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Molecular detection of *Fowl adenovirus* from broiler chicken in Kerala#

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Abstract

Fowl adenovirus affects birds, mainly chicken, duck, geese, turkey, and pheasant. Fowl adenovirus comprises of 5 species and 12 serotypes, which causes a multitude of disease syndromes. The most economically significant diseases caused by Fowl adenovirus include inclusion body hepatitis and hepatitis-hydropericardium syndrome. In the present study, a total of 56 post-mortem samples were collected from broiler birds brought to the Department of Veterinary Microbiology, Department of Veterinary Pathology and some organised farms in Kerala. Post-mortem samples of liver, heart, lungs, kidney, bursa of Fabricius and spleen were collected for molecular detection. DNA was extracted from the pooled tissue samples using phenol chloroform method and subjected to polymerase chain reaction, employing primers targeting the hexon gene. Seven out of 56 samples turned out to be positive in PCR (12 per cent). The study revealed the increasing prevalence of Fowl adenovirus among the broiler population of Kerala.

***Keywords**: Fowl adenovirus, broiler, hexon, IBH-HPS*

Fowl adenoviruses (FAdVs) are double-stranded DNA viruses that lack an envelope and are classified in the genus *Aviadenovirus* of the family *Adenoviridae*. Avian adenoviruses were classified into five species (FAdV-A to FAdV-E) based on their molecular structure, and their serological associations were utilised to further categorise them into twelve serotypes (FAdV-1

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to 8 a and 8 b to 11) (Du *et al*., 2017). Among the various capsid associated proteins, hexon is a major viral protein with a neutralising epitope that is found to be serotype specific (Russell, 2009). The serotypes of FAdV are closely connected to the hexon gene sequence Niczyporuk (2016). In chicken, inclusion body hepatitis (IBH), hepatitis-hydropericardium syndrome (HHS), and gizzard erosions (GE) are the most significant diseases connected with FAdV infection. All the 12 serotypes cause IBH (Winterfield *et al*., 1973). Inclusion body hepatitis is most commonly seen in 3-5-week-old chicks and is characterised by mortality rates nearing 10 per cent. The major lesions of IBH include congestion and enlarged liver with necrosis, petechial haemorrhage, and basophilic intranuclear inclusion bodies (Xia *et al*., 2017). Hepatitis-hydropericardium syndrome, on the other hand, is mostly caused by FAdV serotype 4 (FAdV-4) strains. The mortality rate linked with HHS ranged between 30 to 70 per cent with the most common lesions being IBH, nephritis, and hydropericardium syndrome (Kim *et al*., 2008). In terms of GE, the most common causative agent is FAdV-1 and in few cases FAdV-4, FAdV-8 (FAdV-8a and -8b), and FAdV-11 are also involved. Fowl adenoviruses may easily spread horizontally and have been found in the kidney, trachea, nasal mucosa, and faeces (Grgic *et al*., 2006). The virus might be transmitted vertically through eggs, causing losses in healthy chicks without co-infections. They might, on the other hand, be undiscovered for a while before reactivated in young birds with immuno-suppression. The virus primarily targets organs such as heart, liver, lungs, kidney, spleen, and bursa Fabricius and hence, these tissues are utilised for molecular detection. Many studies on the prevalence and molecular characterisation of FAdV have established the presence of many serotypes of the virus circulating among the poultry population in India. As far as Kerala is concerned, no systematic investigations for the detection of FAdV have been undertaken. However, field veterinarians are reporting many cases with lesions suggestive of IBH and HHS among chicken. As a result, the current study was intended for the molecular detection of FAdV in Kerala, which could be beneficial in the development of suitable disease prevention strategies, including vaccination in future.

For the detection of FAdV, 56 pooled tissue samples (liver, heart, lungs, kidney, spleen, thymus and bursa of Fabricius) were collected from ailing/ recently dead birds submitted to the Departments of Veterinary Microbiology and Pathology, CVAS, Mannuthy. Tissues collected from suspected cases of FAdV outbreaks reported from Thiruvananthapuram, Wayanad, Palakkad, Thrissur and Ernakulam districts of Kerala also formed samples for the study.

Fowl adenovirus inactivated oil adjuvanted vaccine was procured from Ventri Biologicals, Maharashtra and was used as a control for detecting FAdV. Isopropyl myristate was used to separate the oil part from the vaccine as per the protocols of Yang *et al*. (2022). The tissue samples *viz*., heart, liver, lungs, kidney, spleen, and bursa of Fabricius were collected in PBS and stored at -80°C till downstream processing. Total DNA was extracted from the triturated tissue samples and vaccine using phenol – chloroform method. Concentration and purity of the DNA were measured using NanoDrop 2000C (Thermo Scientific). The samples showing DNA concentration above 1000 ng/µL and purity between 1.8 and 2 at 260/280 nm were chosen for PCR.

The primers specific to the hexon gene Niczyporuk (2016) were used for the detection of FAdV. The sequences and other parameters of the primers are depicted in table 1. Primers were custom synthesised (Sigma-Aldrich) and obtained in lyophilised form.

Table 1. *Fowl adenovirus* hexon gene specific primers

Name	Sequences	Product size	
FAdV F	5'-CBG CBT RCA TGT ACT GGT A -3'	897 bp	Zhang *et al*. (2017)
FAdV R	5'-AAT GTC ACN ACC GAR AAG GC-3'		

The optimisation of PCR conditions was achieved through gradient PCR (MJ Mini Bio-Rad thermal cycler), in which modifications of different time-temperature combinations of annealing and extension steps were used. The temperature gradient that provided the best results for amplification was selected for all downstream applications. The reaction was carried out in 0.2 mL PCR tubes which comprises template DNA, emerald Amp GT PCR master mix, primers and nuclease free water. Deoxyribonucleic acid extracted from vaccine was used as the positive control. The negative control consisted of nuclease-free water. The master mix prepared was spun briefly. Polymerase chain reaction was performed in a MJ Mini thermal cycler (Bio-Rad, USA). The amplification reaction occurred under the following conditions: initial denaturation at 95°C for 30 sec, followed by denaturation at 94°C for 2 min, annealing at 62°C for 45 sec, and extension at 72°C for 1.30 min. The final extension cycle was 72°C for 2 min. The PCR reaction was done upto 35 cycles. The PCR products were confirmed by submarine electrophoresis in two per cent agarose gel and visualised under a UV transilluminator and the results were documented in a gel documentation system (Bio-Rad, USA).

Samples were taken from birds with lesions in bursa of Fabricius, heart, liver, lungs, kidney, spleen, and bursa (Dutta *et al*., 2017). The samples were collected during post-mortem from birds that showed symptoms suggestive of FAdV infection, which included friable liver with focal fibrinoid necrosis. In the hepatic parenchyma, pin point or ecchymotic haemorrhages were occasionally detected. Accumulation of fluid in the pericardial sac was the most prominent lesion in the heart. Pulmonary congestion and oedema were noticed in the lungs. Kidneys were swollen, pale with petechial haemorrhages. Spleen was enlarged with haemorrhages. Discolouration and detachment of koilin layer was noticed in gizzard.

The DNA extracted from pooled tissue samples were subjected to PCR using primers targeting the hexon gene of FAdV. The hexon gene was used for primer designing in majority of the reported PCR techniques for the detection of FAdV (Asthana *et al*., 2012). As per Russel (2000), hexon genes were the main targets used for the serotyping of FAdV. Hexon is an essential adenovirus protein with a neutralising epitope and is serotype specific, hence FAdV serotyping is largely established by hexon gene sequencing (Russell, 2009). Hexon contains both group- and type-specific antigenic

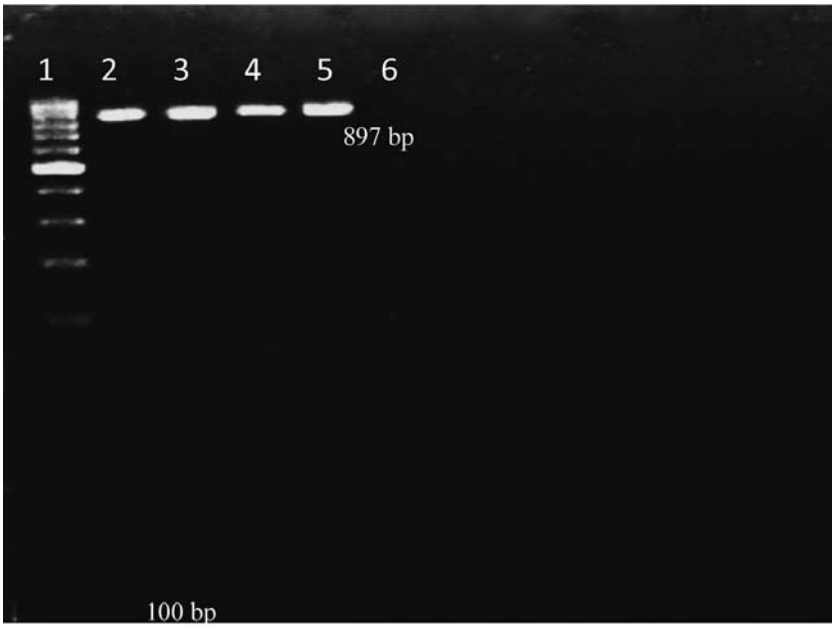

Fig. 1. Agarose gel electrophoresis image of the PCR amplicons of hexon gene (897 bp) (Lane 1: 100bp DNA marker, Lane 2: Positive control, Lane 3-5: Tissue samples, Lane 6: Negative control)

determinants (Toogood *et al*., 1992). Hexon protein has been related to hemagglutination, viral infectivity, antibody neutralisation, and pathogenicity, making it an important virulence factor in FAdV (El-Shall *et al*., 2022). The study analysed DNA extracted from pooled tissue samples were subjected to PCR, and DNA extracted from FAdV oil adjuvanated vaccine was used as the positive control. A total of 56 samples were tested and seven of them turned positive for FAdV, as indicated by an amplicon size of 897 bp corresponding to the hexon gene, in agarose gel electrophoresis (Fig.1.) Per cent positivity was calculated to be 12 per cent. The sequences were submitted in NCBI so the given accession numbers were OR 637463, OR 637464, OR 637465, OR 637466 and OR 637467.

The presence of FAdV was documented in all body secretions, however, the highest concentration was reported in faeces (Morshed *et al*., 2017). In chicken, among the various disease manifestations caused by FAdV, IBH and HPS are economically the most significant ones. Regardless of gender, broilers between the ages of 3 and 6 weeks were more likely to develop HHS (Asrani *et al*., 1997). The signs and lesions of IBH were detected in birds as young as nine days old, with the theory being that these instances were the result of vertical transmission in the first few days of life (Steer *et al*., 2011). The virus had affinity towards hepatic endothelial and lymphatic cells (Asthana *et al*., 2012).

The results indicated the increasing prevalence of FAdV among the broiler poultry population in Kerala. *Fowl adenovirus* is an emerging avian health hazard in India that threatens the poultry industry's survival and the livelihoods of millions of people who rely on it. The virus's potential to cause numerous diseases and its ability for rapid transmission, both by horizontal and vertical means, makes it an important concern. Apart from direct damage, FAdV also potentiates the pathogenesis of other poultry pathogens, especially when co-infected with infectious bursal disease, chicken infectious anemia and Marek's disease viruses. Since, a high prevalence of all the above viral pathogens have been established in Kerala, the results of the present study is definitely a matter of concern to the economy of poultry sector in Kerala.

Summary

In the present study, 12 per cent positivity for FAdV was obtained in PCR, which revealed the increasing prevalence of the virus in Kerala. Regular and systematic diagnosis of FAdV infections in poultry from different parts of Kerala is critical, which enable to develop a vaccine in the state against the disease in future.

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