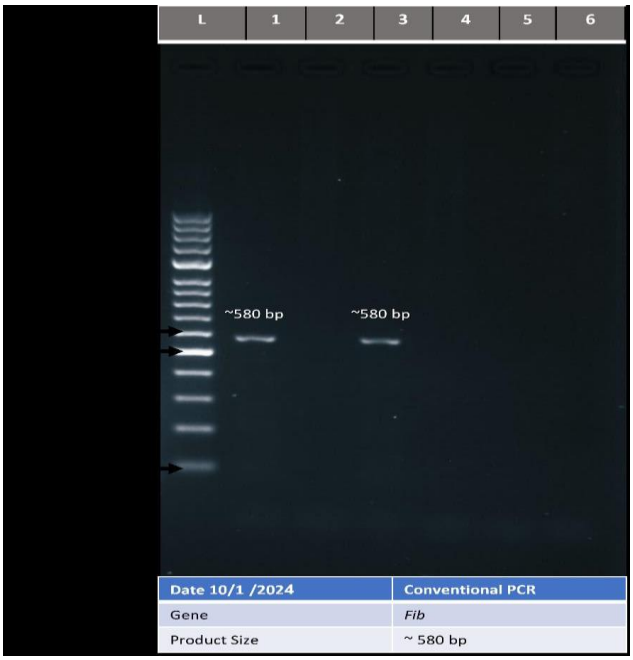

Figure 3. Conventional PCR test of liver tissue from broiler chickens infected with adenovirus. The size of the fragment is approximately 580 bp.

ORIGIN
1 cgacagaaaa ggctcaacgg ctgcaaatcc gcttttaccc catccaaacc gacgacacgt
61 cgacgggcta ccgcgtgcgg tacaacatca atgtgggcga cggttgggtc ctggacatgg
121 ggtcgaccta tttcgacatc aagggaatcc tagaccgagg gccgtccttc aagccctact
181 gcggcacggc ttacaacccg ctggctccca aggagtccat gtttaacaac tggtcggaga
241 cggcacccgg gcagaacgtg tccgcctccg gtcagctgtc caacgtctat accaacacga
301 gcacctccaa agacacgacg gcggcgcagg tgacgaagat ttccggcgtc ttccccaatc
361 ccaaccaggg acccggaaga aatcctctgc gacgggtaga aaacgccaac accggcgtgc
421 tcggtcgctt cgccaagtct cagtacaatt acgcttacgg tgcctacgtc aagcccgtcg
481 ccgccgacgg ttcccagtcc ctcacgcaga ccccctactg gatcatggat aacacgggca
541 ccaattacct gggagcggtg gccgtcgagg actacaccaa cagcctctcg tacccagata
601 ccatagtcgt gccgcctccc gaggactacg acgattataa cataggcacc acgcgtgcgc
661 tcaggcccaa ctacatcggg ttcagggata acttcattaa cctgctgtat cacgactccg
721 gcgtgtgctc gggcaccctc aactcggagc gttcgggcat gaacgtggtg gtcgagctgc
781 ccgaccggaa taccgagctc agctaccagt acatgctggc

Figure 4. Gene sequences used in NCBI-based BLAST searches identify Fowl Adenovirus C from Ross 308 chickens affected by Hepatitis Hydropericardium Syndrome.

DNA sequencing

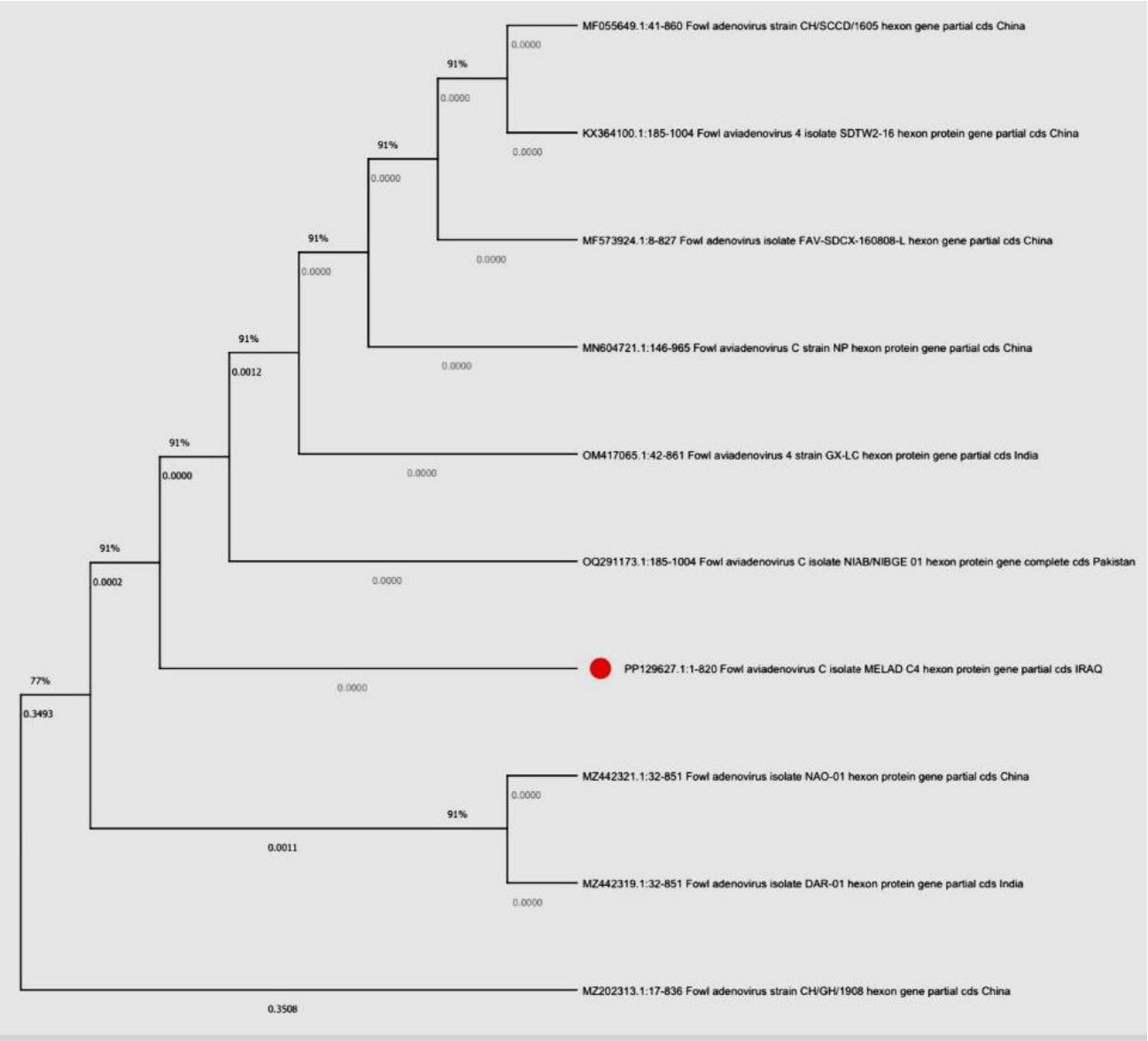

Figure 5. Sequence analysis of the new strain using NCBI-based BLAST search confirmed the identification of Fowl Adenovirus C in Ross 308 chickens as the causative agent leading to hepatitis hydropericardium syndrome.

Analysis results and validation

The nucleotide sequences of the two-flank regions, Fib F and Fib R, were analyzed, revealing two sequences with molecular weights of 6011 and 6287, respectively. The guanidine-cytosine (GC) content for both fragments was determined to be 60%. The sequencing data were cross-checked with existing sequences in the GenBank database using the NCBI BLAST tool to confirm the identity of the virus. This analysis confirmed that the causative agent of HHS in the Al-Shatra district was adenovirus serotype 4 type C, aligning with the gene sequencing data provided by the National Center for Biotechnology Information (NCBI). The samples were identified as Fowl Adenovirus C, supporting the conclusions of other studies, particularly those referencing the Fowl Aviadenovirus C isolate MELAD *hexon* protein gene and partial coding sequences (Figure. 5; Liu et al., 2016). The detailed analysis and validation steps ensured the accuracy and reliability of the molecular diagnosis, providing a comprehensive understanding of the genetic makeup and variation of the adenovirus strain involved in the outbreak.

DISCUSSION

Hydropericardium hepatitis syndrome (HHS) is a fatal disease caused by fowl adenovirus serotype 4 (FAdV-4, Figure 5). The current study unequivocally confirms that HHS is a lethal infectious disease caused by FAdV-4, affecting broilers

aged 24 days on farms located in southern Iraq. This finding is consistent with global reports of HHS outbreaks, highlighting the widespread nature of the disease with no specific geographic limitations (Cheema et al., 1989; Afzal et al., 1991; Asrani et al., 1997; Philippe et al., 2005; Choi et al., 2012; Liu et al., 2016; Niczyporuk, 2016; Cui et al., 2020; Mirzazadeh et al., 2020; El-Shall et al., 2022; Yamaguchi et al., 2022). In Iraq, the disease has been well-documented since the 1990s (Abdul-Aziz and Al-Attar, 1991; Abdul-Aziz and Hassan,1995; Abdulrahman et al., 2022; Al-Taee and Saeed, 2022; Oraibi and Abdalmaged, 2022) and continues to pose a significant threat, with recent cases consistently attributing the causative agent to FAdV-4 (Ye et al., 2016; Li et al., 2017; Mete et al., 2021). The virulence of HHS appears to have increased over time, a trend that the current study substantiates, with reported mortality rates reaching up to 65%. Post-mortem examinations revealed classic HHS lesions, including those depicted in Figures 1, 2, and 3, which are consistent with the findings of other researchers (Naeem et al., 1995; Asrani et al., 1997). This study also emphasizes critical issues by detecting a novel strain responsible for the ongoing epidemic in Iraq. Adenoviruses (FAdVs) may have undergone significant genetic changes over the years through multiple passages, resulting in the emergence of highly virulent strains. The genome of FAdV is composed of double-stranded DNA, encapsulated by protein coats that include two specific proteins, hexon, and fiber (Zhao et al., 2016; Sharif et al., 2020). The *hexon* protein, which houses genes responsible for pathogenicity, is located at the end of the genome, while the central region of the genome shows conserved organization among different FAdVs. Notably, most mutations occur at the genome ends, which are crucial in distinguishing the five genera of the virus (Mittal et al., 2014; Zhao et al., 2016; Niczypouk, 2016; Sharif et al., 2020). Pallister et al. (1996) observed that variations in FAdV virulence could be attributed to a single gene. Ye et al. (2016) reported numerous deletions in serotype 4 FAdV, while Li et al. (2017) identified ORF19 deletions in the FAdV-4 HB 1510 strain as investigated by NBIC.

Furthermore, Yamaguchi et al. (2022) and Chen et al. (2019) indicated that serotype 4 FAdV isolates with truncated ORF19 displayed higher virulence than other isolates, particularly those in Japan and China. These findings hold significant implications for poultry health management, emphasizing the necessity for targeted intervention strategies by avian disease specialists to mitigate the impact of the disease.

Routine monitoring of HHS is necessary because adenovirus infections can severely compromise the efficacy of vaccinations for other poultry diseases, such as Newcastle disease (ND), influenza, infectious bursal disease, and infectious bronchitis. This interference is likely due to the destruction of lymphoid tissues and the induction of immunosuppression, either directly or indirectly (Nakamura et al., 1999; Mase et al., 2010; Yan et al., 2020). Additionally, co-infections with avian Ortho reoviruses (ARVs) have been shown to enhance FAdV-4 replication in specific pathogen-free (SPF) chickens, resulting in significant changes in cytokine levels in co-infected groups compared to those with single infections (Nakamura et al., 1999; Tian et al., 2020). Another critical consideration is the presence of adenoviruses in apparently healthy chickens. The isolation of adenovirus from such broiler chickens in China suggests that the virus can circulate asymptomatically within the host, only to become activated and mutate into more virulent forms under certain conditions (Yan et al., 2020; De Luca et al., 2022). This phenomenon supports the hypothesis that new, more virulent strains may emerge through deletion processes at the genome ends, significantly affecting the *hexon genes* and thus causing more severe disease outbreaks.

Meanwhile, the current study underscores the increasing virulence of FAdV-4 and its substantial impact on poultry health. This finding necessitates ongoing vigilance and advanced research into adenovirus mutations and their epidemiological trends. Such efforts are essential for developing effective strategies to control and prevent HHS, safeguarding poultry populations from this devastating disease. The current findings align with global reports, emphasizing the widespread and escalating threat posed by this adenovirus strain. Molecular genetic analysis revealed notable mutations and deletions in the ORF19 region associated with increased virulence. The finding supports the hypothesis that FAdVs undergo significant genetic modifications over time, leading to the emergence of more virulent strains. The identification of the Melad strain of FAdV-4, unique to this study, underscores the ongoing evolution of the virus and its implications for poultry health management. The study highlights the need for continuous monitoring and advanced research into FAdV mutations and their epidemiological trends. Comprehensive post-mortem, histopathological, and molecular examinations elucidated the severe pathological impact of FAdV-4 on affected broiler chickens, characterized by extensive hepatic necrosis, basophilic intranuclear inclusion bodies, and gelatinous fluid accumulation within the pericardium.

While this study provides significant insights, certain limitations should be noted. The geographic scope is confined to broiler farms in southern Iraq, which may only partially be representative of other regions. The sample size, although significant, may not capture the total genetic diversity of FAdV strains present. The study spans from December 2023 to May 2024, offering a limited temporal snapshot of the impact of the disease and the genetic evolution of the virus. While the focus on FAdV-4 is essential, the potential role of co-infections and other adenovirus serotypes warrants further exploration. However, this study underscores the need for vigilant monitoring, advanced research, and tailored

intervention strategies to combat HHS in poultry. By addressing these challenges, the poultry industry can better safeguard against the devastating impacts of this disease, ensuring healthier flocks and more stable production outcomes.

CONCLUSION

This study confirmed the incidence and outbreak of HHS in broiler farms in southern Iraq, attributing the causative agent to fowl adenovirus serotype 4 (FAdV-4). The findings highlight the significant virulence of the disease, resulting in mortality rates reaching up to 65%. Future research should focus on developing region-specific vaccines tailored to the genetic makeup of prevalent FAdV strains, implementing stricter biosecurity protocols to prevent the introduction and spread of FAdVs, conducting long-term studies to track the evolution of FAdV strains and their impact on poultry health, and integrating disease management strategies combining vaccination with improved management practices.

DECLARATIONS

Finding

This study received no financial support.

Availability of data and materials

All data from the current study are available in this article.

Authors' contributions

Melad Ibrahim Oraibi, Majid Hagi Khaleel, and Amer Abdulameer A. Al-Baldawi conceived the idea, developed the theory, and performed the computations. Majid Haqi Khaleel and Amer Abdulameer A. Al-Baldawi verified the analytical methods. All authors are involved in laboratory work. All authors read and approved the final version of the manuscript.

Competing interests

The authors declare that they have no conflicts of interest.

Ethical considerations

The authors considered ethical concerns and farmers' consent before conducting the surveys. This article was originally written without copying from other sources and submitted to this journal for the first time.

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