Molecular detection and histomorphological analysis of Inclusion body hepatitis in Broiler chicken

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Abstract

Inclusion body hepatitis (IBH) outbreaks have been increasingly reported in different regions of India mainly in broiler flocks. The present study was aimed at studying prevalence and histomorphological alterations in IBH in broiler chickens in Kashmir, India. Liver, Kidney, heart and spleen samples were collected from IBH suspected 100 commercial broiler flocks from different districts of Kashmir. All liver samples were subjected to 564bp FAdV polymerase gene-specific PCR for confirmation. Out of a total of 3246 dead birds screened, liver lesions were seen in 1526 birds giving an occurrence of liver affections as 46.99%. Among these 1526 affected livers, inclusion body hepatitis was detected in 42 cases giving a case prevalence of IBH as 2.75% and occurrence as 1.29%. All the positive cases of IBH belonged to more than 15 days age group with highest case prevalence in 22- 28 days age group (42.8%) and lowest in 15-21 days age group (23.8%). The overall mortality due to IBH was reported as 1.11%. IBH was grossly characterised by enlarged liver with focal necrotic areas hydropericardium, swollen kidneys and congested spleen. Microscopic lesions were characterised by presence of basophilic intranuclear inclusion bodies in the hepatocytes, congestion in the heart, lymphoid depletion in the spleen and glomerular atrophy in the kidneys.

Key Words: Inclusion body hepatitis; Histomorphology; Poultry; Intra nuclear inclusions

Introduction

Fowl adenovirus (FAdV) is widespread across different regions worldwide, affecting chickens and various other bird species (Hess, 2020). FAdVs are non-enveloped, dsDNA viruses belonging to the genus Aviadenovirus within the family Adenoviridae. FAdVs have been grouped into five species FAdV-A to FAdV-E, and 12 different serotypes (FAdV-1 to FAdV-8a and FAdV-8b to FAdV-11) (Meulemans et al. 2004). FAdVs are responsible for causing a variety of diseases in chickens namely inclusion body hepatitis (IBH), hydropericardium hepatitis syndrome (HHS) and adenoviral gizzard erosion (Cizmecigil et al., 2020). IBH is an acute disease in poultry having a significant economic impact due to high mortality and poor production caused by FAdVs belonging to groups D (serotypes -2, -3, -9, and -11) and E (serotypes -6, -7, -8a, and -8b) (Schachner et al. 2016; Steer et al. 201; Mohamed and El-Sabagh, 2023). In the last few years, a large number of outbreaks have been reported in different countries (Schachner et al. 2021; Mohamed and El Sabagh, 2023). IBH affects chicken up to 5 weeks of age and causes a variable mortality ranging from 10 to 30% (Schachner et al. 2016). Although IBH is a primary disease of chickens that can occur without the presence of other predisposing factors (Mirzazadeh et al. 2021), concurrent infection with other immunosuppressive agents like infectious bursal disease virus (IBDV), chicken infectious anemia virus (CIAV), *Escherichia coli* and coccidiosis help in development of the clinical presentation of the disease (Mittal et al. 2014; Niu et al. 2017). The affected birds display a range of clinical signs like decreased feed intake, stunted growth, ruffled feathers, lethargy and ataxia (Kumar et al. 2013). IBH is transmitted both vertically through the embryonated eggs and horizontally as the virus is present in all excretions of the bird with high concentrations in the feaces. The pathological changes include a liver that appears pale, swollen, and friable, with pale necrotic foci; a discolored pancreas exhibiting petechial hemorrhages; swollen, pale kidneys; and a mottled spleen (Cizmecigil et al., 2020; Niczyporuk et al. 2021). Recently, the poultry industry in Kashmir has faced disease outbreaks indicative of fowl adenovirus (FAdV) infection. Therefore, this study aimed to detect the FAdV infections associated with IBH using molecular methods, to study prevalence and histomorphological changes induced by the infection.

Materials and Methods

Sample collection

The study was conducted in various broiler farms operating in central zone of Kashmir. Liver samples were collected from a total of 100 outbreaks based on history, clinical signs and lesions following a thorough post mortem examination of birds. Molecular and pathological studies were conducted in the Division of Veterinary Pathology, FVSc & AH, Shuhama, SKUAST-K.

Pathomorphological studies

The carcasses were subjected to systemic necropsy examination. Liver, heart, spleen and kidneys were thoroughly examined grossly for abnormalities if any. Affected organs (liver, heart, kidney and spleen) were collected in 10% formalin for histopathological examination and processed by routine paraffin embedding technique. The sections were stained with Harris' haematoxylin and eosin technique for routine examination (luna,1968).

DNA extraction

Collected samples were stored in sterile dry containers containing phosphate buffered saline (PBS) and transported to the laboratory in an ice box and stored at −20 °C till used for FAdV identification by PCR. Sterile scissors were used for chopping the collected liver samples into small parts. 10 to 20mg of fresh or thawed liver tissue was added with 600µl of chilled nuclei lysis solution and homogenized for 10 seconds. Cell lysis and protein precipitation was done by adding 3µl of RNase solution to liver tissue nuclei lysate and then mixed. Incubation was done for 15-30 minutes at 37ºC and cooled to room temperature.200µl of protein precipitation solution was added to the mixture, vortexing and chilling on ice for 5 minutes was done. After that centrifugation was carried out at 13,000-16,000 × rpm for 4 minutes. The supernatant obtained after centrifugation was transferred to a fresh tube containing 600µl of room temperature isopropanol and then mixed gently by inversion. Centrifugation was carried out at 13,000-16,000 × rpm for 1 minute. The supernatant obtained after centrifugation was removed and 600µl of room temperature 70% ethanol was added and then mixed. Centrifugation was again done at 13,000-16,000 × rpm for 1 minute. Aspiration of ethanol was done and the remaining pellet was air dried for 15 minutes. The pellet containing DNA was rehydrated by adding 100µl of DNA Rehydration Solution for 1 hour at 65ºC.

Detection of FAdV

PCR was carried out for molecular diagnosis of FAdV in the collected liver samples based on the polymerase gene as per the method described elsewhere (Zhao *et al.* 2015) with slight modifications. The FAdV DNA polymerase gene specific primers used in this study were F-5′-TGCTCGTTGTGGATGGTGAA-3′ and R-5′-CTCCGTGTTGGGCTGGTC-3′. PCR was carried out in a final reaction volume of 25 µl reaction mixture using 0.2 ml thin wall PCR tube. Reactions in the PCR were performed according to the following protocol of initial denaturation at 95°C for 5 minutes followed by denaturation, annealing and extension of 30 cycles at 95°C

for 45s,56ºC for 45s, 72°C for 1.5min, and a final extension step of 10 min at 72°C. Amplicons of the PCR reaction were visualized using 1.0% (w/v) agarose gel by UV transilluminator.

Results

Gross and histopathological studies

Grossly liver showed varying degree of hepatomegaly. The consistency of liver was fatty and friable with multiple petechial or small haemorrhages on the surface (Fig 1A). Some cases also revealed presence of white necrotic foci throughout the surface of liver. Grossly, the heart had accumulated 5 to 10 ml of serous fluid in the pericardium known as hydropericardium. Kidneys were pale, swollen and congested. Spleen was also enlarged and congested. No gross lesions were observed in bursa of fabricius, thymus and other organs in affected birds.

Microscopically, liver capsule was thickened and hemorrhages were present in the liver parenchyma. Liver also revealed varying degrees of vascular congestion, infiltration around the blood vessels and hepatocellular degeneration (Fig 1B&C). Basophilic intra nuclear inclusions were seen in the degenerating hepatocytes with clear halo space around them in most of the affected birds (Fig 12D). Histopathological examination of heart sections revealed disruption of muscle fibres, congestion, myocardial haemorrhage and mononuclear cell infiltration. Spleen revealed severe lymphoid depletion. Kidneys revealed infiltration in the interstitium, glomerular atrophy and sloughing of renal tubular epithelium.

Molecular detection

In the present study, a total of 208 liver tissue samples grossly showing liver lesions suspected of IBH were subjected to PCR. out of 208 samples collected, 42 samples were found positive for IBH polymerase gene giving positive percentage of 20.19% for IBH. The PCR products generated was confirmed for their expected size (564bp) in 1% (W/V) agarose gel in 0.5X TAE buffer by using of horizontal submarine gel electrophoresis apparatus (Fig 2).

Occurrence

Out of a total of 3246 dead birds screened, liver lesions were seen in 1526 birds giving an occurrence of liver affections as 46.99%. Among these 1526 affected livers, inclusion body hepatitis was detected in 42 cases giving a case prevalence of IBH as 2.75% and occurrence as 1.29%. All the positive cases of IBH belonged to more than 15 days age group with highest case prevalence in 22- 28 days age group (42.8%) and lowest in 15-21 days age group (23.8%). The overall mortality due to IBH was reported as 1.11%. Most of the outbreaks were recorded during the summer season.

Discussion

Efforts to improve upon the hepatic condition of broilers to increase productivity is going on across the world (Rafiei-Tari et al 2018; Kairov et al 2020). In several countries, Inclusion body hepatitis (IBH) has become an economically important disease affecting broiler chickens (Zhao et al. 2015; Shaib et al. 2017). Many researchers in India have also reported cases of inclusion body hepatitis-hydropericardium syndrome (Thakor et al. 2012; Mittal et al. 2014; Trivedi et al. 2018). Since 2010, IBH cases have risen, with outbreaks showing an increasing trend in broiler flocks across several Indian states from 2016 onwards. Although FAdV infections have been reported in India, their prevalence continues to draw attention. The significance of adenovirus infections lies not only in the economic losses caused by mortality rates of 10–30% and decreased performance but also in the virus's ability to interact with other pathogens (Hafez, 2011). In present study the overall occurrence of inclusion body hepatitis observed was 1.29% with all cases falling in more than 15 days age group. Higher incidence of this disease has been reported from India in earlier studies. Chitradevi et al. (2021) found the incidence of IBH as 55% in commercial broiler chicken and 33.3% in broiler breeder chicken in eleven different States of India from 2016 to 2019. Gupta et al. (2007) found the incidence of inclusion body hepatitis as 5.26% which was minimal among various liver affections. The occurrence of liver affections caused by IBH is low in Kashmir due to farmers' good management practises and timely vaccinations.

In the present study, PCR was employed for detection of IBH infection and 42 samples were found positive for IBH polymerase gene. The PCR has been extensively used for detection of IBH earlier (Choi et al. (2012); Zhao et al. (2015); Mittal et al. (2014). In the present study IBH resulted in 1.11 % mortality in broilers. Mortality rates in broilers affected by IBH range from minimal to severe, sometimes exceeding 30%, with most cases occurring in birds aged 3 to 5 weeks. A mortality of 3.2 to 10.4% due to IBH was recorded by Kumar et al. (2013) In India. The majority of IBH cases had previously been recorded in fast growing (2-6 week old) broilers which must be due to increased liver stress at that stage. The liver in the current study had varyinsg degrees of hepatomegaly. The liver was fatty and friable, with numerous petechial or small haemorrhages on the surface. Few birds had white necrotic foci on the liver's surface. Some birds had a discoloured dark red and mottled liver or a large area of necrosis in the liver. Histopathological examination of liver revealed presence of intranuclear inclusion bodies within hepatocytes, confirming IBH infection. Two types of inclusions were observed: dense

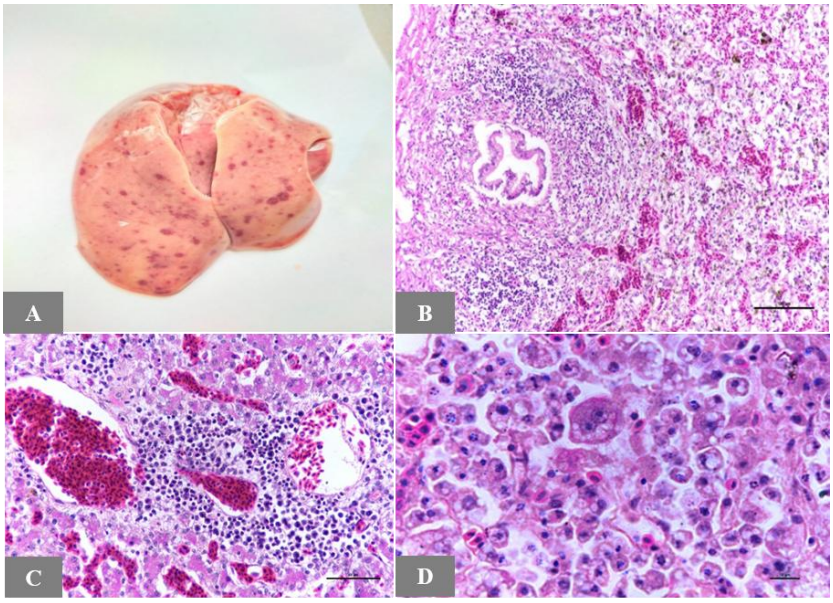

Fig 1: Gross and histopathological changes in Liver in IBH: **A.** Enlarged liver with focal necrosis. **B.** Section of liver Showing heterophilic infiltration and necrosis around bile duct. **C.** Section of liver revealing perivascular infiltration, vascular and sinusoidal congestion and hepatocellular degeneration. **D.** Section of Liver revealing basophilic intranuclear inclusion bodies and karyolysis. *H.E.*

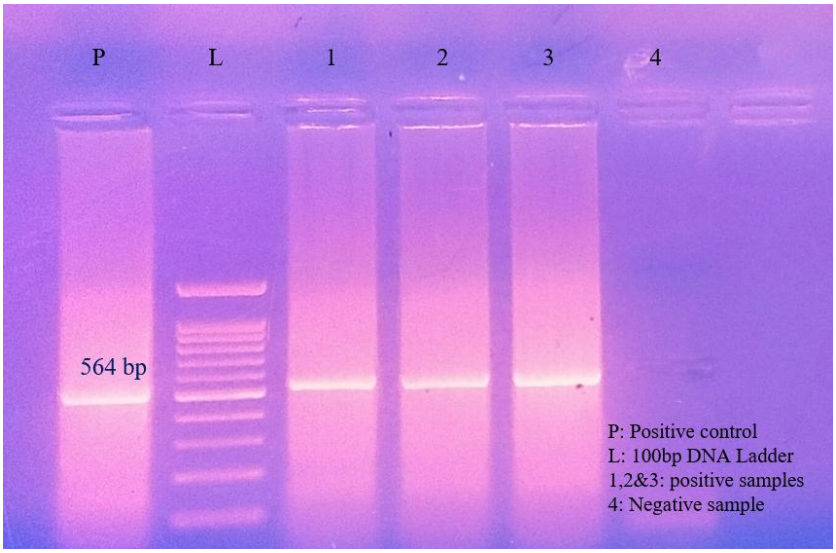

Fig 2: Molecular Detection of Fowl Adenovirus(564bp) 16S rRNA by PCR.

basophilic inclusions occupying most of the nucleus and eosinophilic inclusions surrounded by a halo. The similar lesions described as above were also reported by Sharma et al. (2014) and Das et al (2015). Also most of the birds had moderate to severe accumulations of straw-coloured fluid in the pericardial sac, resulting in hydropericardium. Similar kind of lesions in heart were also described earlier (Sharma et al. (2014) and Das et al. (2015). Mild gross and histopathological lesions were also noted in kidneys and spleen in IBH affected birds. These results are in full agreement with earlier reports (Kumar et al. (2013), Sharma et al. (2014) and Das et al. (2015).

Conclusion

The study concludes that Inclusion Body Hepatitis (IBH) is an important disease in broiler chickens in Kashmir, India, with a prevalence of 2.75%, primarily affecting birds over 15 days old. The disease is characterised grossly by hepatomegaly and hydropericardium and microscopically by presence of intranuclear

inclusions in hepatocytes. Future efforts should focus on enhanced surveillance, vaccine development, and improved biosecurity measures to control IBH outbreaks and minimize losses in poultry production.

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Conflict of interest

The authors declare that they have no known conflict of interest in the conduction of the current study.

Author contribution

SAS and MS conceived the study concept, designed and supervised the study. AHN carried out the study. SAK, BMW and AB carried out histopathological analysis. MSM and MAR helped with the molecular detection. SAS wrote the final manuscript. AAK provided resources. All authors read and approved the manuscript.

Animal Ethics Declaration

Not Applicable

Funding Declaration

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