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Scientific Report

Hepatitis-hydropericardium syndrome in native chickens: A clinicopathological study

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Abstract

Background: Hepatitis-Hydropericardium syndrome (HHS), a significant disease in poultry, is caused by fowl adenoviruses (FAdV). While common in commercial flocks, HHS has not been previously documented in native chicken breeds in Iran. **Case description:** This case report describes HHS in a small backyard flock of native hens in Mashhad, Iran. The affected birds, three 6-month-old hens, exhibited lethargy for one day prior to death. **Findings/treatment and outcome:** Post-mortem examination revealed hydronephrosis, splenomegaly, hydropericardium, and hepatic necrosis. Histopathological examination showed significant lesions in the liver, heart, kidney, and spleen. The liver displayed congestion, hemorrhage, hepatocellular degeneration, necrosis, lymphocytic hepatitis, and large basophilic intranuclear inclusion bodies in the affected hepatocytes, confirmed by Lendrum's phloxine-tartrazine method. Cardiac lesions included pericarditis, myocarditis, edema, and myofiber degeneration and necrosis. The spleen exhibited congestion and hemorrhage, while renal tissues showed tubular epithelial cell degeneration and necrosis with inflammation. PCR assay on the liver samples confirmed FAdV serotype 4. **Conclusion:** These findings show HHS and a FAdV serotype 4 etiology, highlighting the emerging threat of adenoviral diseases to noncommercial poultry settings in Iran.

Key words: Hepatitis-hydropericardium syndrome, Histopathology, Inclusion bodies, Iran, Native chicken

Introduction

Fowl adenoviruses (FAdVs) are double-stranded DNA viruses that commonly infect chickens globally (Shamim *et al*., 2009; Yasmeen *et al*., 2017; Oraibi *et al*., 2024). These pathogens can produce a range of disease depending on the viral strain, host factors, and environmental cofactors (Yu *et al*., 2018; Alavizadeh *et al*., 2024; Oraibi *et al*., 2024; Ghorani *et al*., 2025). Three major FAdV-associated diseases are inclusion body hepatitis (IBH), hepatitis-hydropericardium syndrome (HHS), and adenovirus gizzard erosion (AGE) (Li *et al*., 2017). IBH primarily affects young broiler and layer chickens from three to seven weeks old, causing moderate mortality. Gross lesions in this disease include hemorrhages and necrosis in the liver and kidneys (Qiao *et al*., 2024). HHS, known as hydropericardium syndrome or Angara disease, manifests in acute death accompanied by hepatitis, hydropericardium, and nephritis with mortality often exceeding 50% in affected flocks (Niczyporuk *et al*., 2021; Alavizadeh *et al*., 2024). AGE leads to erosion and hemorrhage of the gizzard with no marked internal lesions (Schade *et al*., 2013). FAdVs can spread both vertically and horizontally within poultry populations, with fecal matter containing the highest viral titers (Mirzazadeh *et al*., 2021).

While FAdV infections are well-documented in commercial broiler and layer operations (Hosseini *et al*., 2021; Oraibi *et al*., 2024; Sun *et al*., 2024), their incidence and impact in backyard small holding poultry flocks is unclear. In recent years, there has been a concerning increase in the incidence of IBH cases across various regions, including Iran (Morshed *et al*., 2017; Alavizadeh *et al*., 2024; Najafi *et al*., 2024; Ghorani *et al*., 2025). This trend highlights the need for further studies to understand the prevalence and economic impact of FAdV infections in both commercial and non-commercial poultry flocks. Here, we reported the first known cases of HHS probably associated with a FAdV infection in native chickens in Iran.

Case description

Three 6-month-old native hens from a small backyard flock in Mashhad were presented for necropsy at the veterinary hospital of Ferdowsi University of Mashhad, Iran, in May 2021. According to their owner, the hens exhibited lethargy for one day prior to death. During

necropsy, the carcasses displayed hepatic necrosis with pale foci, hydropericardium (with no right ventricular hypertrophy) (Fig. 1), hydronephrosis, and splenomegaly. Tissue samples from the liver, heart, kidney, and spleen were collected for histopathological examination. The tissue samples were fixed in 10% neutral buffered formalin solution and routinely processed. The paraffin-embedded samples were cut into 5 µm thick sections and finally stained with hematoxylin and eosin (H&E) for further histopathological evaluation. Moreover, the FAdV serotype 4 *hexon* gene was detected using a PCR assay on the liver samples and specific primers (FAdV-F: 5´-CGA ATA CCT CTC TGA GGA C-3´ and FAdV-R: 5´-CTG GGT AGC GCT GAT ATC G-3´), based on the work conducted by Yin *et al*. (2020).

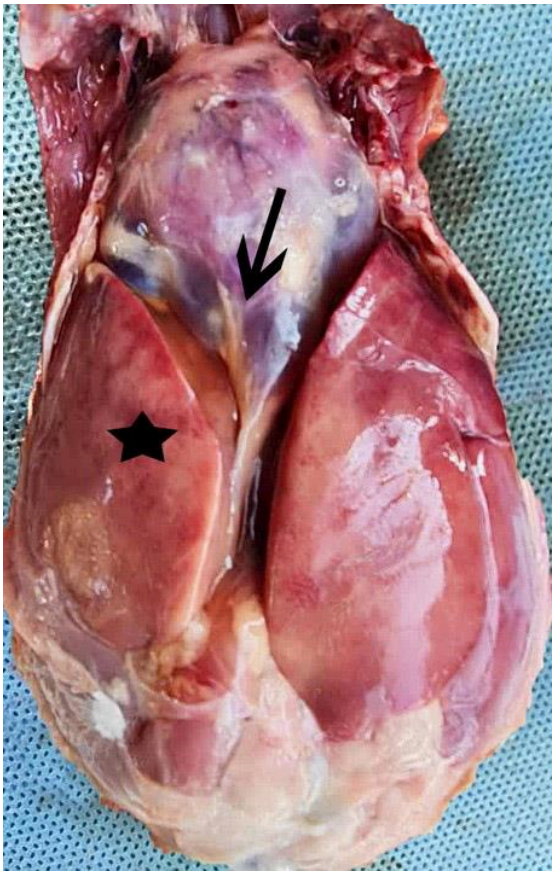

Fig. 1: A straw-colored fluid in the pericardial sac (arrow) with pale necrotic foci in the liver (asterisk) is shown in the native hens

Findings/treatment and outcome

Microscopic examination of the liver revealed congestion, hemorrhage, hepatocellular degeneration and necrosis, hepatitis characterized by infiltration of mononuclear inflammatory cells, particularly lymphocytes, as well as large intranuclear basophilic inclusion bodies probably indicative of an adenovirus infection (Fig. 2a-d). In addition, to confirm the intranuclear inclusion bodies in hepatocytes, Lendrum's phloxine-tartrazine method was performed (Lendrum, 1947), and the presence of the red-colored intranuclear bodies were observed and confirmed in hepatocytes of the liver tissue (Fig. 2d).

In the heart, there were pericarditis and myocarditis with a mild infiltration of mononuclear inflammatory cells. Congestion, edema with separation of myofibers, and degeneration and necrosis of cardiac myofibers, along with fragmentation of muscle fibers were also present (Fig. 3a and b). Interestingly, a large intranuclear basophilic inclusion body was seen in cardiac myocyte (Fig. 3a).

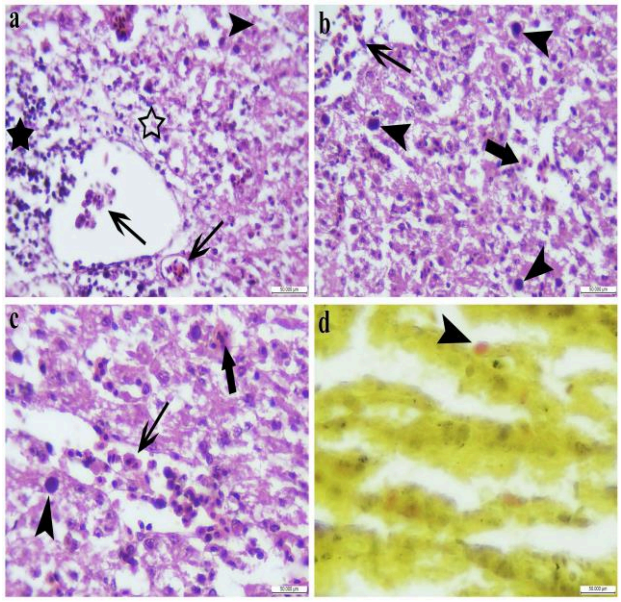

Fig. 2: Photomicrographs of the liver in the native hens. Congestion (thin arrow) and disruption of the liver structure showing hepatocellular degeneration and necrosis (empty asterisk) with infiltration of mononuclear inflammatory cells (asterisk) are observed (**a**), besides congestion (thin arrow) and hemorrhage (thick arrow), large intranuclear basophilic inclusion bodies (arrowheads) are seen in hepatocytes (**b** and **c**), (H&E, scale bar, 50 µm for **a-c**), Micrograph showing red-colored intranuclear inclusion bodies (arrowhead) in hepatocytes (**d**), (Lendrum's phloxine-tartrazine method, scale bar, 50 µm)

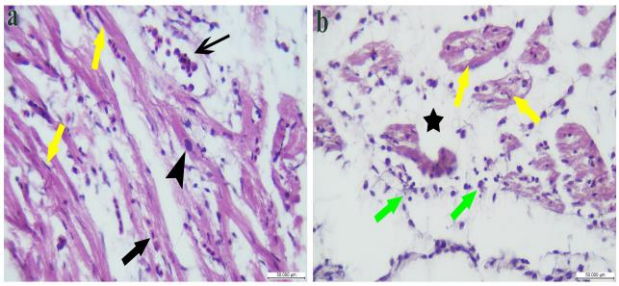

Fig. 3: Micrograph of the heart illustrating congestion (thin arrow), hemorrhage (thick arrow), necrosis of cardiac myocyte with loss of cross striations (yellow arrows), and a large intranuclear basophilic inclusion body in myocyte (arrowhead) (**a**), representative heart sections showing myofiber separation because of edema (asterisk), necrosis and fragmentation of the myofibers (yellow arrows), and infiltration of mononuclear inflammatory cells (green arrows) (**b**), (H&E, scale bar, 50 µm for both)

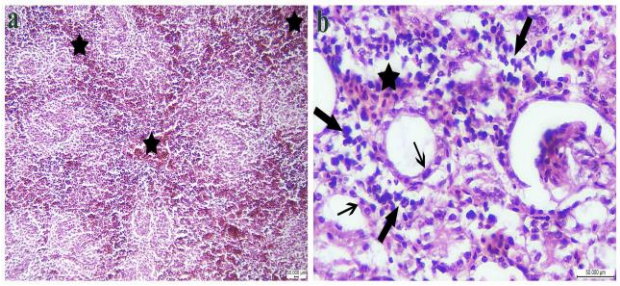

Fig. 4: Photomicrograph of the spleen showing congestion and hemorrhage (asterisks) (**a**), and congestion, hemorrhage (asterisk), and necrosis of tubular epithelial cells (thin arrows) with mononuclear inflammatory cell infiltration (thick arrows) are seen in the kidney of the native hens (**b**) (H&E, scale bar, 50 µm for both)

The histopathological examination of the spleen revealed congestion and hemorrhage (Fig. 4a). Renal lesions included congestion, hemorrhage, degeneration and necrosis of tubular epithelial cells, and infiltration of mononuclear inflammatory cells, as well as atrophy of the glomeruli with an increased urinary space in the renal corpuscles (Fig. 4b). No gizzard erosions were observed. The histologic findings, coupled with the gross lesions, were highly suspicious of HHS caused by a fowl adenovirus.

Moreover, the PCR analysis showed that the virus identified from the liver samples was FAdV serotype 4.

Discussion

This report describes the first suspected cases of HHS associated with a fowl adenovirus infection in native chicken breeds in Iran. While FAdV outbreaks have been reported in commercial broiler and layer flocks in Iran previously (Hosseini *et al*., 2021; Ghorani *et al*., 2025), this appears to be the first such case in noncommercial backyard poultry. In 2021, the first cases of HHS affected by FAdV serotype 4 were confirmed in commercial poultry flocks of Northeastern Iran (Toroghi *et al*., 2022). Then, several outbreaks were reported in poultry flocks of this region. Simultaneous detection of HHS in commercial and backyard flocks of this region highlights the importance of surveillance efforts to detect and control the disease. The clinical course and postmortem findings in the present study aligned closely with descriptions of HHS in prior literature (El-Shall *et al*., 2022; Najafi *et al*., 2024). HHS typically manifests as an acute disease with high mortality in older chickens over 3-4 weeks (El-Shall *et al*., 2022). However, in our cases, the affected hens were considerably older, at 6 months, which is an important observation. The occurrence of HHS in 6-month-old native hens is noteworthy, as it shows that older birds in non-commercial settings can also be susceptible to this disease. The principal gross lesions of hydropericardium, hepatitis with hemorrhage and necrosis in the liver, and nephritis were present in these native hens.

It is worth highlighting that our findings align with a case report of HHS in a backyard chicken flock in California, as described by Mete *et al*. (2021). Their report documented the emergence of FAdV serotype C-4 in a mixed flock of adult chickens. Similar to our cases, the California outbreak affected older birds, with mortality occurring in chickens aged 6 months to 2 years. The postmortem examination in both studies revealed liver lesions, including hemorrhage and necrosis. Histopathological findings were also comparable, with hepatic necrosis and hepatocytes containing large, mostly basophilic, intranuclear inclusion bodies observed in both instances.

Histopathological examination revealed significant lesions consistent with HHS. In the liver, we observed congestion, hemorrhage, hepatocellular degeneration and necrosis. Notably, lymphocytic hepatitis characterized by infiltration of mononuclear inflammatory cells was evident. The presence of large basophilic intranuclear inclusion bodies in hepatocytes is pathognomonic for avian adenoviral infection (Dar *et al*., 2012; El-Shall *et al*., 2022). These inclusions were clearly visible in our samples, strongly showing an adenovirus etiology. They were confirmed by Lendrum's phloxine-tartrazine method, showing red-colored inclusion bodies in a yellow background (Lendrum, 1947). Our observation of predominantly basophilic inclusion bodies also aligns with the findings of Dutta *et al*. (2017), who reported similar basophilic inclusions in their study of HHS in broiler chickens. This contrasts with the reports by Nakamura *et al*. (2002, 2011), who noted that eosinophilic inclusion bodies were more common in their investigations. This discrepancy in inclusion bodies might be attributed to differences in viral strains, host factors, or the stage of infection at which the samples were collected (Fahnert *et al*., 2004).

Cardiac lesions included pericarditis and myocarditis with mild mononuclear cell infiltration. We also noted congestion, edema, degeneration and necrosis of cardiac myofibers, along with fragmentation of muscle fibers. These findings are consistent with the cardiovascular involvement typically seen in HHS (Nakamura *et al*., 2002, 2011; El-Shall *et al*., 2022). Renal histopathology revealed congestion, hemorrhage, degeneration and necrosis of tubular epithelial cells, and mononuclear cell infiltration. Atrophy of glomeruli with increased urinary space in the renal corpuscles was also observed. These renal lesions further support the diagnosis of HHS, as kidney involvement is a hallmark of the disease (Niu *et al*., 2018; Ren *et al*., 2019).

While virus isolation was done in this case, the constellation of clinical signs, gross lesions, and histopathologic findings indicated a fowl adenovirus etiology. This case report is consistent with two recent studies from Iran, highlighting FAdV infections in commercial poultry. A study from Shiraz reported IBH in broilers, with necropsy and histopathological findings similar to ours, including enlarged livers and necrotizing hepatitis with basophilic intranuclear inclusion bodies without hydropericardium (Mirzazadeh *et al*., 2020). They identified FAdV-11 as the causative agent.

Hosseini *et al*. (2021) identified FAdV in broiler flocks in Golestan province, noting high sequence homology with international strains. These studies underscore the widespread nature of FAdV infections in Iran across different flocks and emphasize the need for comprehensive molecular epidemiological investigations to understand the distribution of FAdV strains in both commercial and backyard settings. This global perspective is crucial for understanding the epidemiology and potential sources of FAdV infections in backyard flocks.

Previous studies have shown that virus recovery can be challenging, especially in cases with advanced autolysis (Schachner *et al*., 2018). The use of molecular diagnostic techniques like polymerase chain reaction could have increased the diagnostic sensitivity (Li *et al*., 2017; Alavizadeh *et al*., 2024; Ghorani *et al*., 2025). Notably, while IBH outbreaks are typically associated with serotypes 6, 7, and 8 globally, the most prevalent FAdV serotypes are 11 and 8 b for IBH, and 4 for HHS in Iran (Morshed *et al.*, 2017; Alavizadeh *et al*., 2024; Sun *et al*., 2024; Ghorani *et al*., 2025). However, studies on backyard or domestic flocks are limited (Najafi *et al*., 2024). In the present cases of HHS in native chickens, we showed that FAdV serotype 4 affected non-commercial flocks.

This first documentation of HHS in native chicken breeds in Iran underscores adenoviral diseases pose a threat beyond just commercial poultry operations. Backyard and small-holding flocks may be vulnerable to the introduction of FAdV from wild birds, exposure at live poultry markets, or other sources. A study from Korea demonstrated HHS affects various poultry types, including layers, breeders, and native fowl, with mortality rates ranging from 1.3% to 11.1% across different age groups and bird species (Kim *et al*., 2008). Further epidemiological studies are needed to clarify the prevalence and economic impact of FAdV infections in noncommercial poultry flocks in Iran and other countries. Implementation of basic biosecurity measure and investigations into potential autogenous vaccine strategies may aid in controlling these viral diseases (De Luca and Hess, 2025). Continued surveillance and reporting of adenoviral cases in diverse poultry populations is vital for understanding their evolving threat.

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Conflict of interest

There is no potential conflict of interest with respect to the research, authorship, and/or publication of this article.

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