

# AN INSIGHT INTO FOWL ADENOVIRUS INFECTION IN POULTRY

## Abstract

Fowl adenoviruses (FAdV) are significant infectious agents infecting wide range of poultry host including parrots, falcons and ostrich having a significant negative economic impact on the global poultry industry. Because FAdV strains are so widely dispersed, the majority of avian species are susceptible to infection. Five species and 12 serotypes (A–E; 1–8a and 8b–11) make up the FAdV classification. As FAdV strains are so widely dispersed, the majority of avian species are susceptible to infection. Chicken production can be affected by a number of syndromes brought on by fowl adenoviruses (FAdVs), including Inclusion Body Hepatitis (IBH), Hepatitis-Hydropericardium Syndrome (HHS) and others which can result in significant financial losses across the world. Because fowl adenoviruses (FAdVs) are more common than occasional outbreaks and there are differences across experimental studies, the stand-alone pathogenicity of FAdVs has been a topic of discussion for a while. However, during the past 20 years, a global pattern of FAdV-associated disorders has emerged, with epidemics of inclusion body hepatitis that are more widely dispersed geographically and hepatitis-hydropericardium syndrome occurring more in Asia. When Adenoviral Gizzard Erosion (AGE) first appeared in Asian and European countries, the spectrum of disorders was fully completed. Poultry industry's shift to highly specialised genetic breeds and strict biosecurity measures also contributes to the rising prevalence of diseases linked to FAdV. Additionally, increased biosecurity regulations have resulted in breeding stocks with weak immune systems, putting broilers at danger from vertical FAdV transmission. Therefore, in the future, breeders should

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have appropriate antibodies before production, and vaccination should be employed as a part of prophylactic efforts to safeguard progeny.

**Keywords:** FAdV, Serotypes, Inclusion Body Hepatitis (IBH) and Hepatitis-Hydropericardium Syndrome (HHS).

## I. INTRODUCTION

Fowl adenoviruses (FAdVs) are members of the genus *Aviadenovirus* and family *Adenoviridae*. Inclusion body Hepatitis (IBH) and Hydropericardium Syndrome (HPS), two significant clinical disorders in broiler chickens, breeder flocks and layers, have been linked to the FAdVs, a collection of extremely varied pathogens. Both healthy and sick birds have adenoviruses, which were all characterised by vertical transmission and replication in the nucleus of the host cell. Two genera—*Mastadenoviruses*, which infect mammals, and *Aviadenoviruses*, which infect birds—make up the family *Adenoviridae*. Both healthy and sick birds have adenoviruses, which were all characterised by vertical transmission and replication in the nucleus of the host cell [1]. The avian adenoviruses have a wide range of virion characteristics, including viral shape, genome length, and genome organisation, all of which are crucial for diagnosis. Different serotypes were revealed by various neutralisation assays used to characterise FAdVs, however cross-reactions complicated their easy differentiation. The first FAdV illness, inclusion body hepatitis (IBH), was found to require immunosuppression, leading to the conclusion that FAdVs alone had limited pathogenicity. The necessity of a targeted prophylactic measures are questioned by concurrent studies on the widespread or even ubiquitous presence of FAdVs in chicken flocks.

## II. MORPHOLOGY AND CLASSIFICATION

Adenoviruses are non enveloped viruses belonging to *Aviadenovirus* genus, *Adenoviridae* family and contains non-enveloped and double stranded DNA of almost 43-46 kb in size with icosahedral symmetry [2]. Hexon, penton base, fibre, terminal protein, protein, protein IIIa, protein V, protein VI, protein VII, and protein VIII are the ten primary structural proteins that this virus's genome encodes, along with 11 non-structural proteins (E1A, E1B, E2A (DBP), E3 (ADP), E4, EP, 33/55 K, pol, pIVaII, and 100 K) [3]. A non-enveloped icosahedral virion's major capsid protein is called the hexon protein, and it comprises type, group, and subgroup-specific determinants. Hexon and fibre are the two important structural proteins which are non-covalently attached to the penton base. All mammalian adenoviruses have a single fibre attached to the penton base however fowl adenovirus represents a morphological distinctiveness of having two filaments per penton base. The type-specific g-antigen, which is in charge of the haemagglutinating activities, is located in the head of fibre which is the protein's distal C-terminal region. *Adenoviridae* family is widely dispersed and has been divided into five genera, including *Aviadenovirus*, *Mastadenovirus*, *Atadenovirus*, *Siadenovirus*, and *Ichtadenovirus*. Three of them (*aviadenovirus*, *siadenovirus*, and *atadenovirus*) can infect birds. The viruses within each of these were classified into species, which can then be further subdivided into serotypes. Cross-neutralization, genomic organization, the content of nitrogenous bases such as guanidine and cytosine and host range were used to classify these organisms, with phylogenetic distance serving as the primary criterion. FAdV are divided into 12 serotypes (FAdV-1 to 8a & 8b to 11) and five species (FAdV-A to FAdV-E) within the *aviadenovirus* genus based on cross-neutralization tests and molecular structure, respectively [4]. Avian adenovirus is separated into three different groups which reflects biological diversity. Conventional avian adenoviruses in Group I include those infecting a variety of birds species such as chicken, turkey, geese, ducks, and pigeon. These avian adenovirus group I isolates from birds share a common group antigen. Group II viruses include HEV, the Avian Adenovirus splenomegaly (AAS) virus, and the Marble spleen disease virus (MSDV) of pheasants. Group III virus are

haemagglutinating virus associated with EDS 76 (egg drop syndrome). Inclusion body hepatitis (IBH), Hydropericardium syndrome (HPS), and Gizzard erosion (GE) are the most common diseases associated with FadV infection in chickens. The most common type of FAdVs isolated from IBH cases are FAdV-D and FAdV-E. The FAdV strains linked to HHS are FAdV-4 (FAdV-C), which are extremely harmful to hens. Serotype FAdV-1 has been isolated from the majority of gizzard erosion.

### **III. PATHOGENESIS**

FAdVs are ubiquitous in nature in poultry and 3- 6 weeks old broilers are mostly susceptible to FAdV infection. The infection process begins with the virus attachment to the host cell receptors on the cell membrane surface. FAdV attachment requires the coxsackievirus adenovirus receptor (CAR), a transmembrane protein on the surface of the target cell. This connection enables the virus to enter host cells through endocytosis on the virus fibre knob, where the genome is then delivered to the cell nucleus [5]. The novel FAdV-4's short fibre is bound by the CAR's D2 domain (D2-CAR), which acts as an active domain. Once inside the cell, FAdV will begin its replication programme using the cellular machinery [6]. Apoptosis, autophagy, and an inflammatory cytokine response are a few of the outcomes of this virus-host interaction, which occurs when the viral and host cell components interact at both the protein and nucleic acid levels.

### **IV. INCLUSION BODY HEPATITIS**

FAdV, primarily belonging to species FAdV- D and FAdV- E are the main causes of IBH. FAdVs are believed to be opportunistic pathogens that produced IBH along with concurrent infections with immunosuppressive viruses, such as Infectious Bursal Disease Virus (IBDV) and Chicken Anaemia Virus (CAV) [7]. While rarely affecting layers and broiler breeders, IBH predominantly impacts broilers under five weeks old. Mortality rises during IBH epidemics in 3–4 days and can occasionally surpass 30%. Clinical signs include poor development, lethargy, prostration, ruffled feathers, and huddling behaviour in affected birds. Clinical signs of IBH affected birds is extremely similar to birds with HHS infection but is less severe. Necropsy examination reveals severe hepatitis with liver being enlarged, friable, having a mottled appearance with several necrotic foci. Additionally, several investigations noted petechiae, necrosis, atrophy, and colour changes in the pancreas of infected birds with IBH. Swollen and haemorrhagic kidneys are also observed. Large regions of coagulative necrosis, lymphoid infiltration and basophilic inclusion bodies in the nucleus of the hepatocytes are characteristic histological findings found in the liver of affected chickens. Bursal and thymus atrophy as well as lymphoid tissue depletion in the bursa and spleen are also seen as additional microscopic changes in the lymphoid tissues. The oval or isohexagonal virus particles of around 70 nm in diameter, the granular material, and occasionally lamellae concentrically encircling the virus core make up the ultrastructure of the basophilic inclusions, which is likely connected to the process of virus generation.

### **V. HEPATITIS – HYDROPERICARDIUM SYNDROME**

An economically significant poultry illness is hydropericardium syndrome (HPS), also named as inclusion body Hepatitis-Hydropericardium Syndrome (IBH-HPS), hydropericardium syndrome, Angara disease (in Pakistan), and Litchi heart disease (in India).

In the autumn of 1987, Angara Goth, Karachi, Pakistan, reported having the first HHS cases in broiler chicks [8] even though isolated occurrences were noted as early as 1985, since that time, the illness has spread to Iraq, Mexico, Peru, and Chile [9]. In 1993, Jammu and Kashmir and Punjab's poultry belt in India saw the first reports of HPS. Broilers of 3–6 weeks old are mostly affected [10]. HPS depends significantly on horizontal and vertical transmission. Through embryonic eggs, the virus is vertically transmitted. Contact with infected faecal fomites causes horizontal spread. In field studies, the death rate—the primary clinical hallmark of HPS—was noted to be 60%–70% in Pakistan, 10%–30% in Iraq, and 10%–60% in India, while it ranged from 1.3%–11.1% for Korean cases. Straw coloured fluid accumulates in the pericardial sac around heart acting as the striking gross lesion finding is generally considered as a pathognomonic feature of HHS. Birds are found to be depressed, huddling with ruffled feather and yellow droppings around vents. In almost all cases, lesions are generally found in liver and kidney. On gross examination, liver appears pale, friable with necrotic foci and in some cases petechial haemorrhages are also seen. The kidneys are pale, swollen and congested when examined grossly. Histopathological observations appears similar as in IBH, particularly appears in the liver, showing basophilic intranuclear inclusion bodies and varying degrees of pyknosis, karyorrhexis and karyolysis are observed in the majority of the hepatic cells along with inflammatory as well as degenerative processes. Mononuclear cell infiltration, significant vascular alterations, major oedema leading to muscular bundle disruption, haemorrhages, and degenerative abnormalities were all discovered during the heart's histopathological investigation. Lymphocytolysis and cyst development are the changes in the Bursa of Fabricus, thymus, and spleen that results in the loss of lymphocytes in the medullae of follicles in the bursa of Fabricus and shows pyknotic nuclei in the lymphocytes in the spleen. The pathogenesis of HHS strains is significantly influenced by the virus affinity for lymphoid tissue, which leads to lymphoid organ degradation and lymphocyte depletion, underlining the immunosuppressive potential of virulent FAdV-4 [11].

## **VI. ADENOVIRAL GIZZARD EROSION**

An infectious condition that is on the rise in broilers called Adenoviral gizzard erosion (AGE) has a detrimental effect on flock productivity FAdV serotype-1 (FAdV-1) is mainly described as aetiological agent responsible for gizzard erosion in broilers. Although FAdV-8a or -8b are also occasionally found in infected gizzards [12] but FAdV-1 strains were largely responsible for later outbreaks of the disease. The dissemination of the virus comprises both vertical and horizontal transmission of FAdV-1. Multiple dark or black regions of keratinoid layer erosion, together with swelling and ulceration of the underlying gizzard mucosa, can be seen in infected birds with gizzard lesions under the microscope [13]. Gizzard perforation, proventricular and intestinal lumen, and sanguineous fluid are all apparent in deceased birds. On histological examination, mucosa, submucosa, and muscle layer in the afflicted gizzard shows invasion with macrophages and lymphocytes.

## **VII. DIAGNOSTIC METHODS**

By using electron microscopy (EM), viral infection can be quickly identified due to its distinctive appearance [14]. Another non-specific method is to use hematoxylin and eosin to demonstrate characteristic intranuclear inclusions in suspect cell cultures. The creation of an assays such as Enzyme-linked Immunosorbent test (ELISA) to detect group specificity in

less than 100 mean tissue-culture infective doses of adenovirus per gramme of liver tissue is another approach to detect the presence of virus. For more advanced typing, the virus must first be isolated. Chicken kidney cells and Chicken embryo liver cells are the cells from where FAVs are most frequently isolated. Embryonated eggs are reported as sensitive medium so inoculation through Chorio-allantoic membrane and yolk sac route in embryonated eggs are found to be effective method for fowl adenovirus isolation.

**Molecular Methods:** FAdVs are genetically divided into five distinct types, A to E, which included all 12 serotypes. FAdV has been detected using PCR. To categorise viruses into species A to E and to detect genotypes within species, primers primarily based on the hexon gene are utilised. Nested and Real time PCR have been shown to boost sensitivity in comparison to virus isolation methods and can be used for quantification. Due to molecular detection and sequencing of both the fibre and hexon proteins, we are better able to comprehend how new FAdV strains are evolving over time, which in turn enables appropriate approach to create new strategies for preventing disease outbreaks in poultry farms.

## VIII. PREVENTION AND CONTROL OF ADENOVIRAL INFECTION

FAdVs are extremely resistant to inactivation, remain active for a very long time in the environment, and are transmitted both horizontally and vertically. The management and prevention of FAdV infection is both challenging and complex when FAdVs are co-infected with other highly contagious viruses, such as Infectious Bursal Disease virus (IBDV), Avian leukosis virus (ALV), and chicken Anaemia virus (CAV). To prevent and control infectious diseases, it is crucial to implement sound management procedures and biosecurity measures. Consequently, immunisation against FAdV infection is routinely advised and used with encouraging outcomes. The FAdV serotypes 4 and 8 are most commonly used among 12 serotypes for commercial vaccine preparation. Inactivated vaccines, attenuated live vaccines, and recombinant vaccines are effective in protecting chickens against FAdV infection.

- 1. Inactivated Vaccine:** In this type of vaccine, virus is inactivated and are not strongly influenced by antibodies in host body, compared to live vaccine. The inactivated oil-emulsion FAdV-4 vaccine is reported to provide extensive cross protection against different FAdV serotypes in both vaccinated birds and the offsprings of vaccinated breeder birds. Inactivated fowl adenovirus (FAdV8b + FAdV11) breeder vaccines provides broad-spectrum protection in chicks against IBH infection.
- 2. Attenuated Vaccine:** Virulent FAdV-4 isolates are modified to less pathogenic type by passing the isolate in a quail fibroblast cell line (QT35) or in SPF chicken embryos. These modified isolates were then used to create vaccines that could lessen the immunopathology brought on by a significant challenge. The advantage of attenuated live virus over the inactivated vaccine is that live virus could elicit not only humoral immune response but also cell-mediated immunity.
- 3. Recombinant and Subunit Vaccine:** Animal and human vaccinations have both been developed using recombinant DNA technology. It may be possible to create subunit vaccines using the specific proteins of FAdV, such as hexon, fiber and penton base. No clinical symptoms or obvious lesions were seen in the vaccinated chickens after the challenge with the highly contagious FAdV-4 strain HB1501, and immunisation of

Specific pathogen free (SPF) chickens with recombinant fiber protein could produce a quicker and more potent immune response than the inactivated oil emulsion FAdV-4 vaccine.

## IX. CONCLUSION

FAdV-related disease epidemics have been shown to be on the rise globally, especially over the last 10 to 15 years. The current trends in chicken production, husbandry, and rearing, which adhere to stringently enhanced requirements in biosecurity and environmental circumstances, are strongly linked to the rising prevalence of FAdV-related diseases. Although the pathogenicity of the majority of isolates is still in question, IBH and HHS are currently regarded as emergent poultry illnesses. Future methods of control IBH and HHS should focus on coordinating protective measures and flock status monitoring, which are best accomplished by combined serological monitoring and immunisation. Co-infections with other immunosuppressive virus significantly worsen the severity of infection in the field. Application of current technologies, such as Enzyme-Linked Immunosorbent Assays (ELISA) based on whole virus particles detection is more specific and sensitive test system, while the adequately serotype-specific and sensitive serum neutralisation test imposes severe limitations on large-scale sample processing. In order to meet these requirements, newly created ELISAs based on recombinant proteins show promise as a next-generation test technique that enables FAdV distinction and is applicable for mass screening. As there are several FAdV serotypes, which regularly coexist in nature and result in different degrees of serological cross-reactions. Clinical diagnosis and molecular detection using polymerase chain reaction (PCR) are the best methods for diagnosing diseases in birds. To control fowl adenovirus infection, breeders must implement stringent biosecurity protocols, suitable management practises, and immunisation programmes. Therefore, continuing research into diseases associated with adenoviruses is essential for maintaining the growth of the chicken industry.

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