RESEARCH ARTICLE

Pathogenicity Assessment and Vaccine Efficacy of Fowl Adenovirus Serotype 4 and 11 Responsible for Inclusion Body Hepatitis Hydropericardium Syndrome in Broilers

Kuldip V. Joshi[1]*, Chandrakant J. Dave[1], Harshrajsinh B. Joddha[2], Bharat B. Bhanderi[2], Dinesh J. Ghodasara[1], Digjay V. Kabariya[1]

Abstract

This study aimed to assess the pathogenicity and vaccine efficacy of fowl adenovirus serotypes 4 and 11, causing inclusion body hepatitis hydropericardium syndrome in broilers. The study was carried out on 144-day-old Cobb broiler chicks divided into six experimental groups, each of 24 birds. Group, I birds served as a control without any vaccine or viral challenge, while birds of Group II were vaccinated with commercial vaccine on 6th day. Group III and IV birds were non-vaccinated and challenged with 107 TCID50 of FAdV serotype 4 and 11, respectively, on the 27th day. In contrast, Group V and VI birds were vaccinated on the 6th day and challenged with 107 TCID50 of FAdV serotype 4 and 11 on the 27th day. The challenge of FAdV serotype 4 led to 66.66% mortality, and serotype 11 produced 5.00% mortality in the experimental groups. The challenge of the virus led to the production of clinical signs such as depression, huddling, reduced feed intake, reluctance to move, and terminal gasping before death. Characteristic gross lesions of IBH-HPS were observed in birds that died after the challenge of serotypes 4 and 11 of FAdV. The liver was enlarged with focal areas of necrosis, subcapsular hemorrhages, and mild fatty changes. The heart was flabby, congested, and showed hydropericardium with an accumulation of clear watery to a yellow jelly-like fluid in the pericardial sac. The kidneys were enlarged and hemorrhagic. On microscopic examination, the liver showed multifocal areas of necrosis with infiltration of mononuclear cells, mild fatty changes and large basophilic intranuclear inclusion bodies. The birds that died after the challenge of the virus were positive for the presence of FAdV upon PCR. The vaccine efficacy study indicated that the vaccine provided satisfactory protection against Fowl Adenovirus challenge in experimental groups V and VI.

Keywords: Fowl adenovirus, Inclusion Body Hepatitis, Poultry, Vaccine efficacy.

Ind J Vet Sci and Biotech (2022): 10.48165/ijvsbt.18.4.20

Introduction

Inclusion body hepatitis (IBH) primarily affects broilers up to five weeks of age in the field. IBH-hepatitis hydropericardium syndrome (HPS) is thought to be a secondary infection rather than a primary pathogen as described in studies where IBH-HPS was seen with other concurrent infections such as Mycotoxicosis, *Escherichia coli* infections, Infectious Bursal Disease, Coccidiosis, Chronic Respiratory Disease, and Ranikhet Disease (Mittal et al., 2014). The Fowl Adenovirus (FAdV) belongs to the genus Aviadenovirus of the Adenoviridae family. FAdV is a non-enveloped DNA virus with icosahedral symmetry. FAdVs have been classified into a total of 12 serotypes which are serotypes 1-7, 8a, 8b, 9-11 belonging to 5 species (FAdVA-E). FAdV serotypes 2, 11, 8a, and 8b are involved more frequently in developing inclusion body hepatitis.

On the other hand, FAdV serotype four is predominantly isolated from cases of Hydropericardium syndrome (Schachner et al., 2018). In recent years, the clinical cases of IBH and HPS have been increasing all over the world, resulting in considerable economic losses in many countries, such as the USA, India, Canada, China, Korea, and Japan. In prior studies,

[1]Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Anand-388001, Gujarat, India.

[2]Department of Veterinary Microbiology, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Anand-388001, Gujarat, India.

Corresponding Author: Kuldip V. Joshi, Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Anand-388001, Gujarat, India., e-mail: kuldipjoshi455@gmail.com

How to cite this article: Joshi,V. Kuldip, Dave,J. Chandrakant, Joddha,B. Harshrajsinh,Bhanderi,B. Bharat, Ghodasara,J. Dinesh, Kabariya,V. Digjay. Experimental Pathogenicity Assessment and Vaccine Efficacy of Fowl Adenovirus Serotype 4 and 11 Responsible for Inclusion Body Hepatitis Hydropericardium Syndrome in Broilers.(2022). Article Title. Ind J Vet Sci and Biotech. 18(4), 97-103.

Source of support: Nil

Conflict of interest: None.

Submitted: 25/05/2022 **Accepted:** 29/08/2022 **Published:** 10/09/2022

a great variation has been recorded in the pathogenicity potential of various field serotypes. The present study was designed to assess the pathogenicity of field strains of FAdV serotypes 4 and 11 and the vaccine's efficacy against the challenge of both serotypes in commercial broiler birds.

MATERIALS AND METHOD

Inoculum Preparation and Experimental Design

Confirmed field strains of FAdV 4 and 11 were grown on chicken embryo liver cell line as per the method described by Sohaimi et al. (2019). The TCID50 endpoint was calculated by Reed and Muench formula (Reed and Muench, 1938).

The experimental protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of the College, KU, Anand (India). It was undertaken as per the guidelines of the CPCSEA, Government of India, New Delhi. The experimental study was conducted on 144-day-old Cobb-400 broiler chicks. Briefly, the experimental birds were randomly divided into six experimental groups with 24 birds in each group. Group I birds served as a control without any vaccine or viral challenge, while birds of Group II were vaccinated with commercial vaccine on 6th day by subcutaneous injection. The birds of Group III and IV were non-vaccinated and challenged with 107 TCID50 of FAdV serotype 4 and 11, respectively, on the 27th day. In contrast, Group V and VI birds were vaccinated on 6th day and challenged with 107 TCID50 of FAdV serotype 4 and 11, respectively, on 27th day by deep intramuscular injection in the pectoral muscles by a 26-gauge disposable insulin syringe. The challenge of both serotypes of the virus was carried out with proper preventive measures.

Pathological Study

The pathological study consisted of clinical signs, gross lesions, and histopathological lesions. The experimental birds of each group were observed for the development of abnormal clinical and behavioral signs up to 35th day of study. The presence of clinical signs was noted. A detailed post-mortem examination was performed in the dead birds after the challenge. No gross lesions were noted, and samples of the liver, heart, and kidney were collected in 10% neutral buffered formalin for histopathological examination. The tissues were processed according to standard paraffin embedding techniques in an automatic tissue processor, and 4-5 micron thin sections were prepared. The sections were stained with Haematoxylin and Eosin stains.

Molecular Confirmation

The liver tissue was collected from dead birds after the viral challenge in cryovials and stored at -20°C for confirmation of FAdV using PCR. The total genomic DNA was extracted from the collected liver tissues using QIAamp Fast DNA tissue kit following the manufacturer's instructions. For polymerase chain reaction (PCR) amplification of a 590-bp fragment of the Loop1 region of the hexon gene HexonL1s F5'-ATGGGAGCSACC TAYTTCGACAT-3' and HexonL1As R 5'-AAATTGTCCCKRAANCCGATGTA-3' primers were used following the procedure described by Raue et al. (2005). The amplified product was visualized in a 2% agarose gel with ethidium bromide.

RESULTS AND DISCUSSION

Clinical Signs

Group III and IV experimental birds started showing varying clinical signs after the 3rd DPI (Day Post-Inoculation) of challenged virus serotypes. The birds in Group III challenged with FAdV-4 showed more pronounced clinical signs as compared to the Group IV inoculated with FAdV-11. The major clinical signs observed were dullness, depression and huddling; some of the birds were reluctant to move, and there was a decrease in the feed intake. For the birds that exhibited severe symptoms, death was followed by rapid deep abdominal breathing. Some birds died suddenly without exhibiting any clinical signs. The damage to the liver by the Fowl adenovirus led to reduced hematopoiesis due to decreased protein synthesis by the liver, which is essential for the synthesis of erythrocytes. The decrease in the erythrocytes and hemoglobin may have led to clinical signs of depression, lethargy, inability to move, and terminal deep breathing before death. No clinical signs were observed in Group I, II, V, and VI birds.

The clinical signs observed during the present study are comparable with the findings of Wang and Chang (2000) and Kumar et al. (2003). Others have also observed similar results (Okuda et al., 2004; Thakor et al., 2012; Matos et al., 2016; Xia et al., 2017; Cui et al., 2020). However, Okuda et al. (2004) and Xia et al. (2017) also reported diarrhea and mucoid drooping in addition to the common clinical signs, which were not observed during the present study.

Mortality

The mortality started on the 4th DPI with FAdV-4 in Group III. A total of 16 birds died in Group III from inoculation of FAdV-4, on the other hand, only one mortality was observed in Group IV inoculated with FAdV-11 on 6th DPI. In the present study, mortality due to FAdV-4 was 66.66%, while mortality due to FAdV-11 was 5%. This indicated that the FAdV serotype-4 had a higher pathogenic potential compared to serotype-11. The birds of Group V and VI, which were vaccinated against IBH-HPS, did not show any clinical signs as well as mortality throughout observation.

Previous researchers have reported variable results in the mortality after inoculation of the experimental birds with the FAdV. The experimental groups' mortality ranged from 0 to 100% (Wang and Chang, 2000; Ahmed et al., 2011; Chitradevi et

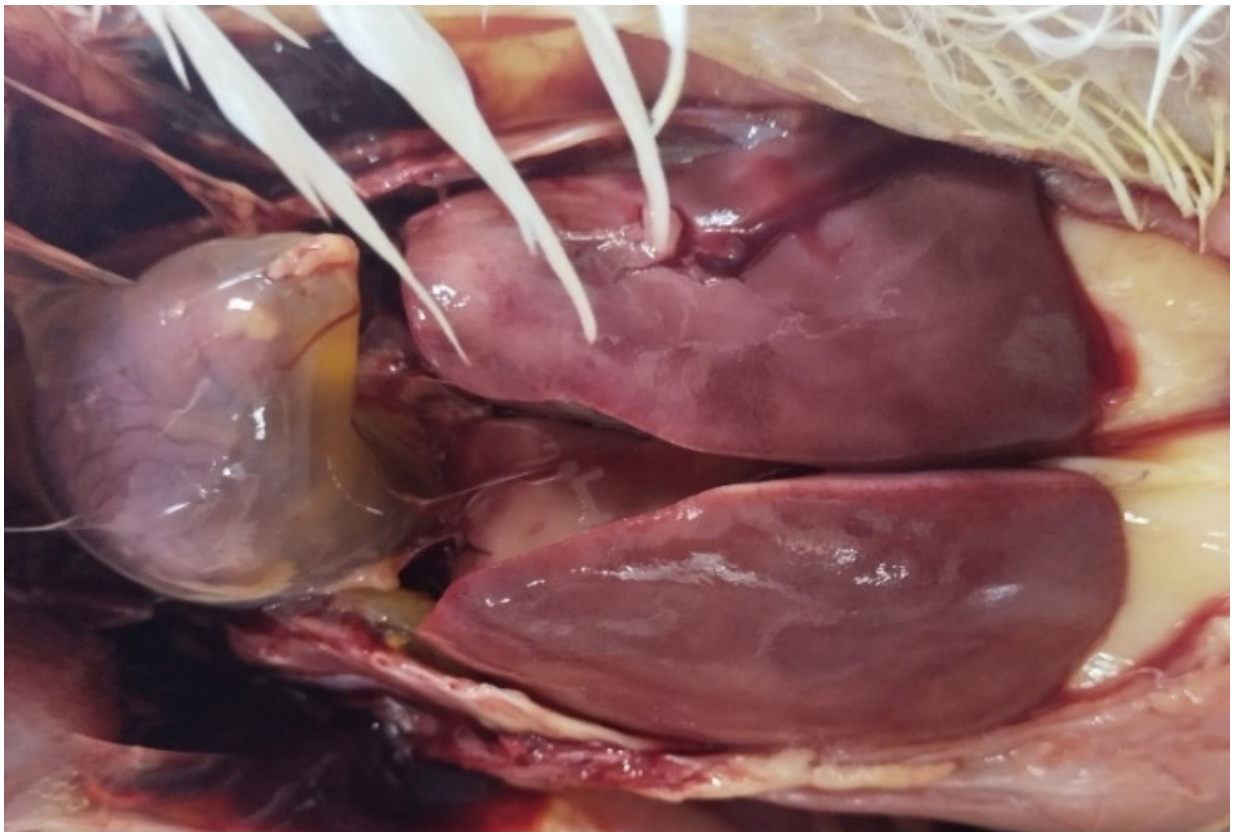

Fig. 1: Development of hydropericardium with hepatic necrosis in birds challenged with FAdV.

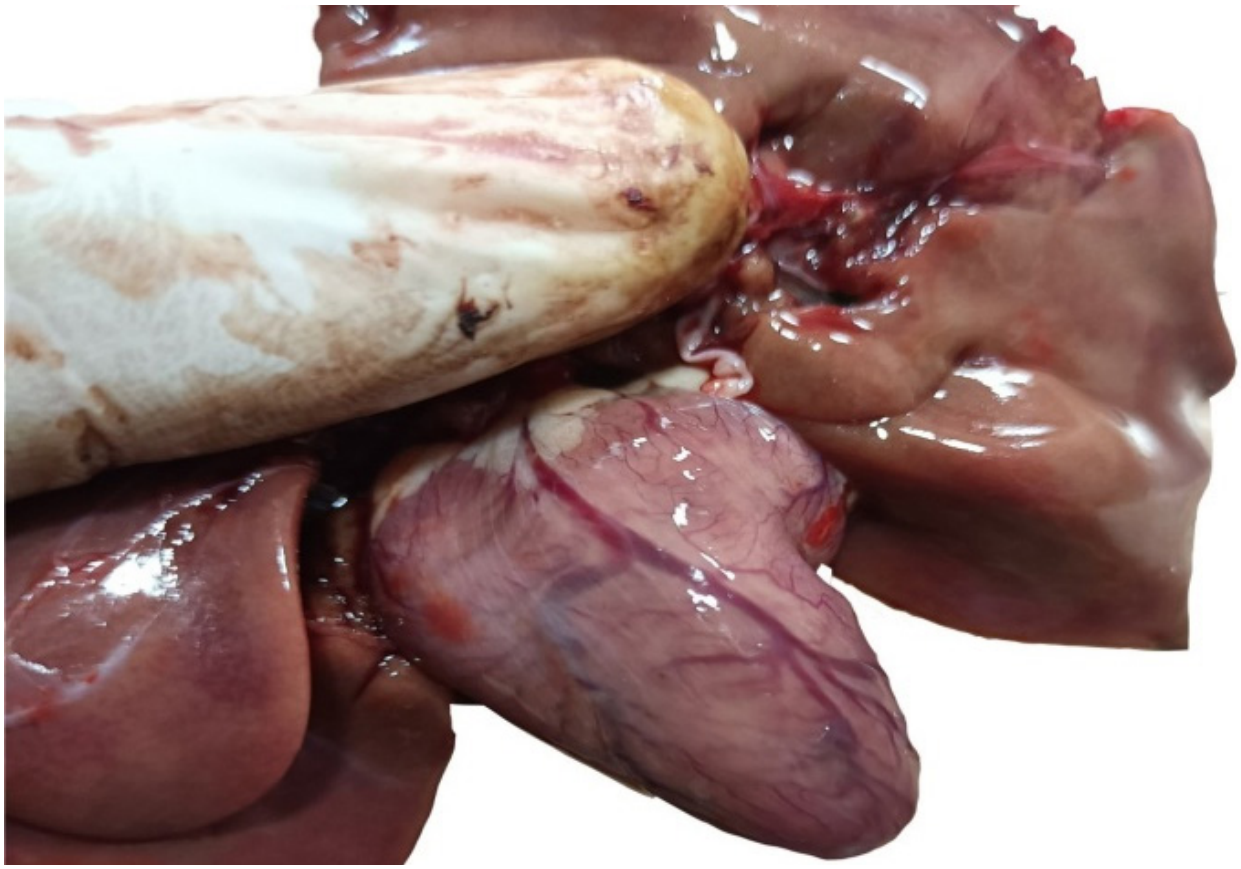

Fig. 2: Flabby misshapen heart with congestion and petechial haemorrhages on the apical surface.

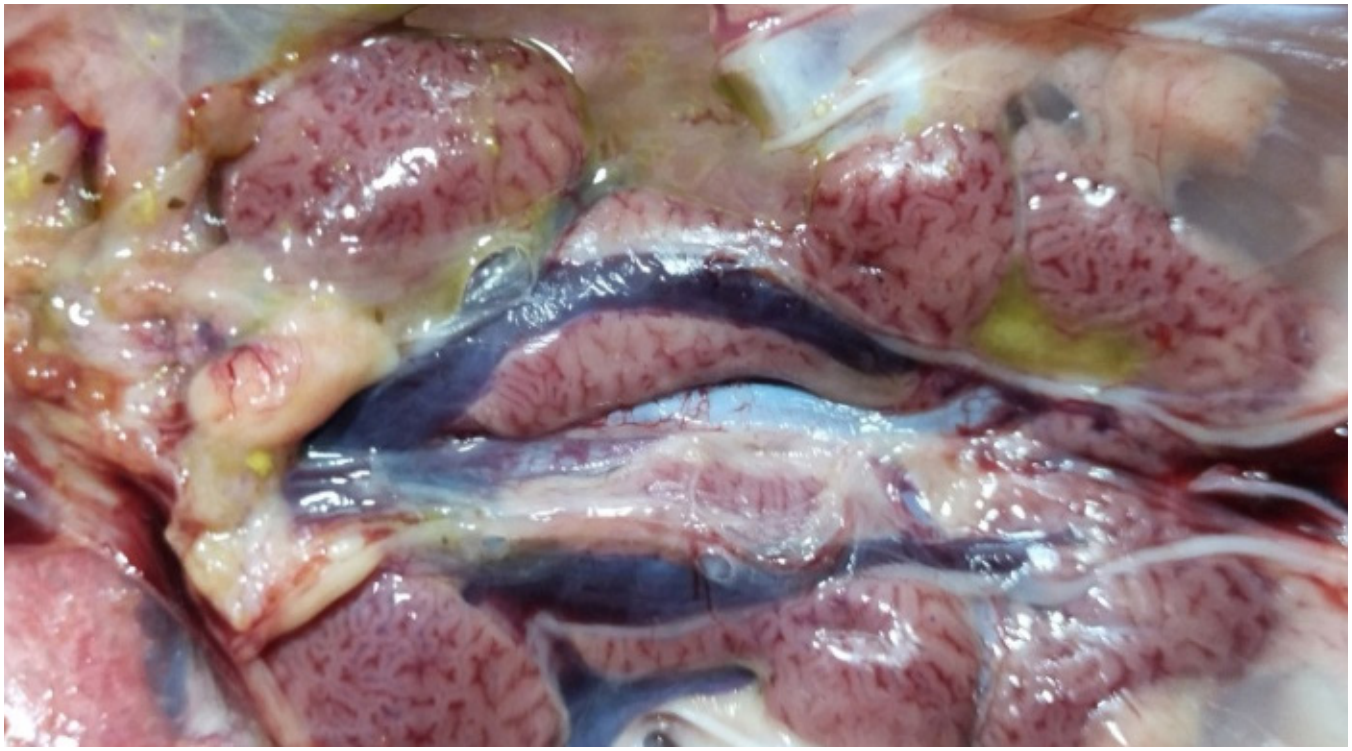

Fig. 3: Experimental birds showing haemorrhages and few foci of necrosis in kidney

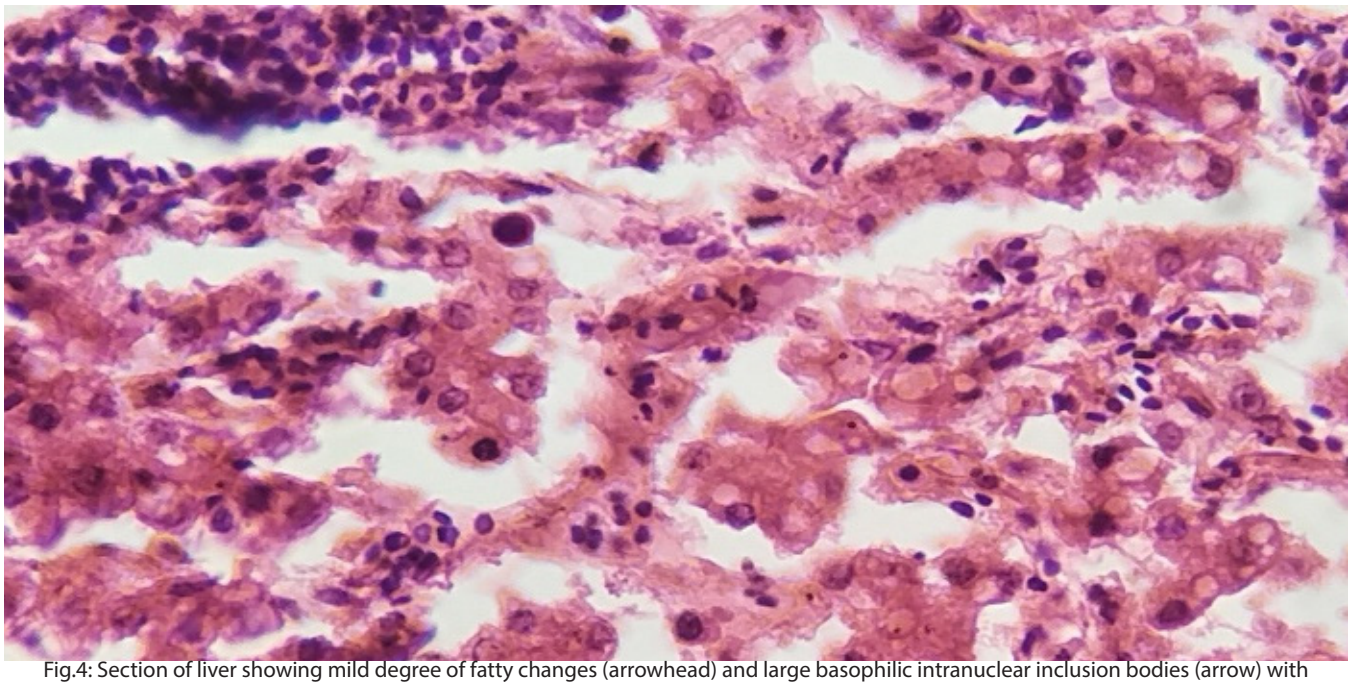

Fig.4: Section of liver showing mild degree of fatty changes (arrowhead) and large basophilic intranuclear inclusion bodies (arrow) with mononuclear cell infiltration (H & E stain, 480X).

al., 2021). Zhao et al. (2015) reported 28.6% and 8.0% mortality for FAdV serotypes 4 and 11, respectively, in SPF chicks. The results of Zhao et al. (2015) are similar to the present study's findings, where serotype 4 of FAdV was responsible for more significant mortality compared to serotype 11. Ahmed et al. (2011) demonstrated more significant mortality with the viral inoculum prepared from liver homogenate as compared to a tissue-cultured virus. The variation in the mortality in the experimentally induced disease may be attributed to the type of inoculum, the concentration of virus, route of inoculation, age of birds, the pathogenic potential of the virus, vaccination against IBH-HPS in the parental stock and susceptibility of the experimental birds.

The vaccine efficacy study indicated that the vaccine could provide satisfactory protection against both FAdV serotype 4 and 11 by preventing mortality by 100% in both

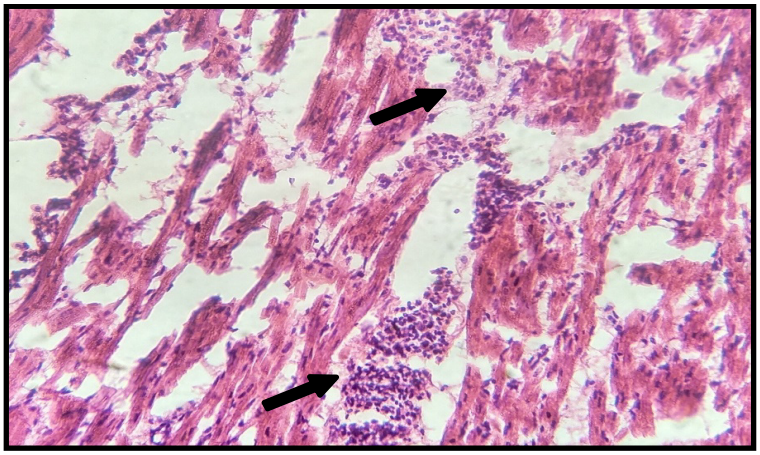

Fig. 5: Section of the heart showing separation of muscle fibers with haemorrhages and infiltration of mononuclear cells in experimentally induced disease (H&E stain, 480X).

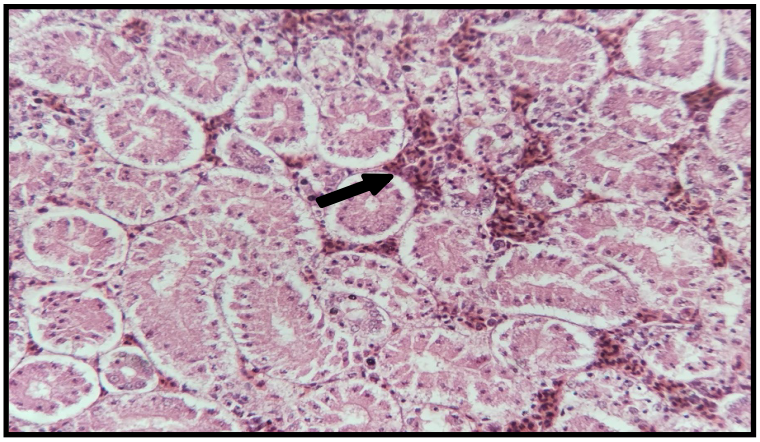

Fig. 6: Section of kidney showing mild degree of intertubular haemorrhages with tubular degeneration and desquamation (H & Estate, 480X).

groups V and VI. Various researchers have reported similar findings (Chandra et al., 2000; Alvarado et al., 2007; Kataria et al., 2013; Lu et al., 2022).

Gross Lesions

The birds who died after the challenge produced characteristic gross lesions of IBH-HPS. The liver showed mild to moderate enlargement with friable consistency. Multiple areas of hemorrhages were observed on the surface of the liver with mild fatty changes and necrosis. The heart showed typical hydropericardium with an accumulation of clear watery to yellow straw-colored jelly-like fluid in the pericardial sac (1). The heart was flabby and congested. The apical surface of the heart in some birds showed petechial hemorrhages (2). The kidney was enlarged in most of the birds with areas of hemorrhages and necrosis (Figure 3). Upon sacrificing the birds at the end of the observation period, one bird from Group V revealed the development of a mild degree of hydropericardium with a little amount of yellowish straw-colored fluid accumulated in the pericardial space. The disease development process may be mild, so the bird did not die.

Similar gross lesions have been observed by Kumar et al. (2003), Ahmed et al. (2011), Dar et al. (2012), Zhao et al. (2015), and De Luca et al. (2020) in the experimentally induced disease. Ahmed et al. (2011) reported bursal edema, which was an inconsistent feature in the present study. Dar et al. (2012) and De Luca et al. (2020) reported the presence of petechial hemorrhages over the surface of the pancreas, which was not seen during the present study.

Histopathology

The liver showed mild to moderate amounts of multifocal coagulative necrosis leading to impaired hepatic architecture. There was an infiltration of mononuclear cells and heterophils in the necrotic areas. The presence of fatty changes was observed in some birds. The sinusoids of the liver were congested. The presence of characteristic basophilic intranuclear inclusion bodies within the hepatocytes near the area of necrosis which completely masked the nucleus (Figure 4). The nuclei of the hepatocytes were shrunken and pyknotic. The heart showed the presence of separation of muscle fibers with infiltration of mononuclear cells (Figure 5). The blood vessels in the myocardium were congested, and areas of hemorrhages were evident. The kidney revealed tubular degeneration, desquamation, and intertubular hemorrhages

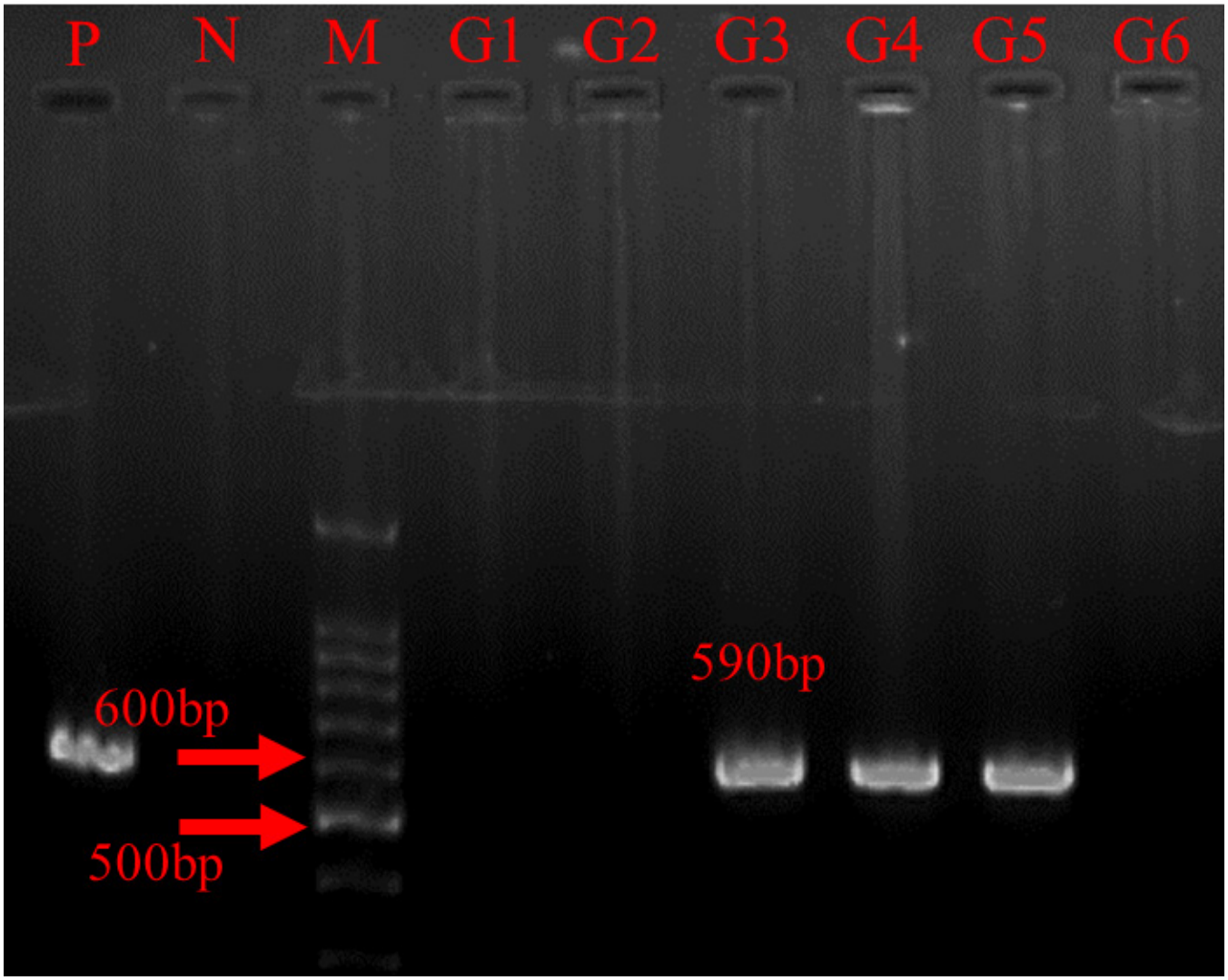

Fig. 7: Agarose gel showing amplified product with FAdV Loop1 specific primers (approximately 590bp).M: DNA ladder-100bp, P: Positive control, N: Negative control, G1 to G6: experimental study groups

(Figure 6). Many earlier researchers had similar observations during their experimental studies (Dar et al., 2012; Zhao et al., 2015; Suoha and Rajkumar, 2021).

Several researchers have reported gross and microscopic lesions of IBH-HPS during natural outbreaks in the field (Thakor et al., 2012; Suohu and Rajkhowa, 2021). The gross and microscopic lesions observed in the experimentally induced disease were similar to the natural outbreaks. Concurrent infections are common during natural IBH-HPS outbreaks, which may explain why some characteristics of natural outbreaks are difficult to replicate in the lab.

Molecular Detection

On PCR, all the birds that died after inoculation and one bird from Group V that showed the presence of hydropericardium were found positive for the presence of FAdV indicated by approximately 590-bp amplification product in agarose gel electrophoresis (Figure 7).

Earlier workers (Alvarado et al., 2007; Chitradevi et al., 2018; Bertran et al., 2021) were able to use different sets of primers designed to amplify the Hexon gene at different sites. The product size after the PCR reaction varied according to the binding site of the primer sets on the gene.

CONCLUSION

The results of experimental pathogenicity testing suggested that the field strain of FAdV serotype 4 was more pathogenic compared to the FAdV serotype 11, as it produced 66.66% mortality against only 5.00% mortality with serotype 11. The gross and microscopic lesions produced during experimental infections closely resembled the natural field outbreaks. All the dead birds were found positive for the presence of FAdV in the liver upon PCR. The vaccine efficacy study revealed that the vaccine could provide satisfactory protection against both the FAdV serotypes. The vaccine could be considered to be 95% effective against FAdV serotype-4 and 100% effective against FAdV serotype 11 in preventing the development of IBH-HPS disease.

ACKNOWLEDGMENT

The authors are grateful to Principal of Veterinary College and authorities of KU, Anand for the facilities and encouragement provided for this work.

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