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Inclusion Body Hepatitis: A Comprehensive Overview of Disease Impact and Control in Poultry

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Abstract

INCLUSION Body Hepatitis-Hydropericardium Syndrome (IBH-HPS) is an economically significant disease affecting poultry worldwide. The causative agent of IBH-HPS is predominantly Fowl Adenovirus (FAdV), with serotypes 4, 11, and 8b being most implicated. The disease is characterized by hepatitis with intranuclear inclusion bodies and hydropericardium, leading to substantial morbidity and mortality in poultry flocks. Epidemiological studies reveal that IBH-HPS outbreaks are often associated with intensive farming practices, suboptimal biosecurity, and immunosuppression induced by co-infections (e.g., infectious bursal disease (IBDV) and chicken anemia virus (CAV) or stressors like aflatoxicosis. Vertical and horizontal transmission routes are critical in the spread of disease. Pathogenesis involves viral replication in hepatocytes, leading to necrosis, inclusion body formation, and systemic inflammatory responses. Clinically, affected birds exhibit depression, lethargy, ruffled feathers, and characteristic gross lesions including pale, friable livers with inclusion bodies and fluid accumulation in the pericardial sac. Laboratory diagnosis relies on histopathology, PCR, and virus isolation techniques to confirm FAdV presence and serotype identification. Both inactivated and live-attenuated vaccines have shown efficacy in reducing disease incidence. However, vaccination strategies must align with the circulating serotypes to ensure optimal protection. Understanding IBH-HPS requires an integrated approach addressing environmental, managemental, and immunological factors. This review underscores current knowledge, the disease's impact on poultry production, and the continued research to enhance diagnostic methods, refine vaccine formulations, and develop comprehensive prevention strategies.

Keywords: Inclusion body hepatitis, hydropericardium syndrome, IBH-HPS, Adenoviruses, Poultry.

Introduction

Hydropericardium syndrome (HPS) was first identified in broiler chickens in Angara Goth, Karachi, Pakistan in the autumn of 1987 and called Angara disease although sporadic cases were recorded as early as 1985 [1]. Soon afterward, the disease emerged in Iraq, Peru, Mexico, Chile, Slovakia, India, South Korea, and China associated with a sudden increase in mortality in healthy 3-5 weeks old flocks [2]. Pulmonary edema, and hepatitis which appear in the form of an enlarged, congested but sometimes pale liver with basophilic intranuclear inclusions inside hepatocytes and enlarged congested kidneys were detected during postmortem examination. This disease is characterized by the accumulation of a clear, straw-colored "jelly-like" fluid in the pericardium, hence the name HPS [1].

The IBH and HPS are clinically similar except the accumulation of straw-colored jelly fluid in the pericardial sac was not found in IBH. Due to the characteristic appearance of the heart, surrounded by hydropericardium, the Indian farmers called this condition "Leechi disease". Different synonyms were given to this syndrome such as hydropericardium-hepatitis syndrome and inclusion body hepatitis-hydropericardium syndrome (IBH-HPS) [1].

The IBH-HPS outbreaks have recently been reported as an economically important disease in several countries, including Egypt [3; 4]. IBH usually affects broiler chickens between 3 to 6

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weeks of age but sometimes appears in layers and breeders. IBH outbreaks cause severe economic losses represented in mortality which generally peaks within 3–4 days and ranges from 10-30% [5] but may rise to 80% in case of immunosuppressed birds especially those infected with (IBD and or CIA) [6]. During outbreaks of IBH, infected broilers exhibit non-specific signs including Poor development, apathy, prostration, ruffled feathers, and huddling behavior. In countries where it is prevalent, IBH-HPS is of considerable economic importance and is regarded as a threat to broiler production [7].

Etiology

Adenoviruses are heterogeneous members of the family *Adenoviridae* which is distributed worldwide. *Adenoviridae* has been recently classified into six genera: *Aviadenovirus (*IBH-HPS), *Mastadenovirus (*affects a wide range of mammalian species), *Atadenovirus (*Egg drop syndrome (EDS_{76}), *Siadenovirus (*hemorrhagic enteritis in turkeys, marble spleen disease in pheasants, and splenomegaly in chickens), and *Ichtadenovirus (*only affect white sturgeon fish)) with the proposed new genus *Testadenovirus* (affecting turtles and tortoises) [8].

The causative agent of IBH-HPS belongs to the *Aviadenoviruses with a* genome of 35-59kbp, linear, non-segmented dsDNA with 53–59 percent of guanosine/cytosine in DNA. The genus is divided into five species (FAdV-A to FAdV-E) based on restriction endonuclease analysis using two enzymes and 12 serotypes (FADV-1 to FADV-8a and FADV-8b to FADV-11) based on genomic restriction digest patterns, cross-neutralization tests and melting curve analysis (Figure 1) [9]. It has been shown that IBH-HPS and gizzard erosion (GE) are the most common diseases associated with FAdV infection in chickens. [10]. All the isolated FadV-12 serotypes have been associated with severe IBH-HPS outbreaks with significant economic losses. [7]. However, the most common relevant isolates associated with IBH-HPS are FAdVs species D and E including serotypes; FAV-2, -3, -11 and FAV-6, -7, -8a, -8b respectively. [9], evidence revealed that FAdVs play a role in IBH primarily belonging to the species D (FAdV-2 and FAdV-11) and E (FAdV-8a and 8b).[5; 11]. The HPS is related to group 1 aviadenovirus, species C, serotype 4 [1] (Figure 2).

The FAdV genome encodes 40 proteins that contain redundant terminal sequences with inverted terminal repeats (ITR). Terminal proteins (TP) are covalently connected to both ends of the viral DNA [12]. The adenovirus genome encodes roughly 13 structural proteins according to Russell [13]. He discovered that the viral particle's capsid is composed of three key proteins: hexon, penton base, and knobbed fiber. The hexon gene, the major capsid protein, is used for serotyping with several neutralizing epitopes and high mutation rates [14]. The remaining minor structural proteins include cement proteins (VI, VIII, IX, IIIa) and core proproteins (VI, VIII, IX, IIIb). An investigation of the FAdV protein profiles revealed 8-12 protein fractions of 13.8 to 110 kDa molecular weights with 7 immunogenic polypeptides with molecular weights ranging from15.8 to 110 kDa. Among the highly immunoreactive components, hexon, penton base, and fiber were determined using western blot analysis. During viral penetration, the penton and fiber structural capsid proteins interact with the host cell receptors [15].

Virulence factors of FAdV

FAdVs virulence factors include structural and de-novo synthesized proteins that facilitate the virus replication and immune escape and immunomodulation of the host defense [16; 17]. With the help of fiber and penton proteins, FAdVs can infect the host cell followed by preliminary interaction with the susceptible epithelial cells with the help of the coxsackie-adenovirus-receptor (CAR) [18].

These CAR receptors are widely distributed in epithelial cells which in turn manage FAdVs to infect respiratory, digestive, hepatic, pancreatic, and the bursa of Fabricius epithelial linings. Some virulence factors are restricted to certain species of FAdV such as a gene that encodes a novel 30-kDa nuclear protein that was called (GAM-1) and open reading frame (ORF-1) of FAdVs, de novo synthesized proteins in chicken embryo lethal orphan (CELO) virus. Also, adenoviral penton protein is another toxic protein that helps with viral infection and causes rounding of the cells. However, FAdV lacks this character, and further studies should be established to confirm this point. Recently, ORFs “0, 1, 1A, 1B, 1C, and 2” of FAdV-9 were identified to be involved in immune evasion and virus replication in chickens [19]. Moreover, FAdVs are characterized by persistence

within the host, and this is due to the ability of the virus to modulate cytokine responses. FAdVs are immunosuppressive by suppressing antiviral IFN-γ response via boosting IL-10 production and influencing viral pathogenesis [16].

Epizootiology and Epidemiology of IBH-HHS

Epizootiology

The extreme contagiousness and pathogenicity of IBH-HPS allow the virus's rapid spread between flocks and farms. The spreading of infection occurs both horizontally and vertically. The vertical transmission of the fowl adenovirus (FAV) from parent to offspring has been considered a key feature and shedding of the virus through eggs lasts for three to six weeks till the development of immunity [20].

Latency of Adenoviridae viruses leads to variations in the time of excretion of the virus either for breeders, which may be latently infected, and the stress of egg production causes the virus to be transmitted through eggs or vertically infected chicks which begin to excrete the virus in feces directly after hatching but more typical chicks begin to shed the virus in feces at 2-4 weeks of age after a decline of maternally derived antibodies (MDA) which in turn activates the latent infection. Contaminated hatching eggs possess the potential risk of spreading the virus between countries [9]. Horizontal transmission is another important route through which the virus spreads from one bird to another by mechanical means and through food and water contaminated with infected feces. [21]. The spreading of the virus through wild birds under natural conditions needs further studies to aid in identifying specific viral reservoirs although the role of wild birds in the disease spreading has been reported [9].

Experimental reproduction of IBH was achieved via subcutaneous inoculation of infected liver homogenate in susceptible birds. Other studies to reproduce the disease experimentally through inoculation of the birds with an infected liver homogenate via subcutaneous or oral route and direct contact with infected birds were attempted. Typical symptoms of IBH were reproduced 2-5 days after subcutaneous inoculation suggesting the possibility of reproduction of the disease experimentally [1].

Epidemiological situation

Over the past two decades, FAdVs were reported to be associated with many IBH and\ or HPS epidemics with severe economic losses globally which in turn had great attention for further research on these viruses for determination of rapid methods of diagnosis and subsequent rapid control measures.

IBH-HPS outbreaks were reported in several countries around the globe such as Alberta and Montreal, New Zealand, North America, South America, Croatia, Japan, South Africa, Iran & Middle East, Spain, Saudi Arabia, China, Brazil, Malaysia, Morocco, Pakistan, Greece, In China, in several studies throughout 2015-2018, the phylogenetic analysis of hexon sequences revealed that serotype 4 was the predominant serotype representing (79.4%) associated with IBH and or HPS outbreaks while other FAdV serotypes such as serotype 8a, 8b, 2 and 10 represent low percent (about 13.9 %), India, Poland, In California, FAdV4 was firstly isolated from a backyard flock reared as mixed chicken of different breeds and ages and United Arab Emirates [2].

In Egypt, the first IBH outbreak in Egypt has occurred in 1984 in two broiler flocks at El-Waady Elgadeed governorate which suffered from high mortality and reached 60% due to mixed infection with infectious bursal disease [22]. In 2002, another two private broiler flocks that suffered from a high morbidity of 23% and a low mortality rate of only 2% were examined and samples from dead birds were fixed and examined histopathologically at the Animal Health Research Institute at Assuit regional laboratory with the detection of basophilic inclusion bodies inside the hepatocytes [23]. From 2003 to 2015, there were no records of IBH outbreaks till Radwan et al. isolated FAdV serotype 8a during their study to isolate the circulating FAdV in commercial broiler flocks aged 35 days in Behira province suffered from IBH with mortality rates of 7-17%. During the post-mortem examination, ascites, and enlarged and fibrotic liver were detected although only severe hepatitis without mortality was recorded after inoculation of 4-day specific pathogen free (SPF) chicks orally with 10^4 $TCID_{50}$ per ml from this isolate [4].

More importantly, the immunosuppressive FAdVs character explains the frequent isolation of IBDV and CIA from the IBH outbreaks. Co-infection of FAdVs with CIA increases the severity

of IBH outbreaks. FAdV-D was detected in 7–21 days old broiler chicken flocks with 2.7–15% mortalities and all IBH-positive flocks seroconverted against CIAV [3]. Between 2017-2018, FAdV-D (serotype 2 and 11) was isolated and characterized from 17 out of 37 broiler chicken flocks with 0.4 to 16.6% mortality rate in 16 positive flocks and this percent increased up to 20% in the 17th flock due to co-infection with IBDV [24]. During 2019 and 2020, a molecular investigation to determine multiple FAdV species circulating across the country revealed the dominance of species D and serotype 8a/E FAdVs in broiler flocks than in layers across the Nile Delta governorates and the new detection of FAdV serotypes 1, 3, and 8b [25]. Additionally, serotype 2 (FAdV-2) was isolated from the livers of 7.5% broiler flocks of 3–5 weeks old showing varying rates of mortality (8–14%) and non-specific signs of depression and ruffled feathers in Sharkia governorate, Egypt [26]. Another FAdV species E serotype 8a was also isolated from 2 out 15 broiler flocks of 19–40 days old with 5–10% mortalities [27].

During 2022, FAdV serotype 2 was isolated alone without immunosuppressive agents from broiler flocks that from increased mortalities and poor performance in Assuit province [28]. From January 2021 to March 2022 (Beheira and Menofia governorates, Egypt,) fowl adenoviruses species D serotype 2/11 was reported to be the dominant serotype associated with 26 outbreaks of commercial broiler chicken farms of different breeds showing non-specific signs such as ruffled feather, depression, ascites, with varying degrees of mortality rates ranging 10-28% [29]. In 2021, FAdV-4 genotype was isolated for the first time from HHS outbreaks encountered in a Cobb 500 broiler flock aged 32 days suffered from a high mortality rate (15%), greenish diarrhea, varying degrees of hepatitis with necrotic foci, an edematous kidney and a flabby heart surrounded by straw-colored fluid filling the pericardial sac in Alexandria governorate, Egypt [30]. Recently, FAdV-D serotype 2/11 was isolated from flocks from different Egyptian provinces; Alex, Mars-Matroh, and Behira [31]

The above-mentioned previous records indicate that the most predominant FAdV serotypes associated with IBH outbreaks in Egypt are FAdV-D serotypes 2, 3, 11 and FAdV-E serotypes 8a and 8b. Consequently, further studies on the FAdV serotypes circulating in Egypt should be intensified for a better understanding of the FAdV criteria to establish the most effective control measures and subsequently overcome the severity of its economic losses.

Pathogenesis

Predisposing factors

The fact that FAdVs are primary or opportunistic pathogens is controversial. Some reports support the hypothesis that said FAdVs are opportunistic pathogens as FAdVs are frequently isolated from chickens that had been infected with immunosuppressive agents such as IBDV, CIAV and sometimes with reovirus or parvoviruses which was confirmed by some researchers that have succeeded in reproducing severe forms of FAdV diseases following experimental immunosuppression by chemicals or fungal mycotoxins which is more closely related to the field situation [32].

Other reports from IBH epidemics from Australia, New Zealand, and Canada depend on experimental FAdV infections by various routes have excluded the immunosuppressive effects of FAdV, and confirmed the primary role of FAdVs as the primary etiology of IBH in broiler chickens [33].

Virus entry and replication

Following entrance, FAdVs replicate primarily in the enteric epithelium (cecal tonsils) 12 hours after infection or respiratory tract epithelium, and this assures the hypothesis of Kohn (1962) that considered the alimentary rather than the respiratory tract to be the main site of viral replication. FAdVs enter the cell then the interaction between fiber and cell receptors, mostly (CAR), occurs. Viral replication begins with intracellular transport and folding of viral proteins with the help of the most important nonstructural proteins known as 100K, according to [34].

Following replication, viremia occurs within 24 hours and the virus spreads to other organs including the respiratory and digestive tracts as well as renal tubular epithelium and bursal epithelium [35]. Recently, Matos et al. categorized the disease development process into 3 stages beginning with the incubation period as the first stage lasting 1-3 days post-infection during which the virus replicates inside organs such as the liver and spleen

but with no clinical indications or gross lesions followed by the second stage expressing the degenerative changes within organs, mainly hepatic lesions, taking 4-7 days post-infection ending with the convalescent stage (12 days post-infection) evidenced by a decline in the severity of clinical symptoms with the beginning of the regeneration of cells and a reduction in viral load in the target organs [33; 35].

Fowl adenoviral replication can be detected through cellular and abnormal biochemical parameters. The formation of basophilic intranuclear inclusion bodies is characteristic of cellular changes [33] while abnormal biochemical parameters have been observed during the examination of sera taken from infected birds during the acute phase of IBH such as hypoglycemia and increased pancreatic lipase activity and have been correlated to predominant hepatic and pancreatic necrotic lesions [35]. After the virus completes its replication cycle, the virus leaves the infected cell after lysis and begins to descend through respiratory or fecal excretions as well as through egg contents if chickens are in egg production [36] and causing disease even mortalities in their progeny depending upon the immune status of the birds.

Clinical disease in poultry

Clinical findings

IBH-HPS mostly affects broiler chickens 1-5 weeks old. However, reports of the disease were as early as 2 to 4-day-old broiler chickens [11] and adult broiler breeders and layers [37]. The acute disease is characterized by a sudden rise in flock mortality by 3-4 days post-infection that gradually lower by 5-6 days post-infection [38]. The mortality usually ranges between 5 to 10% but may be as low as 1% to as high as 30% in the case of IBH and may reach 80% in the case of HPS, especially in young birds [1; 5] depending on the age, the levels of maternally derived antibodies, co-infections (mainly IBDV and CIAV), and the immune status of infected birds [32].

It is difficult to diagnose the IBH-HPS clinically since the affected birds show non-specific signs. The affected birds exhibit ruffled feathers, huddling together, off food with the abrupt onset of mortality in broiler chicks aged 3–6 weeks gives rise to suspicion of HPS; however, the typical hydropericardium in HPS cases is a significant lesion to differentiate it from IBH cases [39].

Gross lesions and histopathology

The most prominent lesion in both spontaneous and artificial infections of IBH is observed in the liver which appears pale, friable, and enlarged with minute white foci but may be associated with petechial or ecchymotic hemorrhages in certain cases. Other parenchymatous organs such as the kidneys are also affected and appear swollen with prominent glomerulonephritis. Additionally, atrophied bursa of Fabricius and thymus and aplastic bone marrow were frequently observed [5]. Accumulation of clear, straw-colored fluid in the pericardial sac is a pathognomonic lesion for HHS. Other differences between IBH and HHS include enlarged friable liver and kidneys with dilated tubules and pulmonary edema [40] (Figure 3). In HHS severe epidemics, ascites and extensive necrosis of the pancreas have been reported so, the fatality rate is extensively greater in HHS than in IBH [41].

Definitive diagnosis of HPS can be based on histopathological lesions in the liver. The lesions consist of small multifocal areas of coagulative necrosis, the infected cells appeared highly degenerated and vacuolated, and other hepatocytes appeared highly destructed forming necrotic foci with mononuclear cell infiltration and detection of basophilic intranuclear inclusion bodies (INIBs) with a clear, pale halo with virus particles inside those intranuclear bodies [42] (Figure 4). Based on the presence and severity of liver lesions the disease stages include incubation (1–3 days postinfection), degeneration (4–7 days postinfection), and convalescence (14 days postinfection) stages. FAdV-8b and FAdV-11 were reported to present in the liver, kidney, and gizzard of infected birds during the degenerative stages and persist in the gizzard until convalescence [33]. Subcapsular hemorrhages, hyperemia, degenerated and exposed tubular epithelium, necrosis, moderate interstitial lymphoplasmacytic nephritis, and glomerulonephritis were the most reported renal pathology in IBH infection [43].

In the lungs, congestion, edema, and infiltration by inflammatory cells with the presence of hemorrhagic exudate in the bronchi and alveoli and moderate infiltration of macrophages into the pulmonary parenchyma. Chickens and ducks suffered from HHS showed lesions of myocarditis

[44]. Significant immunosuppression was reported in SPF birds experimentally infected with virulent FAdV-4 causing B and T cell depletion in lymphoid organs [32] and follicular atrophy in the bursa of Fabricius with lymphocyte depletion and hemorrhages can be seen [41]. Also, lymphocyte depletion, vascular congestion, and hemorrhagic focal areas in the spleen [43].

Diagnosis of aviadenoviruses

The diagnosis of FAdV infections was traditionally based on clinical history, postmortem, and histopathology lesions. Viral isolation in embryonated SPF eggs and cell culture can be used to identify FAdVs. Viral antigens or virus nucleic acids in infected tissues can be identified using molecular techniques such as polymerase chain reaction (PCR), or virus-specific antibodies in serum samples can be measured through different serological tests [32]. Molecular diagnostic techniques and serological testing are the most often used assays in diagnostic and experimental laboratories worldwide.

Clinical chemistry analysis

Changes in enzymatic systems and metabolite concentrations caused due to the IBH-HPS pathogenesis were detected using biomarkers of the liver and pancreas functions developed for strain pathogenicity and IBH pathogenesis [35]. In HPS and IBH-affected birds, the serum levels of both albumin and β-globulins decreased because of liver damage observed in IBH-HPS infected birds. The reduction of albumin results in lower colloidal plasma osmotic pressure and accumulation of fluid into the pericardial sac. Subsequently, the blood glucose and plasma protein levels decreased, and uric acid, potassium, calcium, and triglyceride concentrations elevated.

Leukocytosis, erythrocytosis, and an increased hemoglobin concentration were also reported with low total leukocytic and erythrocytic count, hemoglobin values, and an increase in heterophil percent in both natural and experimental infections in broiler chickens. Heart failure may be caused by injury to the liver or the heart muscle, which is indicated by an increase in SGPT activity. Because of degeneration and necrosis in the cardiac muscle fibers, the damaged chickens had much higher aspartate aminotransferase, alanine aminotransferase, and creatine phosphokinase activity than the healthy chickens [45] .

Serology

Serological tests can detect antibody response due to routine vaccination or infection; therefore, they are critical for surveillance FAdV studies. The agar gel immunoprecipitation (AGID) or immune fluorescence technique (IFA), and enzyme-linked immunosorbent assays (ELISA) are being used to detect FAdV [46]. ELISA identifies group-specific antibodies according to the tested antigen, however, the test fails to differentiate between FAdV serotypes due to the masking effects of group-specific antibodies [9]. Recently, serotype-specific diagnosis can be established through specific ELISA and/or Virus neutralization tests (VNT) were developed to determine the FAdV fiber-specific antibodies for FAdV-1, -2, -4, -8a, -8b and -11 [37; 47; 48].

Immunohistochemical methods are beneficial to determine the tissue tropism of FAdV by using virus-specific antibodies. These virus-specific antibodies were first detected in serum on day 14 and peaked at days 21, 28, 35, and 42 based on statistically significant levels [49]. Antibody response against FAdV is detected as a result of vaccination as well as infection and it is possible to discriminate between them using the recombinant non-structural proteins 33 and 100 K [9]. Homologous antibody response can be detected with the help of partial hexon protein with good sensitivity [50]. Immunity against FAdV infection is not only humoral but also mucosal but the presence of humoral antibodies is not important and indicates the status of local immunity at mucosal surfaces.

Isolation and identification of aviadenoviruses

A. Primary tissue culture

Cell culture and SPF embryonated chicken eggs (SPF ECE) can be used to isolate FAdVs. Samples for FAdV isolation are feces, pharynx, cecal tonsils, kidneys, and the liver in case of IBH. Susceptible cell lines include chick embryo liver cells (CEL), chick kidney cells (CK), chicken embryo fibroblast, Leghorn male hepatocellular carcinoma (LMH), and chicken hepatoma (CH-SAH) cell line. FAdV infection causes rounding and detachment of cells, which appear refractile under a light microscope. chicken cells are ideal for FAdV propagation, however, homologous species cell lines are suggested to isolate FAdV from avian species [9].

B. Isolation of fowl adenovirus on SPF ECE

Although inoculation of SPF embryonated eggs is insensitive for primary aviadenoviruses isolation, embryo propagation onto yolk sac or CAM is an alternative in laboratories lacking cell culture techniques. Liver homogenates of affected chickens from the three poultry farms confirmed positive with conventional PCR were used for inoculation of 9 days SPF ECE. Some of the embryos inoculated died after 3–4 days post-inoculation. The inoculated embryos were haemorrhagic, and showed enlarged livers, with either yellow to reddish foci or diffuse greenish necrosis (Figure 5). Histological examinations showed acute hepatitis with necrotic hepatocytes and the presence of basophilic intra-nuclear inclusion bodies within hepatocytes (Figure 6). For confirmation of an adenovirus virus isolation, electron microscopy, and immunocytochemistry (ICH) are reliable for detecting adenoviruses within infected cells. Finally, the VNT serotyping of the isolated virus can be conducted using common reference antisera of different serotypes [32].

C. Molecular techniques

PCR in combination with other procedures that depend on the nucleotide differences within the hexon gene using specific primers is used to identify FAdV on the level of species (A to E) and genotypes within species. Designation of specific primers for the stereotyping of FAdVs is another rapid and reliable differentiating method. Nested and real- time PCRs are another quantitative and more sensitive method than virus isolation and are beneficial for virus quantification [51]. The evolution of FAdV strains over time has been clarified by sequencing of the hexon and/or fiber genes which allows the development of more accurate strategies to control IBH-HPS outbreaks in poultry farms [52]. Hexon as well as fiber gene sequencing can be applied to differentiate FAdV serotypes [11].

High-resolution melt curve (HRM) analysis of the hexon gene is another faster and more accurate technique that can genotype FAdVs within hours [53]. In the early 2000s, FAdV sequence data witnessed a breakthrough thanks to the initial whole genome sequencing and the molecular typing of the hexon gene. The L1 loop of hexon and the knob of fiber genes encode virulence factors, so they are considered the FAdV infectivity markers. However, the fiber-2 protein is a determinant for FAdV pathogenicity, and the fiber-1 protein is responsible for viral replication and assembly, despite virulence capability rather than infectivity. Meanwhile, the hexon protein is essential for infectivity and tissue tropism [52].

Immunity and vaccines

The common group- specific antigen of aviadenoviruses differentiates them from other genera [54]. The FAdVs type- specific antibodies are detectable after one week and peak titers are detectable by 3 weeks post-infection. Protective immunity against the same infecting serotype is developed after 45 days or even after 8 weeks from re-infection including neutralizing and precipitating antibodies, hence the birds are protected from reinfection [54]. The presence of humoral antibodies does not prevent virus excretion and infection through natural routes may occur despite the presence of maternal antibodies. With regard to protection, neutralizing antibodies, as well as local immunity, are essential [9]

Immunosuppressive effect of aviadenoviruses

Immunosuppressive agents are challenging the poultry industry with a serious impact factor in the form of mixed infections and vaccination failure. Recently, FAdVs, the causative agent of HHS, have been considered relatively new cellular and humoral immunosuppressive agents [32]. FAdV-4, FAdV-8b, and FAdV-11 are considered primary pathogens in case of HHS and IBH causing thymus and bursal atrophy. The B and T cell depletion in lymphoid organs due to bursal follicles atrophy and degeneration of lymphocytes with hemorrhages were reported after experimental FAdV4 infection of SPF birds is suggested to cause immunosuppression [1]. This suggestion is further supported by the detection of FAdV-8b and FAdV 11 in the bursa and thymus of infected birds during the degenerative stage of IBH [33]. Although FAdV-1 isolate is considered pathogenic, the isolate was detected in bursa and/or gizzard between 2- and 7-days post-infection. Additionally, the reported FAdV-associated immunosuppression increases the susceptibility of IBH-infected flocks to other avian pathogens [4].

Vaccination

Different generations of FAdV vaccines were adapted to combat that problem. In endemic areas, for prevention and control of IBH-HPS, it is recommended to vaccinate primary breeders with

autogenous vaccines with strict biosecurity measures to exclude vertical transmission and to ensure high levels of protective maternal-derived antibodies from the breeding flocks to their chicks. Many countries develop live and inactivated vaccines to control IBH and HHS and vaccination with certain genotypes/serotypes may become more applicable [55]. The FAdV- 4 s and -8As are the most commonly used serotypes in vaccine preparation due to several reports indicating their primary pathogenicity [5].

In Pakistan, broilers are routinely immunized against the disease using FAdV- 4, the common serotype associated with HHS, with apparent protection [5]. Progenies are also vaccinated at 10 days of age, especially if they descend from breeder flocks lacking serotype-specific FAdV antibodies or uniformity of maternal antibodies. Fortunately, a formalin-inactivated liver homogenate vaccine was adopted for broilers aged 10–15 days as a prophylactic vaccination with a satisfactory protection level in the field in Pakistan. Inactivated vaccines have been driven from different routes including liver homogenate, from embryos and cell cultures [56].

In India, an inactivated oil-emulsified vaccine made from an adenovirus using chicken embryo kidney cell culture provided 100% protection in broiler chickens against IBH-HPS [57]. However, the vaccine lacks cross- protection due to the isolation of heterologous strains from diseased birds. Similarly, a live FAdV8b vaccine was applied 1-3 times in breeders between the ages of 9 and 18 weeks through drinking water in Australia; however, heterologous IBH outbreaks were reported in their progenies [33]. Conversely, an inactivated oil- emulsion FAdV- 4 vaccine was reported to provide broader protection against FAdV serotypes experimentally and in progenies of vaccinated breeders in the field [58]. A multivalent inactivated vaccine was also developed to include species FAdV- D and FAdV- E and used to immunize a grandparent flock at 10 and 17 weeks of age completely protected their progenies for 50 weeks of their life [59].

Though an inactivated FAdV- 4 vaccine is commercially available for breeder chicken flocks in Egypt; however, data about its cross-protection against the prevalent FAdVs D serotype 2 and 11 and E serotype 8 a,b is still unknown. Zhang et al. developed a non-pathogenic chimeric virus rHN20 strain based on the hexon gene of FAdV-4 (the key gene for FAdV-4's pathogenicity) as a live vaccine. The vaccine induced detectable high levels of neutralizing antibodies and protected chickens against FAdV-4 with no reporting of clinical signs or lesions in the liver, and significantly reduced virus titers in chicken tissues [60].

In a comparative study between the structural proteins of FAdV-4, fiber 2 and the 100 K non-structural protein expressed as recombinant proteins induced a higher level of protection against HHS [61] while only 40% protection was reported using the latter considering the recombinant vaccine as a new tool for controlling FAdVs IBH and HHS [61]. Cellular immunity is vital for protection due to the lack of neutralizing antibodies before the challenge and vaccination of broiler breeders against both CIAV and FAdV- 4 conferred higher protection [9]. Interestingly, the vaccination of the knob domain containing fusion protein with subsequent efficient protection against the lethal challenge of FAdV-4 in chickens. It also highlights a bright application of knob domain-based subunit vaccines to control FAdV-4 in the future [62].

Intervention strategies to prevent or minimize IBH and HHS in chicken flocks.

Biosecurity practices are the basis for the prevention of aviadenoviruses infection including strict management practices and restricted entry and/or personal protection of visitors and workers are important tools to control IBH and HHS [63]. The persistence of aviadenoviruses inside premises in addition to their heat (up to 70°C) and disinfectant (e.g. lipid solvents) resistance are challenges to prevent HHS. However certain disinfectants such as glutaraldehyde and calcium hydroxide liquid combination are effective against FAdV1 at 21°C [63].

FAdVs are effectively transmitted vertically through the embryonated egg as reported by Yamaguchi et al. who succeeded in the isolation and identification of serotypes (FAdV-1) causing gizzard erosion, and 5 (FAdV-B) as well as 8b (FAdV-E) from apparently healthy broiler and layer breeder chickens of different ages (1–20 weeks of age) in Japan [64], as well as FAdV serotype 7 that was isolated from parental chickens and their offspring [69] Therefore, to prevent infection and vertical transmission of FAdVs, optimum disinfection and vaccination of breeders, are essential. Co-infection with CIAV, IBDV, REO,

REV, and ALV are common so, controlling and/or eliminating these immunosuppressive diseases is also critical in reducing IBH and HHS as they increase the pathogenicity of FAdV [39; 65].

Future prospectives of research efforts

Future research efforts key focus on IBH in poultry will be on understanding the pathogenesis of IBH, including the role of immunosuppressive factors such as co-infections and environmental stressors. Advancements in molecular diagnostics are expected to provide rapid and accurate detection of adenoviruses responsible for IBH, enabling early intervention. Tailored vaccine development remains a promising avenue, with research likely to focus on safer, more effective recombinant vaccines tailored to regional viral strains. Studies on immune-modulating strategies, such as immunostimulants, may provide additional tools for disease prevention. The integration of big data in monitoring outbreaks could improve prediction models and biosecurity measures.

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Not applicable.

Figures and Tables

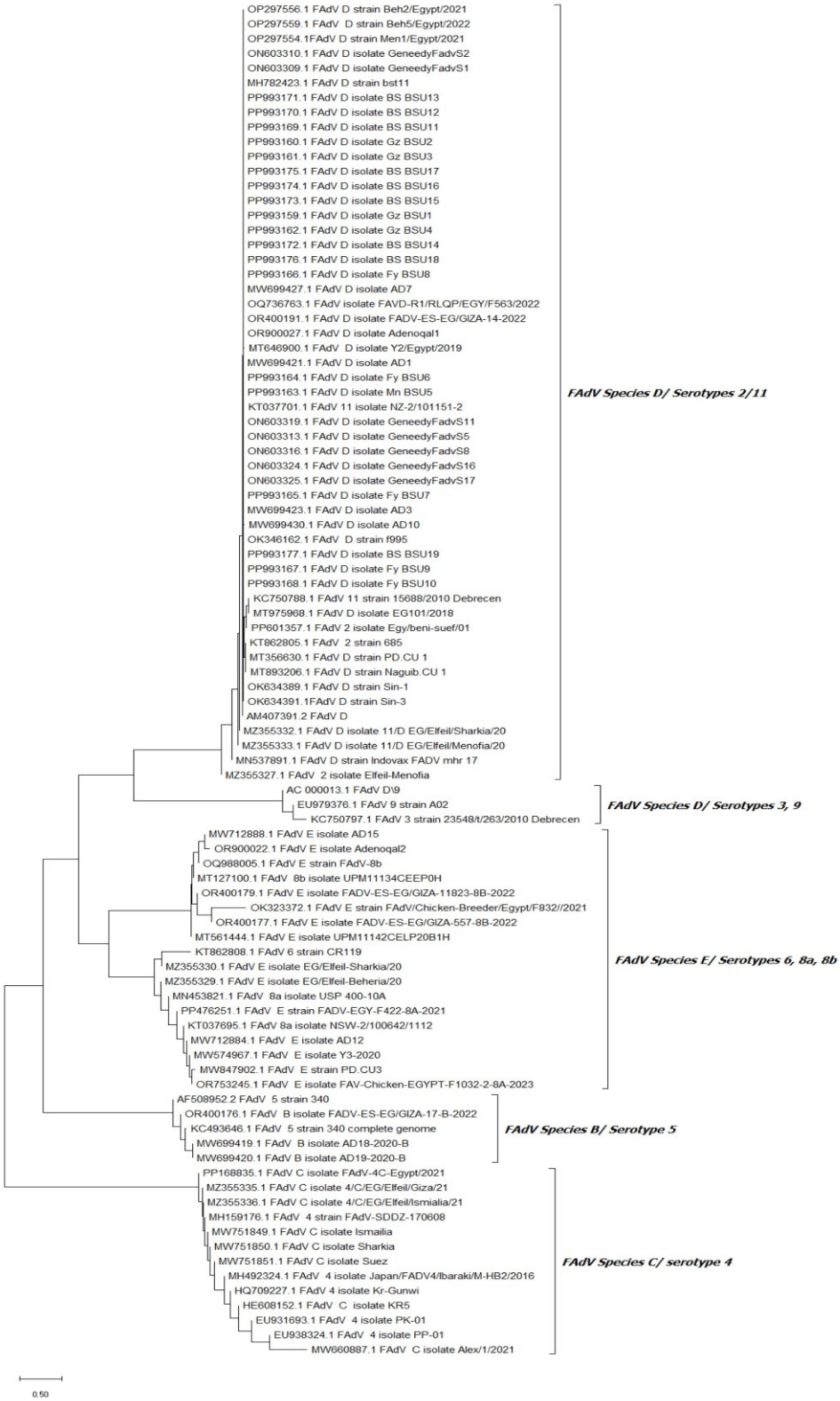


Fig. 1. Genetic characterization of Aviadenoviruses serotypes in poultry. An evolutionary history was inferred using the neighbor-joining method and 92 nucleotide sequences were analyzed. For each sequence pair, all ambiguous positions were removed. A total of 3350 positions were included in the final dataset and evolutionary analyses were conducted in MEGA X [66].

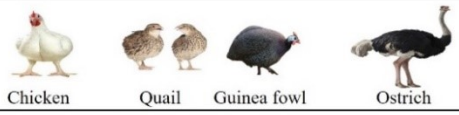
Species	Serotypes	Strains/Isolates	Common diseases	Host range
A	1	112, CELO, Phelps, QBV, OTE, H1, Fontes, PLA2	Chick embryo lethal orphan, Adenoviral gizzard erosion, Quail bronchitis	 Chicken Quail Guinea fowl Ostrich
B	5	340, M2, Tipton, TR22	Inclusion body hepatitis	 Chicken Pigeon
C	4	341, 506, Da60, H2, J2, K31, ON1, K1013, KR5	Hepatitis-hydropericardium syndrome	 Chicken Psittacine
	10	C-2B, CFA20, M11, SA2		Unknown
D	2	685, GAL-1, H3, IDA1, P7, SR48	Inclusion body hepatitis	 Chicken Pigeon Ostrich
	3	75, H5, SR49		
	9	93, A2		
	11	161, 380, UF71		
E	6	168, CR-119, WDA6		
	7	122, X11, YR36		
	8b	764, B3, CFA3		
	8a	58, T8, TR59	Inclusion body hepatitis, Adenoviral gizzard erosion	

Fig. 2. Aviadenoviruses species (FAdV-A to FAdV-E) clinical diseases and host range, modified from Fitzgerald, 2020 [67].

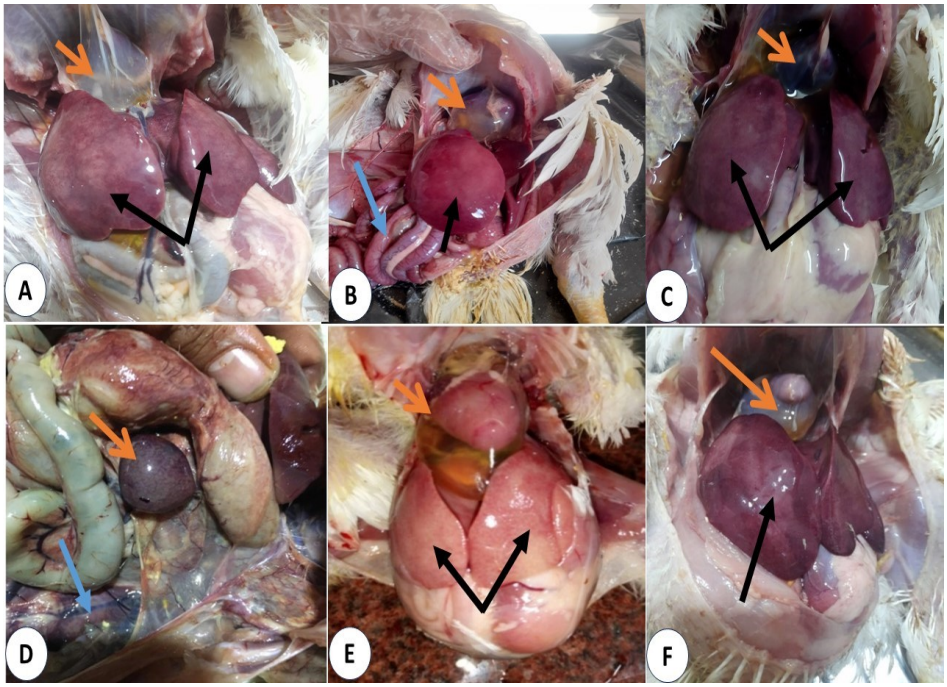


Fig. 3. Growth pathology of naturally infected chickens with FAdVS (HPS). A; beginning of hydropericardium (orange arrow) with necrosis and enlargement of liver (black arrows). B; General hyperemia (blue arrow) with enlarged congested liver (black arrows) and hydropericardium (orange arrow). C; Enlargement of liver with petechial hemorrhages (black arrow) and hemopericardium (orange arrow). D; A spleen showing some degree of enlargement and areas of necrosis (orange arrow) with nephritis (blue arrow). E; Severe hydropericardium in a small chick (orange arrow) and pale liver (black arrow). F: severe hydropericardium leading to heart atrophy (orange arrow) with sever enlarged congested liver in an adult chicken (black arrow).

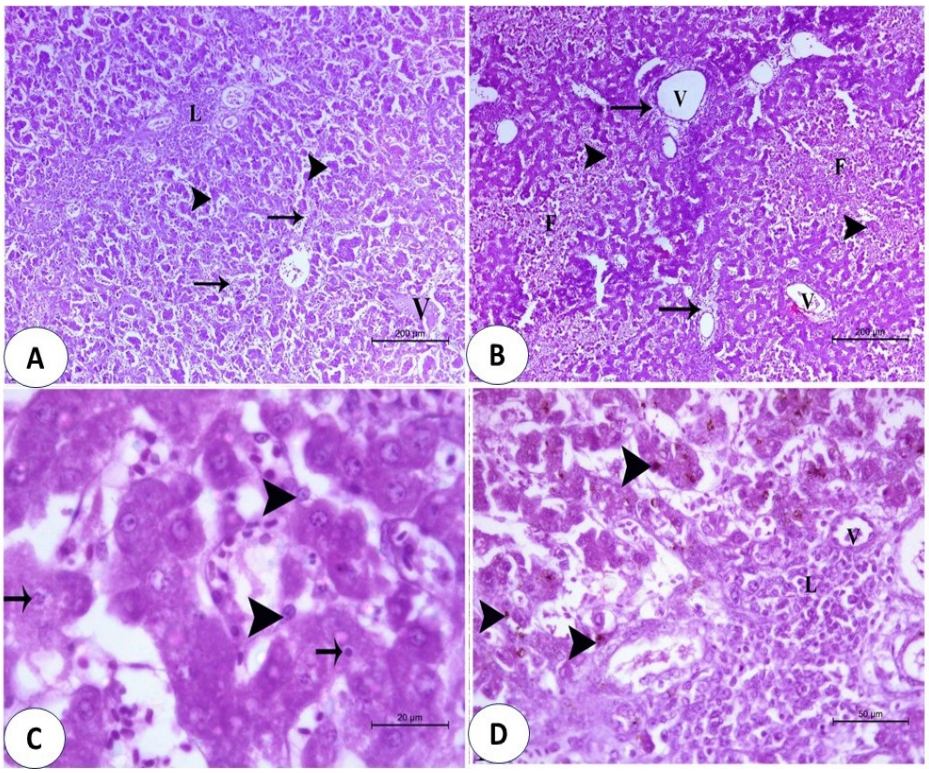


Fig. 4. Histopathological picture of liver from FAdV naturally infected chicken. A: The liver tissue showing severe congestion of central veins and portal vessels (V), degeneration of hepatocytes (arrowhead) forming necrotic foci (F). Note, massive fibrosis around the central vein and the portal area (arrow). (H & E staining, ×100).

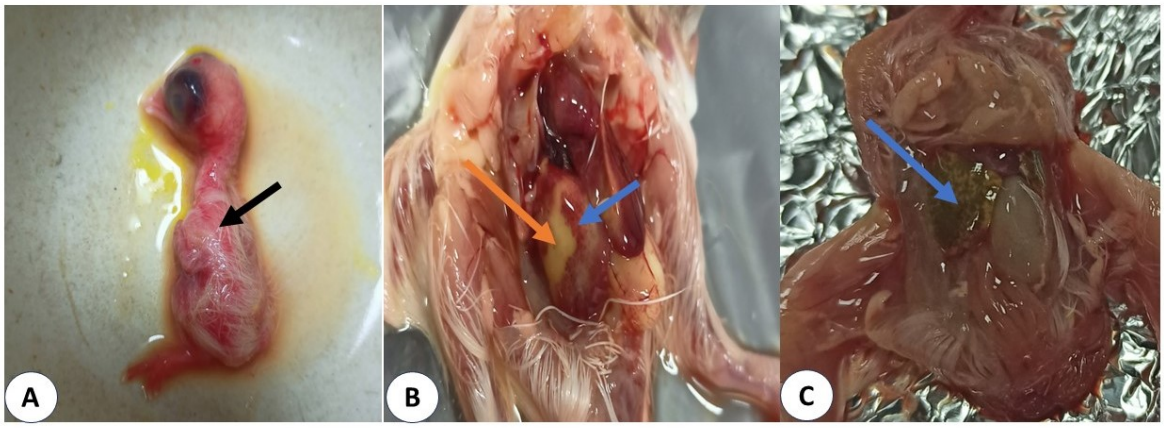


Fig. 5. Gross pathology of liver of SPF ECE inoculated with fowl adenovirus A; Embryos were hemorrhagic (black arrow) B; Embryos showed enlarged livers, with yellow (orange arrow) to reddish foci (blue arrow). C: Embryos showed diffuse greenish necrosed liver (blue arrow).

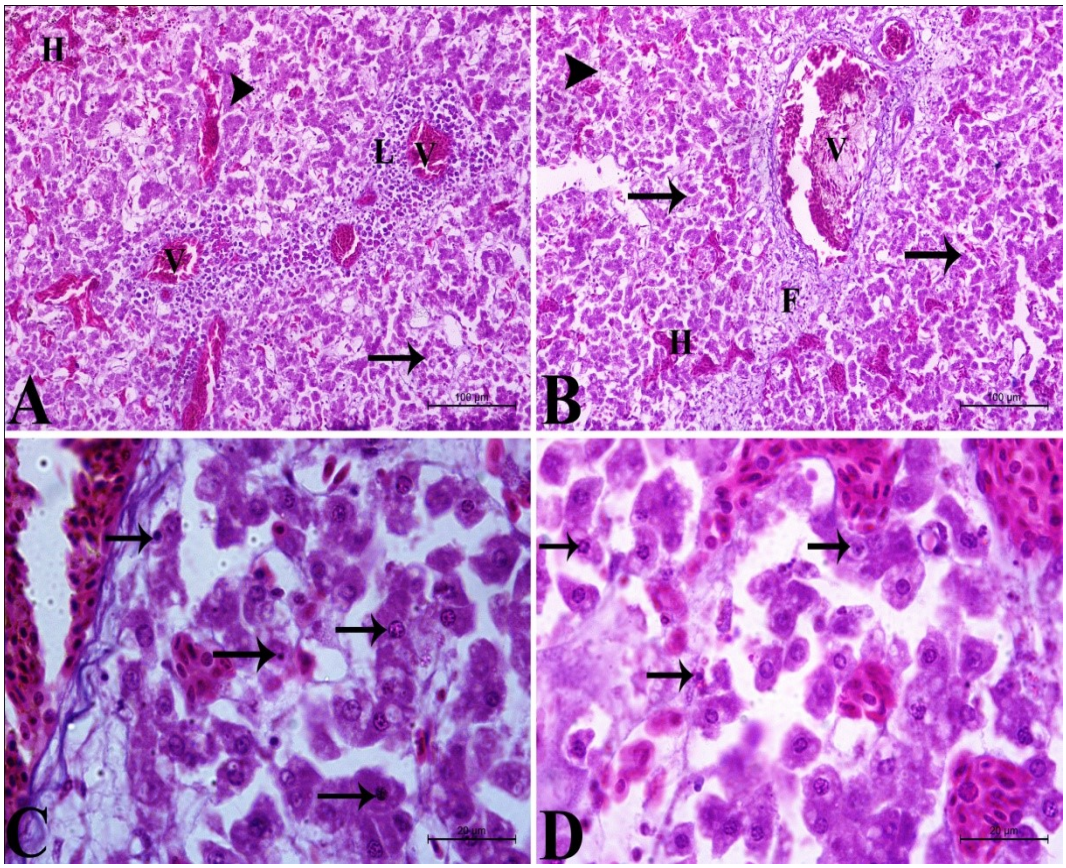


Fig. 6. Histopathological picture of the liver in Adenovirus-infected embryo chicks showing; severe congestion of central and portal vessels (V) enclosed by massive infiltration of lymphocytes around these portal vessels (pre-vascular cuffing) (L) necrosis of hepatocytes (arrow) and widening with hyperplasia of the phagocytic cells in the hepatic sinusoids (arrowhead). Note, that severe hemorrhage (H) occurred all over the hepatic tissue (H & E staining, ×200) (A). sever dilatation and congestion of central veins and portal vessels (V) with marked thrombus formation, severe hemorrhage (H) occurred all over the hepatic tissue, coagulative necrosis of hepatocytes (arrow), and widening with hyperplasia of the phagocytic cells in the hepatic sinusoids (arrowhead). Note, fibrosis around central vein and the portal vessels (F). (arrowhead) (H & E staining, ×200) (B). showed different forms and shapes of intra-nuclear inclusion bodies (arrow). Note, the infected cells appeared highly degenerated (H & E staining, ×1000) (C&D).

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التهاب الكبد الوبائي ذو الجسيمات الضمنية: نظرة شاملة على تأثير المرض والسيطرة عليه في الدواجن

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قسم أمراض الدواجن، كلية الطب البيطري، جامعة بني سويف، بني سويف 62511، مصر.

الملخص

يعد التهاب الكبد الوبائي ذو الجسيمات الضمنية- متلازمة استسقاء القلب (IBH-HPS) مرضًا ذو أهمية اقتصادية تؤثر على الدواجن في جميع أنحاء العالم. العامل المسبب لـ IBH-HPS هو بشكل رئيسي فيروس أدينو الدجاج(FAdV) ، حيث تعتبر الأنماط المصلية 4 و 11 و هى 8bالأكثر ارتباطًا بالمرض. يتميز المرض بالتهاب الكبد مع أجسام مدمجة داخل النواة واستسقاء القلب، مما يؤدي إلى معدل مرضي ووفيات كبير في قطعان الدواجن. تكشف الدراسات الوبائية أن تفشي IBH-HPS غالبًا ما يرتبط بممارسات الزراعة المكثفة، وانخفاض مستوى الأمان الحيوي، و نقص المناعة الناتج عن العدوى المشتركة (مثل مرض الجمبورو (IBDV) وفيروس فقر الدم في الدجاج (CAV) أو الضغوط مثل التسمم بالأفلاتوكسين. تعتبر طرق الانتقال العمودي والأفقي حاسمة في انتشار المرض. تشمل آلية المرض تكاثر الفيروس في الخلايا الكبدية، مما يؤدي إلى نخر، وتكوين أجسام مدمجة، واستجابات التهابية جهازية. سريريًا، تظهر الطيور المتأثرة علامات الخمول، والريش المبعثر، ووجود أعراض تشريحية مميزة تشمل كبدًا شاحبًا وهشًا مع أجسام مدمجة وتراكم السوائل في الكيس التاموري. يعتمد التشخيص المختبري على علم الأمراض النسيجي، وتقنية تفاعل البلمرة المتسلسل (PCR)، وتقنيات عزل الفيروس لتأكيد وجود FAdV وتحديد النمط المصلى. لقد أظهرت اللقاحات المعطلة واللقاحات الحية المضعفة فعالية في تقليل حدوث المرض. ومع ذلك، يجب أن تتوافق استراتيجيات التطعيم مع الأنماط المصلية المنتشرة لضمان الحماية المثلى. يتطلب فهم التهاب الكبد الوبائي ذو الجسيمات الضمنية (IBH-HPS) اتباع نهج متكامل يتناول العوامل البيئية والإدارية والمناعية. فى هذا المقال المرجى نحاول أن نغطى المعرفة الحالية وتأثير المرض على إنتاج الدواجن، والبحث المستمر لتعزيز أساليب التشخيص، وتحسين تركيبات اللقاح، وتطوير استراتيجيات الوقاية الشاملة.

الكلمات الدالة: التهاب الكبد الوبائي ذو الجسيمات الضمنية، متلازمة استسقاء التامور، (IBH-HPS)، فيروس أدينو الدجاج ، الدواجن.