Histopathology and Molecular Characterization of Fowl Adenovirus in Commercial Broiler Chicken: A Case Report

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Abstract
Fowl adenovirus disease is a member of the Aviadenovirus genus, Adenoviridae family and is a highly contagious disease associated with hydropericardium syndrome and inclusion of body hepatitis in commercial broiler, resulting in huge economic losses to the poultry industry worldwide. In the present study fowl adenovirus (FAdV) was detected and characterized from broiler cockerel brought for necropsy examination.

Grossly, the bird revealed hydropericardium and enlarged friable liver and petechial haemorrhages over liver parenchyma. Microscopically fatty degeneration in liver and intranuclear inclusion bodies in hepatocytes were observed. A definitive diagnosis was carried out by amplifying Fowl adenovirus gene of FADV by PCR. Resulting amplicon was sequenced and sequence identified as fowl adenovirus.

Keywords: Fowl Adenovirus, PCR, Histopathology, Post mortem examination.

Introduction

Adenoviruses isolated from healthy or sick birds are found to be widespread throughout avian species causing demonstrable economic losses in commercial poultry farming[7]. Adenoviruses isolated from chicken are called fowl adenovirus (FAdVs), which are non-enveloped dsDNA viruses belonging to genus Aviadenovirus and family Adenoviridae.[5] FAdVs are subdivided into 12 serotypes based on serum cross-neutralization tests (FAdV1 to FAdV-8a and FAdV 8b to FAdV 11)[8]. The diseases associated with FAdV infection in chicken are the inclusion body hepatitis (IBH), hepatitis hydropericardium syndrome and egg drop syndrome[10].

Group I describes adenovirus infection of chickens and turkeys that is generally sub-clinical or accompanied by mild symptoms[7]. Primary disease signs include increased mortality, decreased feed intake, ruffled feathers, rales, coughing, lacrimation and depression [13]. IBH cases describe the liver as the main affected lesion becoming enlarged, friable and with petechial haemorrhages over its parenchyma. Liver histopathology reveals hepatic necrosis along with intra-nuclear inclusions within hepatocytes. Group II pathogenicity describes HE in turkeys displaying dark red/black distended intestines full of bloody contents prominently affecting ages 6-11 weeks old[2,12]. Spleen enlargement is a common factor with mottling and friability[1].

Liver enlargement and congestion of lungs may also occur. Gut lesions are also usually affected by severe blockage of intestinal mucosa, deterioration and hemorrhage of villi[12,14].

Group III affects laying hens and is characterised by abnormal egg production known as egg drop syndrome (EDS) virus. EDS primary symptoms are demonstrated after 7 - 9 days along the loss of eggshell pigment; thinning of egg shell or formation of shell-less eggs[8]. Following infection, oedema of the uterine mucosa and exudate in the lumen are observed. Inclusion bodies are observed in all regions of the oviduct, though they stand prevalent in epithelial cells of pouch shell gland of uterus[16,20].

Molecular characterization of FAdVs is performed by polymerase chain reaction using gene specific primers. Hexon is the major protein of the adenovirus that possesses the neutralizing epitope and is therefore called serotype specific.[6] Therefore, the most common gene used for FAdV detection is the hexon gene[19].

In this study, commercial broiler cockerel was presented to Department of Veterinary Pathology, Nagpur Veterinary College for necropsy examination and was investigated for FAdV infection. History, signs and gross lesions were reported. The liver samples were collected for histopathology and molecular detection by PCR. Further typing of FAdV positive samples was performed by hexon gene sequencing.

Material and Methods

Clinical case and sample: Commercial broiler cockerel aged 31 day old showing clinical signs of decreased body weight, depression and mild diarrhea was presented for necropsy examination. Grossly, livers were pale, swollen and friable with petechial haemorrhages on its parenchyma.

Liver sample was collected and kept in 10 % formalin for histopathological examination and part of it kept in -80 ^{0}C for further molecular diagnosis by PCR.

Histopathology: Liver tissues were taken and fixed in 10% formalin for histopathological investigation. After 72 hours of fixation, samples were dehydrated, embedded in paraffin wax and sectioned (4 micronmetre) for hematoxylin and eosin (HE) staining. PCR was conducted using the PCR

Master Mix (2X) (Thermo Scientific, India) in 25 microlitre reaction volume and 5 microlitre DNA template with hexon gene specific primers. The PCR reaction is heated at 95 ◦C for 5 min as initial denaturation step. Then 40 cycles of denaturation occur at 95 ◦C for 45 seconds, primer annealing at 60 ◦C for 60 seconds and elongation at 72 ◦C for 60 seconds followed by a final elongation step of 10 minutes at 72 ◦C. The PCR product was analyzed by agarose gel electrophoresis under UV transilluminator.

PCR assay: Liver samples were homogenized with normal saline and then clarified by centrifugation. The supernatants were collected and kept at -80 °C until used for DNA extraction. DNA was extracted from the supernatant of the liver homogenate using a Viral Genespin viral DNA/RNA extraction Kit (iNtRON, South Korea) according to the manufacturer's instructions.

Results and Discussion

Gross and Histopathological analysis: Grossly, the heart had serous fluid accumulation in the pericardium. Liver was friable, pale and had petechial haemorrhages over its parenchyma which were similar to IBH lesions described previously[3].

Microscopically, intranuclear inclusion bodies were observed in hepatic parenchyma. Vacuolation of hepatocytes and necrosis with inflammatory cell infiltration, predominantly heterophil infiltration was observed. These observations were in agreement with previous reports [4].

Molecular Detection: Molecular detection of FAdV was performed through PCR assay using FAdV hexon primers for amplification of the expection 590 base pair (bp) amplicon that is a type specific domain in loop 1 of the hexon gene of fowl adenovirus[17]. Hexon is known to be the neutralizing epitope[6]. The presence of FAdV was confirmed by PCR amplification of a 590 base pair segment from liver sample of cockerel which was in agreement with previous studies.

Conclusion

With the advent of higher numbers of viral diseases affecting poultry flocks, some vertically transmitted viral affection remain important as it causes immunosuppression in the affected flock. IBH is one amongst the diseases that is considered as economically important disease in many regions of the world.

Based on histopathological, gross lesions and PCR analysis, we characterized FAdV from commercial cockerel bird. Finally, preventive measures against FAdV infection in commercial poultry farms should be given serious and prudent attention.

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