RESEARCH ARTICLE

Isolation and Molecular Detection of Fowl Adenovirus causing Inclusion Body Hepatitis utilizing Specific Pathogen-Free Embryonated Chicken Eggs

Mehak Tikoo[1], Shubhangi Warke[1]*, Prashant Sonkusale[2], Rohan Fulsundar[2]

Abstract

Indian poultry industry is rapidly growing with an increase in demand among consumers, thus, prevailing control and prevention measures of poultry diseases need to be improved along with the implementation of efficient disease diagnostics. Fowl adenovirus (FAdV) is responsible for causing inclusion body hepatitis (IBH) in broiler chickens, incurring huge economic losses in the poultry industry. A total of 11 pooled liver homogenate samples from an outbreak of IBH in a poultry farm of Katol near Nagpur were collected and processed by conventional PCR based on the detection of hexon gene and isolation in SPF embryonated chicken eggs. Out of 11 samples, 05 (45.45%) were found to be positive for FAdV by PCR. Inoculated embryos were haemorrhagic with prominent lesions in the liver. All the viral isolates showing characteristic pathological lesions were detected positive by PCR. Histopathology revealed basophilic intranuclear inclusion bodies in the liver of infected embryos. Continuous surveillance of FAdVs in broiler chicken flocks is necessary for early detection of diseases.

Key words: Chorioallantoic membrane (CAM), Chicken embryonated eggs, Hexon gene, Histopathology, Inclusion body hepatitis.

Ind J Vet Sci and Biotech (2025): 10.48165/ijvsbt.21.4.10

Introduction

The poultry sector has witnessed substantial growth in the past few years in many countries and continues to grow rapidly with increasing demand from consumers. This increased demand has led to the threat of transmission of diseases and thus incurring economic losses due to a lack of effective control strategies (Lebdah *et al.,* 2022). Fowl adenovirus (FAdV) is the causative agent of a wide range of diseases such as inclusion body hepatitis, hydropericardium syndrome, gizzard erosions and many respiratory manifestations (Dutta *et al,* 2023; Shankar *et al.,* 2022; Islam *et al.,* 2023). FAdVs are non-enveloped double-stranded DNA viruses with a 43-45 kb genome size belonging to the *Aviadenovirus* genus of the *Adenoviridae* family. FAdVs are categorized into 12 serotypes (1,2,3,4,5,6,7,8a,8b,9,10,11) and 5 species (A-E) based on restriction enzyme digestion and sequencing (Kumar *et al.,* 2022; Islam *et al.,* 2023; Rashid *et al.,* 2024).

Inclusion body hepatitis is a significant disease of broiler chickens, amounting to high mortality and huge economic losses in the poultry industry (Alzuheir *et al.,* 2021). Previously, virus isolation has been used to identify and observe the cytopathic effects of FAdVs (Shinde *et al.,* 2020). Molecular detection based on amplification of the hypervariable region (L1-L4) of the hexon gene is useful in the detection of inclusion body hepatitis in affected poultry birds (Safawat *et al.,* 2023; Nunez *et al.,* 2024). Combining virus isolation with PCR may be useful in the detection and characterization of fowl adenovirus responsible for causing inclusion body hepatitis in broiler chickens. The present study aimed to isolate fowl adenovirus from infected broiler chickens utilizing Specific pathogen free (SPF) embryonated chicken eggs, followed by subsequent molecular detection using a PCR approach.

[1]Department of Veterinary Microbiology, Nagpur Veterinary College, MAFSU, Nagpur-440006, Maharashtra, India

[2]Department of Veterinary Pathology, Nagpur Veterinary College, MAFSU, Nagpur-440006, Maharashtra, India

Corresponding Author: Dr. Shubhangi R. Warke, Professor & Head, Department of Veterinary Microbiology, Nagpur Veterinary College, MAFSU, Seminary Hills, Nagpur-440006, India. e-mail: shubhangi_warke@rediffmail.com

How to cite this article: Tikoo, M., Warke, S., Sonkusale, P., & Fulsundar, R. (2025). Isolation and Molecular Detection of Fowl Adenovirus Causing Inclusion Body Hepatitis utilizing Specific Pathogen-Free Embryonated Chicken Eggs. Ind J Vet Sci and Biotech, 21(4), 50-54.

Source of support: Nil

Conflict of interest: None

Submitted 12/04/2025 **Accepted** 29/04/2025 **Published** 10/07/2025

Materials and Methods

This study was conducted during the year 2024-2025 using liver tissue samples collected from a broiler chicken flock affected with an outbreak of inclusion body hepatitis in Katol near Nagpur, Maharashtra (India). The samples were processed at the Department of Veterinary Microbiology, Nagpur Veterinary College, Nagpur.

Sample Collection and Processing

A total of 11 pooled liver tissue samples weighing 10 g each were collected from an outbreak of inclusion body hepatitis in a flock of 1000 broiler chickens, showing several clinical signs, comprising of depression, variable degrees of dullness and drowsiness, decreased body weight, reduced feed intake, respiratory distress and ruffled feathers. During post-mortem examination significant gross lesions were observed in the infected broiler chicken, which were characteristics of IBH. Liver tissue samples were collected in sterile zip-lock bags and transported to the laboratory on ice. The samples were weighed at 10 mg and homogenized in 1 mL of sterile phosphate-buffered saline solution (PBS; pH 7.4) followed by centrifugation at 12000×g for 20 min at 4°C. The supernatant was collected and filtered using a 0.45 µm pore size syringe filter, and an antibiotic-antimycotic solution (Gibco, USA) was added to the filtered liver tissue inoculum and incubated at room temperature for 45 min. The liver tissue inoculum was then stored at -20 °C until further processing (Sohaimi *et al.,* 2018; Sadekuzzaman *et al.,* 2024).

DNA Extraction and Amplification of the Hexon Gene

Liver tissue samples were subjected to DNA extraction by using Wizard® Genomic DNA purification kit (Promega, USA) as per the manufacturer's instructions with slight modifications. DNA samples obtained were subjected to the amplification of the hexon gene using published primers (Muelsman *et al.,* 2001). A PCR reaction of 20 µL comprising 10 µL of 2X PCR master mix, 0.75 µL of forward and reverse primers each (10 pmol), 2 µL of DNA template and the remaining 6.5 µL nuclease-free water was made. The reaction was run using thermocycling conditions as follows: Initial denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at 94°C for 45 seconds, annealing at 61°C for 1 min, extension at 72°C for 1 min and a final extension step at 72°C for 10 min. The PCR products were run on a 1.5% agarose gel prepared in 1X TAE buffer containing 1 µL/mL ethidium bromide and electrophoresed at 5V/cm of the gel. The bands were visualised in a gel-documentation system (Syngene, UK).

Isolation of FAdV in SPF-Embryonated Eggs via Chorioallantoic Membrane Route

Virus isolation was performed with 2 successive passages in 10-day-old embryonated chicken eggs. Liver tissue inoculum (100 µL) was injected into 10-day-old SPF free chicken embryonated eggs via chorioallantoic membrane (CAM) route. Samples showing characteristic lesions of inclusion body hepatitis and confirmed by PCR were injected into 05 SPF free chicken embryonated eggs each, and the negative control containing sterile PBS (pH 7.4) with antibiotics was injected into 05 chicken embryonated eggs. The inoculated eggs were incubated at 37°C in an incubator with 50-55 % humidity for 10 days and candled twice daily for checking viability. Embryos which died after or during the initial 24 h were discarded as such, while other embryos which survived after 48 h were necropsied. The embryos surviving till day 10 post-infection were necropsied, and the allantoic fluid and liver tissue samples were collected for DNA extraction and histopathology, respectively (Sadekuzzaman *et al.,* 2024; Chavan *et al.,* 2024).

Histopathological Examination

Liver tissue samples were collected and preserved in 10% neutral buffered formalin, followed by dehydration, cleaning and impregnation in paraffin wax before being microtome into 4 µm thick sections. Haematoxylin and eosin stains were used to stain the embedded tissue using standard protocols (Suvarna *et al.,* 2018). Histopathological observations were performed under a light microscope (Nikon Eclipse) and imaging was done with Mosaic 2.0 computational imaging software.

Confirmation of Virus Isolates by PCR

FAdV virus isolates were confirmed by hexon gene by conventional PCR using the published primers (Muelsman *et al.,* 2001) with some slight modifications.

Results and Discussion

Clinical Signs and Gross Lesions of FAdV

The infected birds manifested variable clinical symptoms such as huddling, drowsiness, dullness, respiratory depression, anorexia, yellowish diarrhoea, weight loss and death occurring after 4-5 days of onset of the disease. Post-mortem lesions revealed friable, enlarged liver with a pale appearance and presence of grey to white necrotic foci along with petechial haemorrhages. The heart was congested and pericardial effusion was seen. Kidneys were enlarged, pale and congested. Spleen was congested, swollen and mottled in appearance (Fig. 1). Mortality in the affected flock was reported to be 6% in the present study. Birds of the age group of 21-42 days were most affected. Previous studies by Schachner *et al.* (2018), Abghour *et al.* (2019) and Niczyporuk *et al.* (2022) reported similar findings.

Molecular Detection of the Hexon Gene

Out of the 11 liver tissue samples subjected to hexon gene loop 1 PCR, 05 (45.45%) produced an amplicon size of 897 bp (Fig. 2A), confirming infection with fowl adenovirus. Similar findings were reported by Sadekuzzaman *et al.* (2024) and Safwat *et al.* (2022), revealing a prevalence rate of 46.51% and 40% of FAdV, respectively, by conventional PCR.

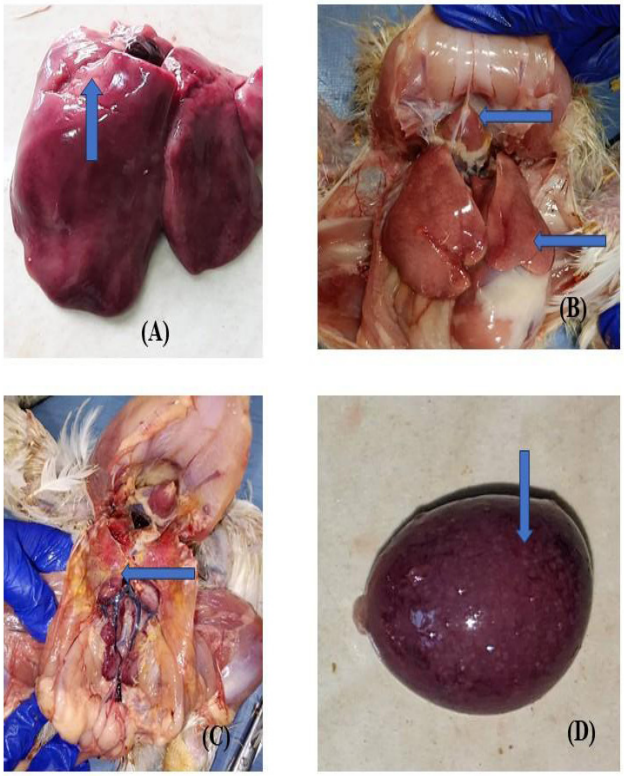

Fig. 1: Gross lesions of inclusion body hepatitis in affected chickens (A) Greyish-white focal necrosis observed in liver (arrow) (B) Enlarged, congested and straw-coloured fluid in pericardial sac (arrow) Pale appearance of liver with necrotic foci and petechial haemorrhages (arrow) (C) Enlarged and congested kidneys with prominent tubules (arrow) (D) Congested and swollen spleen with mottled appearance (arrow)

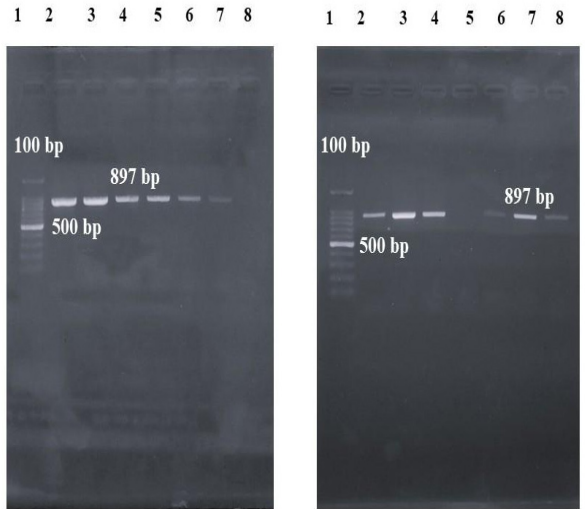

Fig. 2: Amplification of the hexon gene by conventional PCR. **(A)** PCR amplification of infected flock (left side). Lane 1: 100bp DNA Ladder, Lane 2: Positive control, Lane 3: Sample no. 2, Lane 4: Sample no. 3, Lane 5: Sample no. 5, Lane 6: Sample no. 8, Lane 7: Sample no. 11, Lane 8: Negative control. **(B)** PCR amplification of infected embryonated eggs (right side). Lane 1: 100bp DNA Ladder, Lane 2: Sample no. 2, Lane 3: Sample no. 3, Lane 4: Positive control, Lane 5: Negative control, Lane 6: Sample no. 11, Lane 7: Sample no. 5, Lane 8: Sample no. 8

Pathological Changes in SPF Embryonated Eggs

Some of the inoculated eggs died after 48 h and were necropsied to reveal thickening of the chorioallantoic membrane as well as subcutaneous haemorrhages visible all over the body of the embryos. Most of the inoculated embryos survived till day 10 post-infection and showed friable, enlarged livers with prominent necrotic foci. Kidneys were enlarged and congested. The control eggs inoculated with PBS showed normal growth with no gross lesions characteristic of inclusion body hepatitis observed in the internal organs, such as liver, kidney, heart and CAM (Fig. 3). Studies by Safwat *et al.* (2022) and Sohaimi *et al.* (2018) reported thickening of CAM, petechial haemorrhages, friable, enlarged, and reddish to yellow necrotic foci seen in liver.

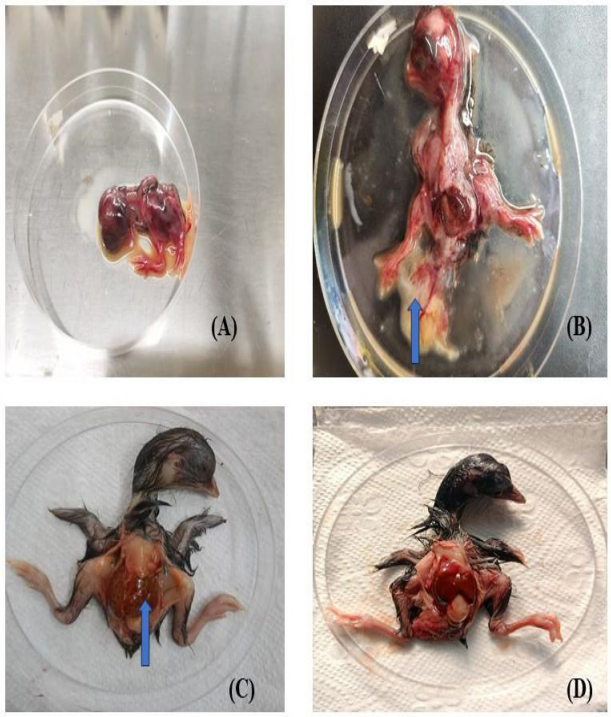

Fig. 3: Pathological lesions of fowl adenovirus homogenates seen in 10-day old inoculated SPF embryonated eggs (A) Subcutaneous haemorrhages seen all over the body of embryo 2 days pi (B) Thickening of chorioallantoic membrane seen in infected embryo 4 days pi (arrow) (C) Enlarged liver with congestion and presence of necrotic foci 10-day pi (arrow). Stunted growth with poor fathering is seen. (D) Uninfected embryo with no pathological changes in internal organs. Normal growth and good feathering are seen.

Histopathological Findings

Histopathological examination of liver tissue samples obtained from FAdV-infected embryonated eggs revealed severe hepatic necrosis and presence of basophilic intranuclear inclusion bodies in hepatocytes (Fig. 4). Earlier studies by Wang *et al.* (2023), Thabet *et al.* (2023) and Chavan *et al.* (2024) reported hepatic cell necrosis and large basophilic intranuclear inclusion bodies in hepatocytes.

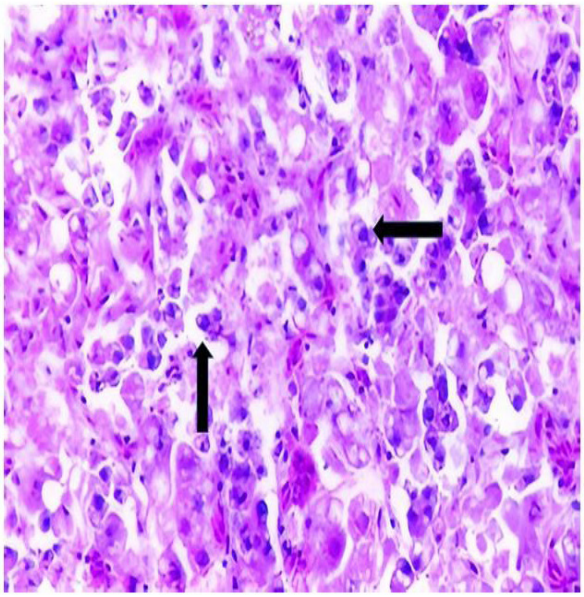

Fig. 4: Microscopic lesions seen in the infected embryo liver stained with Haematoxylin and Eosin stain (40X). Basophilic intranuclear inclusion bodies seen in the liver (arrow)

Confirmation by PCR

Infected allantoic fluid and liver tissue samples subjected to conventional PCR targeting the loop 1 fragment of the hexon gene, confirmed all the isolates positive for FAdV, producing an amplicon size of 897 bp (Fig. 2B).

Conclusion

The study suggested that FAdV infections, predominantly inclusion body hepatitis, were common in the broiler chicken flock of Nagpur. Successful isolation of fowl adenovirus with manifestation of significant gross and microscopic lesions was observed using SPF-embryonated eggs. Virus isolation, histopathology and molecular approaches such as PCR were efficient in the detection of fowl adenovirus responsible for causing inclusion body hepatitis in broiler chickens. Further research is needed to characterize FAdV serotypes associated with IBH in broiler chickens. Implementation of farm control and prevention measures is necessary to prevent future outbreaks of FAdVs.

Acknowledgement

The authors are thankful to the Associate Dean, Nagpur Veterinary College, Nagpur and the Department of Veterinary Microbiology for their support and facilities provided for this research.

References

Abghour, S., Zro, K., Mouahid, M., Tahiri, F., Tarta, M., Berrada, J., & Kichou, F. (2019). Isolation and characterization of fowl aviadenovirus serotype 11 from chickens with inclusion body hepatitis in Morocco. *PloS one, 14*(12),1-14.

Alzuheir, I.M., Jalboush, N.H., Fayyad, A.F., & Daibes, R.A. (2021). Phylogenetic analysis and pathological characterization of fowl adenovirus isolated during inclusion body hepatitis outbreak in Tubas, Palestine. *Veterinary Research Forum: An International Quarterly Journal, 12*(4), 511-514.

Chavan, V.G., Awandkar, S P., Kulkarni, R.C., Chavhan, S.G., Suryawanshi, R.D., Jadhav, R.K., Kulkarni, M.B., & Agnihotri, A.A. (2024). Isolation and molecular characterization of fowl adenovirus from inclusion body hepatitis cases in Western India during 2019-2021. *Iranian Journal of Veterinary Research, 25*(1), 62-68.

Dutta, B., Pathak, D.C., Barman, N.N., Hazarika, N., & Goswami, S. (2023). Temporo-spatial sero-epidemiology of fowl adenovirus (FAdV) infection causing inclusion body hepatitis-hydropericardium syndrome (IBH-HPS) in broiler population of Assam. *Indian Journal of Animal Research, 57*(1), 102-107.

Islam, M.N., Rahman, M.M., Rahman, M.K., & Alam, J. (2023). First evidence of fowl adenovirus induced inclusion body hepatitis in chicken in Bangladesh. *Canadian Journal of Infectious Diseases and Medical Microbiology, 2023*(1), 7253433.

Kumar, A., Ranjan, K., Prasad, M., Mahajan, N.K., & Maan, S. (2022). Molecular detection, phylogenetic analysis and differentiation of fowl adenovirus from chickens with inclusion body hepatitis in Haryana. *Indian Journal of Veterinary Science and Biotechnology, 18*(2), 113-117.

Lebdah, M., Alshaya, D.S., Jalal, A.S., Mousa, M.R., Radwan, M.M., Samir, M., Adel, A., Albaqami, N.M., El-Saadony, M.T., El-Tarabily, K.A., & El Basrey, Y.F.H. (2022). Molecular characterization of aviadenovirus serotypes and pathogenicity of the identified adenovirus in broiler chickens. *Poultry Science, 101*(12), 101918.

Meulemans, G., Boschmans, M., Berg, T.P., & Decaesstecker, M. (2001). Polymerase chain reaction combined with restriction enzyme analysis for detection and differentiation of fowl adenoviruses. *Avian Pathology, 30*(6), 655-660.

Niczyporuk, J.S., & Kozdruń, W. (2022). Current epidemiological situation in the context of inclusion body hepatitis in poultry flocks in Poland. *Virus Research*, *318,* 198825.

Nuñez, L., Santander-Parra, S., Catroxo, M., Astolfi-Ferreira, C.S., Loor-Giler, A., & Ferreira, A.P. (2024). Fowl adenovirus 8a isolated from chickens with runting and stunting syndrome induces inclusion body hepatitis and hepatitis-hydropericardium syndrome in chicken embryos. *Veterinary World, 17*(11), 2556-2566.

Rashid, F., Xie, Z., Wei, Y., Xie, Z., Xie, L., Li, M., & Luo, S. (2024). Biological features of fowl adenovirus serotype-4. *Frontiers in Cellular and Infection Microbiology, 140,* 1370414.

Sadekuzzaman, M., Miah, M.S., Parvin, R., Haque, M.E., Islam, T.R., Sigma, S.H., Hossain, M.G., Hayat, S., Hossain, M.T., & Islam, M.A. (2024). Pathological investigation, molecular characterization and first-time isolation of the predominant serotypes of fowl adenovirus (FAdV-D and E) from commercial poultry in Bangladesh. *Frontiers in Microbiology, 15,* 1490255.

Safwat, M.M., Sayed, A.S.R., Ali Elsayed, M.F., & Ibrahim, A.A.E.H. (2022). Genotyping and pathogenicity of fowl adenovirus isolated from broiler chickens in Egypt. *BMC Veterinary Research, 18*(1), 325.

Schachner, A., Matos, M., Grafl, B., & Hess, M. (2018). Fowl adenovirus-induced diseases and strategies for their control - A review on the current global situation. *Avian Pathology, 47*(2), 111-126.

Shankar, K.S., Priyanka, E., Mathivanan, B., Kumar, B.P., Mukhopadhayay, S.K., Mondal, S., & Kannaki, T.R. (2022).

Molecular epidemiology of fowl adenovirus (FAdV) from inclusion body hepatitis (IBH) incidences from Indian broilers revealed the prevalence of serotypes of FAdV-D and E. *Indian Journal of Animal Research, 56*(1), 1-6.

Shinde, D.B., Anil, LT., Santosh, S.K., Neeti, S., Ashok, R., Vinay, R., Prasad, W., Teguh, Y.P., & Satish, S.T. (2020). Molecular and pathotypic characterization of fowl adenovirus associated with inclusion body hepatitis in Indian chickens. *The Indian Journal of Animal Sciences, 90,* 982-986.

Sohaimi, N.M., Bejo, M.H., Omar, A.R., Ideris, A., & Isa, N.M. (2018). Hexon and fiber gene changes in an attenuated fowl adenovirus isolate from Malaysia in embryonated chicken eggs and its infectivity in chickens. *Journal of Veterinary Science, 19*(6), 759-770.

Suvarna, K.S., Layton, C., & Bancroft, J.D. (2018). *Bancroft's Theory and Practice of Histological Techniques. E-Book*. 7th edn., Elsevier Health Sciences. China, pp. 1-603.

Thabet, A.M., Alzuheir, I.M., Fayyad, A.F., Kheimar, A.M., & Jalboush, N.H. (2023). Occurrence and phylogenetic analysis of fowl adenovirus E in broiler flocks from Gaza Strip Palestine. *The Journal of Infection in Developing Countries, 17*(04), 565-570.

Wang, T., Meng, F., Chen, C., Shen, Y., Li, P., Xu, J., Feng, Z., Qu, X., Wang, F., Li, B., & Liu, M. (2023). Pathogenicity and epidemiological survey of fowl adenovirus in Shandong Province from 2021 to 2022. *Frontiers in Microbiology, 14,* 1166078.