AN INSIGHT INTO FOWL ADENOVIRUS INFECTION IN POULTRY

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**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. Most avian species are prone to infection due to the widespread distribution of FAdV strains. FAdVs are divided into 12 serotypes and five species (A–E; 1–8a and 8b−11). Most avian species are prone to infection due to the widespread distribution of FAdV strains. Infection with fowl adenoviruses (FAdVs) can result in a number of syndromes in the production of chicken, including inclusion body hepatitis (IBH), hepatitis-hydropericardium syndrome (HHS), and others, causing enormous economic losses around the globe. Given the prevalence of the viruses compared to rare outbreaks, and differences between experimental investigations, the stand-alone pathogenicity of chicken adenoviruses (FAdVs) had long been contested. 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 as opposed to countries in the Middle East and Latin America. The range of illnesses was finally completed by the emergence of FAdV-induced gizzard erosion (AGE) in Asia and Europe. The chicken industry's shift to highly specialised genetic breeds and strict biosecurity Smeasures 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, to safeguard progeny, preventative efforts should include enough antibodies in breeders prior to production and - if necessary - immunisation.

Keywords: FAdV, Serotypes, Inclusion Body Hepatitis and Hepatitis-hydropericardium syndrome.

1. **INTRODUCTION**

 Fowl adenoviruses (FAdVs) belong to the family Adenoviridae and genus Aviadenovirus. The FAdVs are a very diverse group of pathogens and have been incriminated as etiological agents for a number of clinical conditions in broiler chickens, breeder flocks and layers; inclusion body hepatitis (IBH) and hydropericardium syndrome (HPS) are the important ones. 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 are quite variable with regard to a number of virion features, such as viral shape or genome length and genome organisation, which are important for diagnostic purposes. 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. Simultaneous reports on the wide spread, or even ubiquity,of FAdVs in chicken flocks questioned the need for a targeted prophylaxis.

1. **MORPHOLOGY AND CLASSIFICATION**

 Adenoviruses are non enveloped viruses belonging to Aviadenovirus genus, Adenoviridae family possessing linear genome of double stranded DNA of approximately 43-46 Kb in size with icosahedral symmetry [2]. The genome of this virus encodes for 10 primary structural proteins (hexon, penton base, fibre, terminal protein, protein, protein IIIa, protein V, protein VI, protein VII, and protein VIII) and 11 non-structural proteins [E1A, E1B, E2A (DBP), E3 (ADP), E4, EP, 33 K, 52/55 K, pol, pIVaII, and 100 K] [3]. The main capsid protein of a non-enveloped icosahedral virion, on which type-, group-, and subgroup-specific determinants are found, is called the hexon protein. Hexon and fibre, two important structural proteins, are non-covalently attached to the penton base, which is why the structure is called a penton. The FAdV reflect a morphological uniqueness of having two filaments per penton base, whereas all mammalian adenoviruses only have a single fibre connected with the penton base. The fibre head is the protein's distal C-terminal portion and contains the type-specific g-antigen, which is in charge of the haemagglutinating functions. Adenoviridae family is distributed worldwide and has been recently classified into five genera, including Aviadenovirus, Mastadenovirus, Atadenovirus, Siadenovirus, and Ichtadenovirus. Three of them can infect birds (aviadenovirus, siadenovirus, and atadenovirus), and viruses with in each were classified into species, which can be further classified into serotypes. Cross-neutralization, genomic organisation, guanidine and cytosine (G+C) content, and host range were used to classify these organisms, with phylogenetic distance serving as the primary criterion. FAdVs are divided into 12 serotypes (FAdV-1 to 8a and 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 isolated from a variety of avian species, such as chickens, turkeys, geese, ducks, and pigeons. 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). It has been shown that hepatitis inclusion body (IBH), hydropericardium syndrome (HPS), and gizzard erosion (GE) are the most common diseases associated with FadV infection in chickens. The most often found FAdVs isolated from IBH cases are FAdVD and FAdV-E. The FAdV strains linked to HHS are FAdV-4 (FAdV-C), which are extremely harmful to hens. FAdV-1 (FAdV-A) has been isolated from the majority of gizzard erosion.

1. **PATHOGENESIS**

 FAdVs are ubiquitous in nature in poultry and 3- to 6-week-old broilers are most susceptible to FAdV infection. The FAdV infection process begins with the virus attaching to the host cell by connecting to certain receptors on the cell membrane surface. Coxsackievirus and adenovirus receptor (CAR), a transmembrane protein on the target cell surface, serves as a specific receptor for FAdV attachment. This attachment allows the virus to enter host cells via the fibre knob of the FAdV through endocytosis, where the genome is then transported to the cell nucleus [5]. The D2 domain of CAR (D2-CAR) serves as an active domain that binds to the new FAdV-4's short fibre. Once inside the cell, FAdV will begin its replication programme using the cellular machinery [6]. During this time, the viral components and those of the host cells interact (virus-host interaction) at both the protein and nucleic acid levels, resulting in apoptosis, autophagy, and inflammatory cytokine response, among other effects.

1. **INCLUSION BODY HEPATITIS**

 FAdVs mostly of the serotypes FAdV-2, FAdV-11 (species Fowl aviadenovirus D), and FAdV-8a and FAdV-8b (species Fowl aviadenovirus E) are responsible for IBH. FAdVs were thought to be opportunistic pathogens that caused concurrent infections with immunosuppressive agents, such as infectious bursal disease virus (IBDV) and chicken anaemia virus (CAV), that led to IBH. IBH primarily affects broilers up to five weeks of age, while it has irregularly been documented in layers and broiler breeders as well. Mortality during IBH outbreaks peaks within 3–4 days and can reach 10% and occasionally be as high as 30% [7]. Affected chickens may exhibit poor development, lethargy, prostration, ruffled feathers, and huddling behaviour as clinical indications. Clinically, IBH is extremely similar to HHS but is less severe. A severe form of hepatitis that causes the liver to be enlarged, friable, have a marble-like pattern, and have necrotic foci can be seen during post-mortem examination. Additionally, several investigations noted petechiae, necrosis, atrophy, and colour changes in the pancreas of birds with IBH. Swollen and haemorrhagic kidneys are also observed. Large regions of cellular necrosis and degeneration, lymphoid infiltration, and inclusion bodies are characteristic histological findings found in the liver of chickens with IBH. Atrophy of the bursa and thymus as well as lymphoid depletion in the bursa and spleen may be seen as additional abnormalities in the primary and secondary lymphoid tissues. The oval or hexagonal virus particles, which are 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.

1. **HEPATITIS – HYDROPERICARDIUM SYNDROME**

 Hydropericardium syndrome (HPS) also known as hydropericardium–hepatitis syndrome/Angara disease (in Pakistan) /Litchi heart disease (in India) or inclusion body hepatitis - hydropericardium syndrome (IBH–HPS), is an economically important poultry disease. The first reported cases of the syndrome near Karachi, Pakistan, in 1987 [8] were rapidly followed by massive outbreaks across the region,including a further spread to India, where it severely affected the intensive poultry husbandry during the early 1990 [9]. In India, HPS was first reported in the poultry belt of Jammu and Kashmir and Punjab in 1993. The main type of birds affected are broilers, aged 3–6 weeks [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. Mortality generally ranges from 40-60%. Typically, the mortality of HPS starts at three weeks of age and causes between 20-80% mortality. Fluid accumulation in the pericardial sac constitutes the most prominent gross pathological finding which is generally perceived as a pathognomonic feature of HHS. Birds are found to be lethargic,hurdling with ruffled feather,yellow droppings. In almost all cases, lesions are found in liver and kidney. The kidneys are pale, swollen and mottled appearance. Hemorrhage into the renal cortex may be found in some of the birds. Histopathological changes, similar to IBH, are principally found 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 while inflammatory as well as degenerative processes are also common in other organs, mainly in the heart, kidneys, lungs and intestine. Importantly, tropism for lymphoid tissue constitutes an important aspect of the pathogenesis of HHS strains, resulting in degeneration of lymphoid organs alongside with lymphocyte depletion, altogether highlighting the immunosuppressive potential of virulent FAdV-4 [11].

1. **ADENOVIRAL GIZZARD EROSION**

 Adenoviral gizzard erosion (AGE) in broilers is an emerging infectious disease with negative impact on flock productivity. Ono et al. (2001) who, for the first time, characterized the adenovirus as FAdV serotype-1 (FAdV-1) by polymerase chain reaction-restriction fragment length polymorphism as aetiological agent from condemned gizzards in the slaughter house. Although FAdV-8a or -8b were occasionally found in infected gizzards [12] but FAdV-1 strains were largely responsible for later outbreaks of the disease. Both, vertical as well as horizontal transmission of FAdV-1, and the subsequent clinical and pathological manifestation of AGE have been described in literature. Macroscopically, affected birds show gizzard lesions characterized by multiple brown or black areas of erosion of the keratinoid layer as well as inflammation and/or ulceration of the gizzard mucosa underneath [13]. In dead birds, sanguineous fluid in the gizzard as well as the proventricular and/or intestinal lumen and gizzard perforation can be observed. Histologically, infiltration with inflammatory cells such as macrophages and lymphocytes are observed in gizzard mucosa, submucosa and/or muscle layer in the affected gizzards.

1. **DIAGNOSTIC METHODS**

 By using electron microscopy (EM), viral infection can be quickly identified due to its distinctive appearance [14]. Staining suspicious cell cultures with hematoxylin and eosin to show typical intranuclear inclusions is another non-specific approach. The creation of an indirect enzyme-linked immunosorbent test (ELISA) able to detect less than 100 mean tissue-culture infective doses of adenovirus per gramme of liver tissue was a breakthrough in terms of group specificity. For more advanced typing, the virus must first be isolated. Chicken kidney (CK) and chicken embryo liver (CEL) cells are the cells where FAVs are most frequently isolated. If embryonic eggs are injected through the yolk sac method, embryonated eggs have also been reported to be a sensitive medium.

1. **MOLECULAR TECHNIQUES**: FAdVs were genetically divided into five distinct genotypes, A through E, which included all 12 serotypes. FAdV has been detected using PCR. To categorise viruses into species A through E and to detect genotypes within species, primers primarily based on the hexon gene are utilised. Nestled and real-time PCRs have been shown to boost sensitivity in comparison to virus isolation and can be used for quantification. As a result of the molecular analyses and sequencing of both the fibre and hexon proteins, we are better able to comprehend how FAdV strains have changed over time, which in turn enables us to create strategies to prevent disease outbreaks in poultry farms.
2. **PREVENTION AND CONTROL OF ADENOVIRAL INFECTION**

 FAdVs are extremely resistant to inactivation, remain for a very long time in the environment, and are transmitted both horizontally and vertically. The management and prevention of FAdV infection is more 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 in commercial vaccines preparation. Inactivated vaccines, attenuated live vaccines, and recombinant vaccines have been proven to be effective in protecting chickens against FAdV infection.

1. **Inactivated Vaccine:** Unlike live vaccinations, inactivated vaccines can cause an antibody response in pullets with high and uniform titres that remain for a long time, leading to long-lasting immunity. The inactivated oil-emulsion FAdV-4 vaccine was reported to be able to provide extensive crossprotection against different FAdV serotypes in both vaccinated birds and the offspring of vaccinated breeder birds. Inactivated and live bivalent fowl adenovirus (FAdV8b + FAdV11) breeder vaccines could provide broad-spectrum protection in chicks against IBH.
2. **Attenuated Live Vaccine:** Virulent FAdV-4 isolates were modified to become less pathogenic by adapting to growth 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. **Recombinent 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 viral proteins of FAdV, such as hexon, fiber-2, 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 SPF chickens with recombinant fiber-2 protein could produce a quicker and more potent immune response than the inactivated oil emulsion FAdV-4 vaccine.
4. **CONCLUSION**

 FAdV-related illness 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 viruses as CIAV, IBDV, ARV, ALV, and Marek's disease virus significantly worsen the severity of infection in the field. The use of current assays, such as enzyme-linked immunosorbent assays (ELISAs) based on whole virus particles, is currently a compromise at the expense of specificity and sensitivity of the test system, whereas the adequately type-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. This is due to the variety of FAdV serotypes, which frequently co-exist in the field and cause varying degrees of serological cross-reactions. Clinical diagnosis and molecular detection using polymerase chain reaction (PCR) are the best methods for diagnosing both diseases in birds. To control these illnesses, breeders must implement stringent biosecurity protocols, suitable management practises, and immunisation programmes. Therefore, continuing research into diseases associated with adenoviruses is essential to maintaining the growth of the chicken industry.

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