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Molecular confirmation of fowl adenovirus associated with inclusion body hepatitis in upgraded variety native chicken in Tamil Nadu

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Abstract

Fowl adenoviruses (FAdV) have been associated with many diseases in chickens including inclusion body hepatitis (IBH), Hydropericardium syndrome (HPS), gizzard erosion and pancreatitis. The present study was carried out to confirm fowl adenovirus infection associated with inclusion body hepatitis in upgraded variety chicken in Namakkal district, Tamil Nadu. Affected chicken in the flock showed reduced feed intake, ruffled feathers, and diarrhea. The recorded mortality was 3.0%. Necropsy was conducted and liver tissue from five dead birds were collected for DNA extraction and histopathological examination. DNA was extracted, PCR amplification of L1 loop region of 897bp hexon gene using hexon A and hexon B primer was carried out and all the five samples were found positive for fowl adenovirus by PCR. Isolation of FAdV in primary chicken embryo liver cells and cytopathic effects including cell rounding, detachment, and honey comb appearance of liver cells was observed. Histopathological analysis of liver tissue showed the presence of basophilic intranuclear inclusions. This study confirms the presence of FAdV infection associated with inclusion body hepatitis in upgraded variety native chicken flock.

Keywords: Fowl adenovirus, Upgraded variety native chicken, Inclusion body hepatitis, PCR, Histopathology

Introduction

Poultry farming contributes significantly to food security and rural livelihoods, particularly through the rearing of native (ungraded) chicken varieties, which are well adapted to local agro-climatic conditions and low-input management systems. Despite their perceived hardiness, native chickens are increasingly affected by infectious diseases that compromise productivity and survivability. Among these, inclusion body hepatitis (IBH) has emerged as an important viral disease affecting chickens across diverse production systems. Inclusion body hepatitis is an acute disease characterized by sudden mortality, depression, and hepatic lesions, primarily affecting young chickens. The disease is caused by fowl adenoviruses (FAdVs), which belong to the genus *Aviadenovirus* under the family *Adenoviridae* (Hess, 2000) [14]. FAdVs are double-stranded DNA and non-enveloped viruses, and are widely prevalent in worldwide in poultry populations. Several studies have demonstrated the association of FAdVs with IBH, hydropericardium-hepatitis syndrome, gizzard erosion, and other disease conditions in chickens (Sharma *et al.*, 2023; Matos *et al.*, 2024) [15, 5].

Although IBH was traditionally considered a disease of commercial broiler and breeder flocks, recent investigations have reported its occurrence in backyard and native chicken populations, indicating wider circulation of FAdVs than previously recognized (Dutta *et al.*, 2022; Ramakrishnan *et al.*, 2024) [3, 12]. Native chickens are often reared under semi-intensive or backyard systems with limited biosecurity and minimal vaccination coverage, which may facilitate both horizontal and vertical transmission of FAdVs (Smyth and McNulty, 2008) [16]. Subclinical infections in such flocks may serve as reservoirs, posing a risk to organized poultry production systems.

Diagnosis of IBH is conventionally based on clinical signs, gross pathological changes, and

histopathological demonstration of characteristic intranuclear inclusion bodies in hepatocytes. However, these methods are not definitive, as similar hepatic lesions may be caused by other viral or toxic etiologies. To overcome these limitations, molecular diagnostic techniques, particularly polymerase chain reaction (PCR), have been widely adopted for the sensitive and specific detection of FAdV DNA from clinical samples (Meulemans *et al.*, 2004; Sharma *et al.*, 2023) [6, 15]. PCR targeting the hexon gene has proven especially useful for confirmation and molecular epidemiological studies of FAdV-associated IBH.

Despite increasing reports of IBH in commercial poultry, molecular confirmation of fowl adenovirus infection in ungraded native chickens remains poorly documented, particularly in India. Therefore, the present study was undertaken to molecularly confirm fowl adenovirus associated with inclusion body hepatitis in ungraded variety native chickens, thereby contributing to the understanding of FAdV circulation and emphasizing the need for molecular surveillance in native poultry populations.

Materials and Methods

Study area and birds

The present investigation was carried out in an upgraded native chicken flock from Namakkal district, Tamil Nadu. Affected birds showed clinical signs such as reduced feed intake, ruffled feathers, and diarrhoea. The recorded mortality in the affected flock was 3.0%.

Sample collection and necropsy

A total of five recently dead birds were subjected to detailed necropsy examination. Gross lesions were recorded with special reference to liver changes suggestive of inclusion body hepatitis. Liver tissue samples were aseptically collected from all five birds for molecular and histopathological examination.

Histopathological examination

Representative portions of liver tissue were fixed in 10% neutral buffered formalin, processed routinely, embedded in paraffin, sectioned at 4–5 µm thickness, and stained with hematoxylin and eosin (H&E). The stained sections were examined under a light microscope for characteristic histopathological lesions, including basophilic intranuclear inclusion bodies and hepatic cellular degeneration.

Polymerase chain reaction (PCR)

Genomic DNA was extracted from liver tissue samples using a commercial Qiagen DNA extraction kit following the manufacturer's instructions. The extracted DNA was quantified and stored at –20 °C until further use. PCR was performed for the detection of fowl adenovirus (FAdV) targeting the L1 loop region of the hexon gene. Amplification was carried out using hexon A and hexon B primers, which amplify an expected product size of 897 bp. The PCR cycle conditions were standardized as per Meulemans *et al.*, (2001) [8]. PCR products were resolved by electrophoresis on 1.5% agarose gel containing ethidium bromide. The amplified products were visualized under a UV transilluminator and documented using a gel documentation system.

Virus isolation in chicken embryo liver cells

Virus isolation was attempted from PCR-positive liver samples using primary chicken embryo liver (CEL) cell cultures (Barua and Rai, 2003) [1]. The inoculated cultures were observed daily for cytopathic effects (CPE) such as cell rounding, detachment, and honeycomb appearance of liver cells.

Results and Discussion

In this study, the affected upgraded native chicken flock showed clinical signs including reduced feed intake, ruffled feathers, and diarrhoea. The overall mortality recorded was 3.0%. These findings are consistent with earlier reports indicating that fowl adenovirus (FAdV) associated inclusion body hepatitis generally causes low to moderate mortality but significant economic losses due to poor performance and sudden deaths, particularly in young chickens (McFerran and Adair, 2003; Schachner *et al.*, 2018) [7, 13]. Necropsy of five dead birds revealed enlarged, pale, friable livers with focal to diffuse hemorrhages. Such hepatic lesions are considered characteristic of inclusion body hepatitis and have been widely documented in FAdV infections (Nakamura *et al.*, 2011; Matos *et al.*, 2016) [10, 5]. The absence of significant lesions in other organs supports the primary hepatic tropism of the virus. Histopathological examination of liver sections revealed hepatocellular degeneration, necrosis, and the presence of prominent basophilic intranuclear inclusion bodies within hepatocytes (Fig.1). These intranuclear inclusions are pathognomonic for adenoviral infection and represent sites of viral replication (Ojkic *et al.*, 2008) [11]. Similar histological findings have been consistently reported in IBH outbreaks in commercial and native chicken flocks in India and other countries (Dahiya *et al.*, 2002; Mittal *et al.*, 2014) [2, 9].

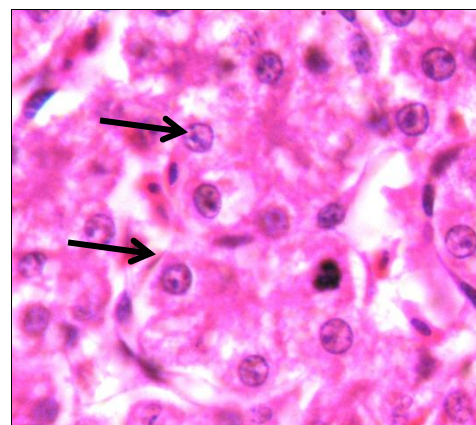


Fig 1: Basophilic intranuclear inclusions in hepatocytes

PCR amplification targeting the L1 loop region of the hexon gene yielded an expected amplicon size of 897 bp in all five liver samples, confirming the presence of fowl adenovirus infection (Fig.2). The hexon gene is highly conserved and widely used for molecular detection and characterization of FAdV strains (Meulemans *et al.*, 2001; Schachner *et al.*, 2016) [8, 14]. The detection of FAdV by PCR corroborated the gross and histopathological findings observed in the present study.

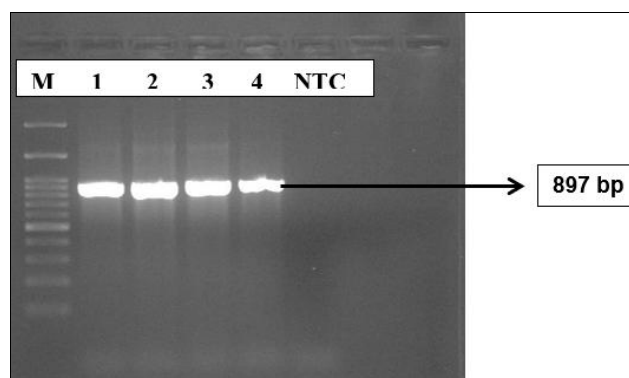


Fig.2. Agarose gel electrophoresis showing 897 bp amplified PCR product of hexon gene of fowl adenovirus isolated from upgraded variety native chicken (M- 100bp DNA ladder, Samples: Lane 1 to 4 and NTC- No template control)

PCR positive liver samples inoculated into primary chicken embryo liver (CEL) cell cultures produced characteristic cytopathic effects, including cell rounding, detachment, and honeycomb appearance of hepatocytes. These cytopathic changes are typical of FAdV replication in primary liver cell cultures and have been described by several investigators (McFerran *et al.*, 1976; Hess, 2000) [7, 4]. Successful virus isolation further confirmed the etiological role of FAdV in the observed outbreak. The confirmation of FAdV associated inclusion body hepatitis in an upgraded native chicken flock highlights the susceptibility of non-commercial and improved backyard poultry to adenoviral infections. Vertical transmission from breeder flocks and immunosuppression due to concurrent infections or management stress have been implicated as major predisposing factors for IBH outbreaks (Toro *et al.*, 2000; Schachner *et al.*, 2018) [17, 13]. The findings emphasize the need for regular surveillance, molecular diagnosis, and implementation of appropriate biosecurity and vaccination strategies to prevent economic losses in native and upgraded poultry systems.

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Conflict of Interest

Not available

Financial Support

Not available

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