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Research Article

COMPREHENSIVE PATHOLOGICAL STUDY OF INCLUSION BODY HEPATITIS-HYDROPERICARDIUM SYNDROME IN BROILER CHICKENS OF BIKANER

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ABSTRACT: The broiler chicken of various age groups presented for necropsy examination as well as samples collected from organized poultry farms suspected of Inclusion body hepatitis-Hydropericardium syndrome (IBH-HPS) were included in this study. Total 147 dead broiler chickens were examined and samples were collected from 104 suspected birds based on gross lesions were selected for histopathological and molecular studies. Inclusion body hepatitis-Hydro pericardium syndrome affects multiple organs specifically liver and heart observed in broiler chicken. The histological diagnosis of IBH-HPS in the present study was made based on intranuclear basophilic inclusion bodies found in hepatocytes. Oedema, degeneration as well as diffuse accumulation and proliferation of mono nuclear cells in the different organs were observed. Gross studies are reliable methods for diagnosing of IBH-HPS in poultry in the field conditions, however PCR amplification of Hexon gene was carried out for the confirmation of the adenovirus.

Keywords: Adenovirus, Bikaner, Gross, Histopathology, PCR and Poultry.

INTRODUCTION

According to archaeological data chickens were domesticated from the red jungle fowl (*Gallus gallus*) in the Indus Valley about 5400 B.C. [1]. The Indian poultry sector is among the fastest-growing segments of agriculture, expanding at an approximate annual rate of 8%. Enhanced production methods over the last forty years have led to significant gains in egg and broiler production-poultry meat now makes up about 51% of the nation's total meat output, with nearly 5 million metric tonnes produced in 2022–2023 [2]. The rapid expansion of the poultry industry driven by increases in flock size and shifts in husbandry practices has significantly altered disease patterns. Infections now cause both direct and indirect financial losses, making robust disease prevention and control measures essential for sustainable growth [3, 4, 5].

Despite advances in diagnostics and vaccines, infectious diseases remain a major challenge. Although the layer market grows at about 6–7% per annum and the broiler market at 8–10%, serious infections, particularly by avian adenoviruses, continue to hamper productivity. Fowl adenoviruses (FAdVs) are double-stranded, non-enveloped viruses belonging exclusively to birds. They are classified into five species and multiple serotypes and while they can be isolated from both healthy and diseased birds, their pathogenic roles—especially in conditions like inclusion body hepatitis (IBH) and hydropericardium syndrome (HPS)—vary [7, 8, 12]. Inclusion body hepatitis first noted in broilers by its sudden onset of death, necrotizing hepatitis, and intranuclear inclusion bodies found in liver of affected bird which is typically affects birds between 3 and 6 weeks of age but may also occur in younger or older birds [6, 10]. Mortality ranges from 5–30% in natural outbreaks and rising to as high as 80% when hydropericardium hepatitis syndrome (HPS) is involved [13,14,15]. Clinically the affected

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birds exhibit lethargy, ruffled feathers, inappetence and greenish diarrhoea with post-mortem findings most notably including a pale, friable liver and in cases of HPS, accumulated clear or amber-coloured fluid in the pericardial sac. FAdVs spread vertically (parent to offspring) and horizontally (via the Oro-faecal route and mechanical transmission) [16,17,18].

Diagnosis of adenoviral infections employs a combination of post-mortem examination, histopathology (revealing intranuclear inclusion bodies), electron microscopy, and serological tests. Recently, PCR techniques combined with DNA sequencing or restriction enzyme analysis have further streamlined FAdV typing and diagnosis [11, 19]. The adenovirus fiber protein plays a pivotal role in viral infectivity and tissue tropism by mediating cell attachment through its distinct knobbed, rod-like structure. Its type-specific epitopes not only aid in viral adherence but also serve as targets for neutralizing antibodies, distinguishing FAdVs from most other icosahedral viruses [20, 25, 26].

Due to the abrupt onset of increased mortality lasting five to seven days, both IBH-HPS and IBH have inflicted significant financial losses on the Indian broiler sector. Despite the severe impact of IBH-HPS over the years, no commercial vaccines have been widely available-apart from the limited use of autogenous vaccines. Although vaccines exist for many other poultry diseases, effective immunization against IBH-HPS remains insufficient. Currently, literature documents two vaccine types for IBH-HPS: oral live attenuated vaccines and parenterally administered inactivated whole viral cell culture vaccines. Early use of autogenous liver vaccines posed risks, including the inadvertent spread of the virus and other pathogens, and their commercial production is not viable. Furthermore, cell culture vaccines remain confined to the laboratory, as no cell line supports sustained IBH-HPS virus cultivation. In this context, the development of subunit vaccines containing recombinant viral proteins is promising, as the penton base and fiber—key for virus attachment and penetration—may serve as potent vaccine candidates [20].

Recent IBH-HPS outbreaks in broiler farms around Bikaner, Rajasthan, underscore the need to reexamine the disease's pathogenicity, morbidity, mortality patterns, and lesion profiles. This study was thus planned to investigate whether the virus has maintained its pathogenic characteristics or undergone changes over time.

MATERIALS AND METHODS

During the study period total 147 carcasses of broiler chickens were received for post-mortem examination in the Department of Veterinary Pathology, College of Veterinary and Animal Science, Bikaner from various poultry farms in and around Bikaner, Rajasthan. On gross examination 104 broiler chickens were suspected for IBH from them samples viz., liver, heart, kidney, lung, and spleen were collected. The study was approved by Institutional Animal Ethical Committee (IAEC) No. CVAS/IAEC/2024-25/35. Gross examination of the various organs will be done at the time of postmortem. Tissue pieces were fixed in 10% neutral buffered formalin, dehydrated in ascending grades of alcohol, cleared in xylene followed by impregnation and embedded in paraffin wax (58-60ºC). 4-5 µm thickness were cut and stained with standard hematoxylin and eosin (H&E) staining method and examined under the light microscope [21]. Tissue sample were collected in HiViral™ Transport Kit (A) and maintain appropriate cold chain for the detection of Hexon gene (900 bp) using the polymerase chain reaction (PCR). Forward and reverse sequence of the primer mention in the table 1 [9]. DNA was extracted from tissue samples by using QIAamp DNA isolation kit (#56304) as per the manufacture's instruction with slight modifications.

RESULTS AND DISCUSSION

Liver

Gross pathological changes on liver are shown in Fig. 1a to 1f. Among birds gross changes in liver included petechial haemorrhages The overall appearance of the liver was slightly pale, enlarged, swollen and mottled. Liver was enlarged, congested, friable and haemorrhagic. In some cases liver was pale, enlarged and dark red coloured with severe fatty liver and presence of necrotic foci. In some of the cases diffuse type of necrosis was present on the surface of the liver. Histopathological alterations observed in the liver section of broiler chicken infected with the IBH-HPS virus are shown in Fig. 2a to 2f. Histopathological alterations in the liver were distinguished by significant congestion and vacuolar degeneration of the hepatocytes. Several hepatocytes had enlarged hyperchromatic nuclei. Basophilic intranuclear inclusion bodies were seen in several hepatocytes, leading to the margination of nuclear chromatin material. Focal areas of necrosis was evident by infiltration of heterophils, with a few lymphocytes, in the hepatic parenchyma. The degree of lipid alterations varied from modest to high in the later stages of illness. In several instances, multiple tiny vacuoles in the cytoplasm were seen, and as the disorders advanced, fatty alterations were evident in numerous cases. Liver injury was distinct

Table 1. Details of primers used in amplification of L1 loop of hexon gene.

Primer		Sequencing	References
Forward	Hexon LA	CAARTTCAGRCAGACGGT (R=A/G)	[9]
Reverse	Hexon LB	TAGTGATGMCGSGACATCAT (M=A/C, S=C/G)	

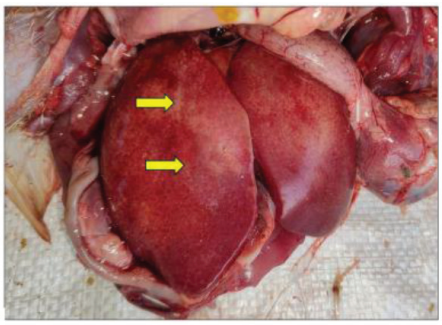

Fig. 1(a). Gross photograph of liver showing noticeably enlargement, congestion with accentuated lobular pattern and focal area of necrosis in the IBH-HPS affected broiler chicken.

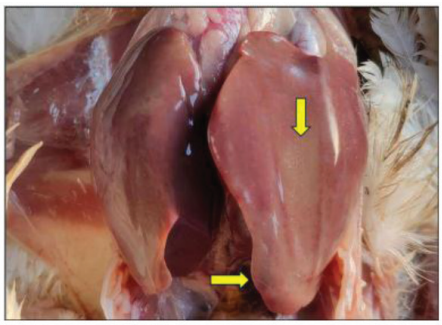

Fig. 1(b). Gross photograph of liver showing pale, enlarge and rounding border due to fatty liver.

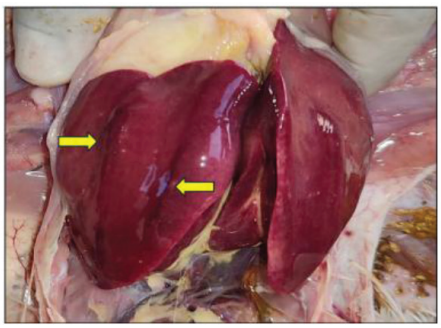

Fig. 1(c). Gross photograph of liver. showing congestion, streak of linear haemorrhagic with diffuse area of necrosis in IBH-HPS affected broiler chicken.

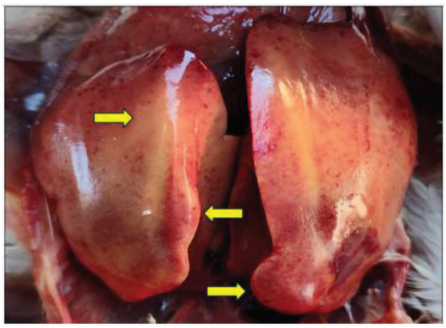

Fig. 1(d). Gross photograph of liver showing a pale, enlarged appearance with a focal area of necrosis and haemorrhage in IBH-HPS affected broiler chicken.

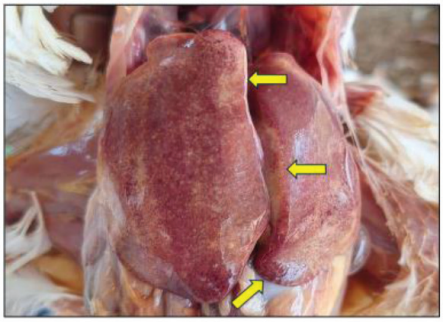

Fig. 1(e). Gross photograph of liver showing enlargement, exhibiting focal necrosis, fatty liver and rounding of border in IBH-HPS affected broiler chicken.

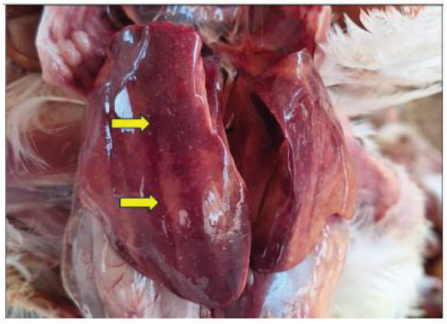

Fig. 1(f). Gross photograph of liver is enlarged, streak of linear haemorrhage and fatty liver along with focal areas of necrosis.

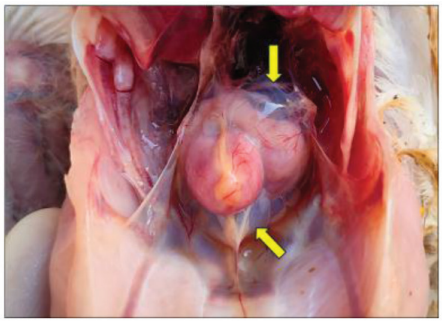

Fig. 1(g). Gross photograph of showing the accumulation of clear fluid in the pericardium of the heart in IBH-HPS affected broiler chicken.

Fig. 1(h). Gross photograph of kidney showing the congestion in IBH-HPS affected broiler chicken.

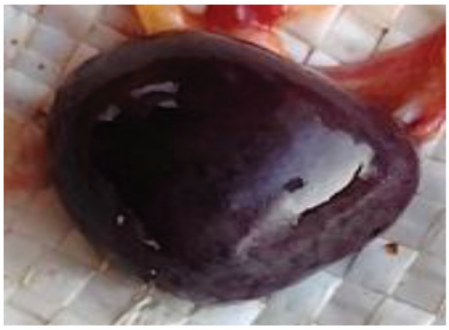

Fig. 1(i). Gross photograph of spleen showing the congestion with dark discoloration in IBH-HPS affected broiler chicken.

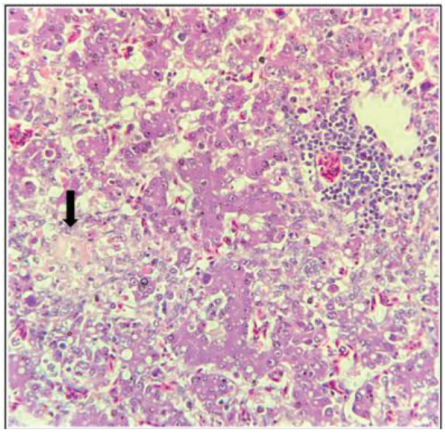

Fig. 2(a). Histopathology of liver showing focal area of necrosis (black arrow) with haemorrhage and infiltration of mononuclear cells (H & E, 400X).

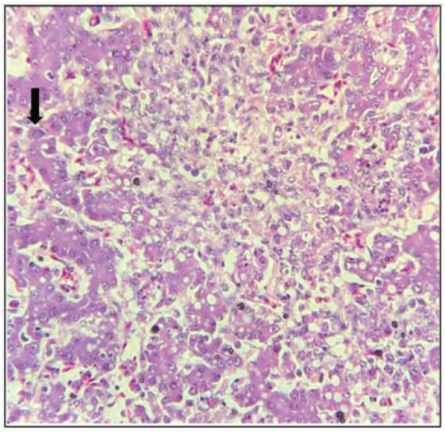

Fig. 2(b). Histopathology of liver showing focal necrosis with infiltrated mononuclear cells and the basophilic intranuclear inclusion body in hepatocytes (black Arrow) (H & E, 400X).

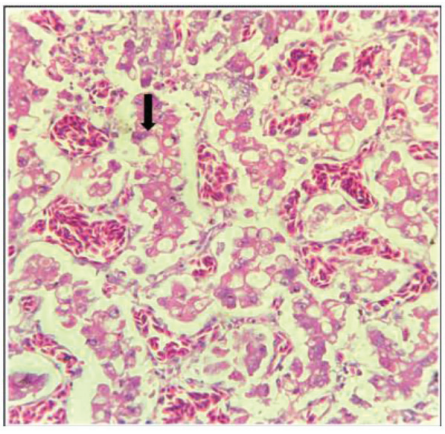

Fig. 2(c). Histopathology of liver showing the congestion and fatty changes (black arrow) in the hepatocytes (H & E, 400X).

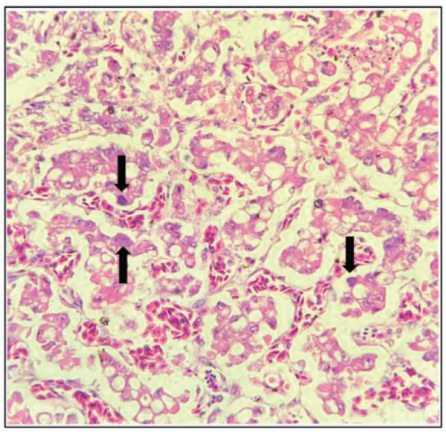

Fig. 2(d). Histopathology of liver showing the congestion, fatty changes with scattered intranuclear basophilic inclusion body (black arrow) in hepatocytes (H & E, 400X).

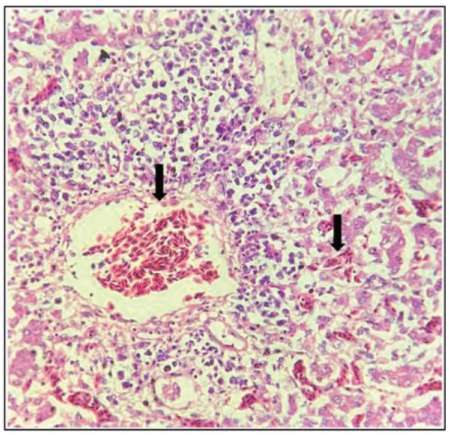

Fig. 2(e). Histopathology of liver showing haemorrhage, congestion with infiltration of mononuclear cells (H & E, 400X).

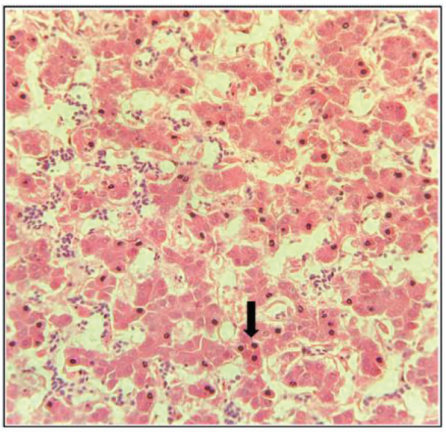

Fig. 2(f). Histopathology of liver section shows the degeneration, fatty changes with infiltration of mononuclear cell and pyknosis of nucleus (black arrow) of hepatocytes (H & E, 400X).

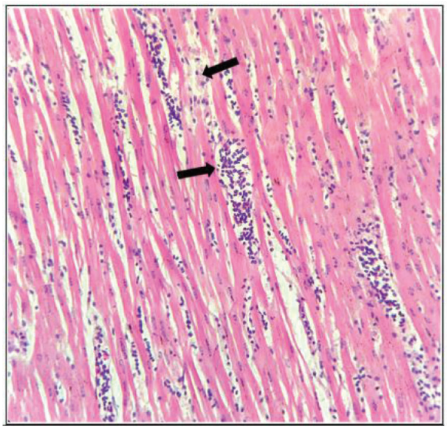

Fig. 2(g). Histopathology of heart showing degeneration of myocardial fibres and infiltration mononuclear cells (H & E, 400X).

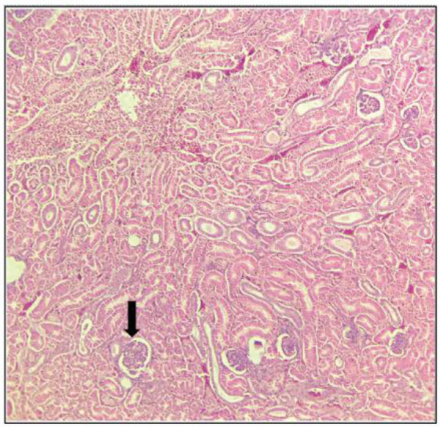

Fig. 2(h). Histopathology of kidney showing congestion & degeneration in the bowman's capsule (black arrow) as well as in tubular epithelial (H & E, 100X).

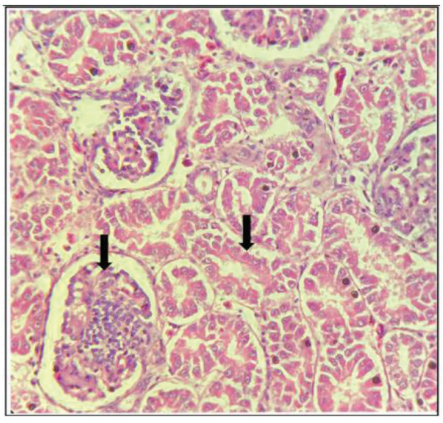

Fig. 2(i). Histopathology of kidney showing degeneration of bowman's capsule and tubular epithelium (black arrow) with infiltration of mononuclear cells in the bowman's capsule (H & E, 400X).

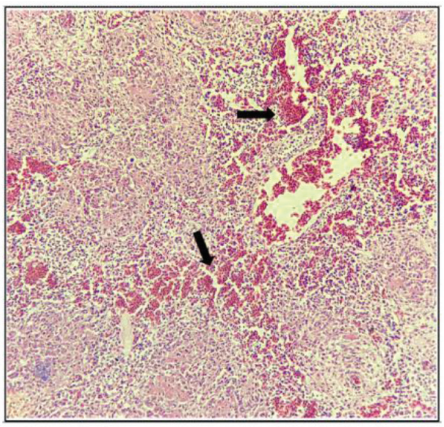

Fig. 2(j). Histopathology of spleen showing depletion of white pulp with haemorrhage (black arrow) (H & E, 400X).

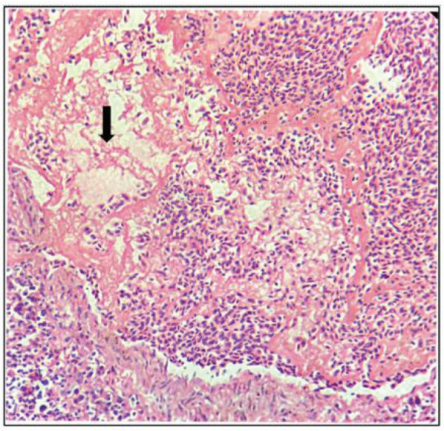

Fig. 2(k). Histopathology of spleen showing oedema (black arrow) accompanied by depletion of splenic pulp (H & E, 400X).

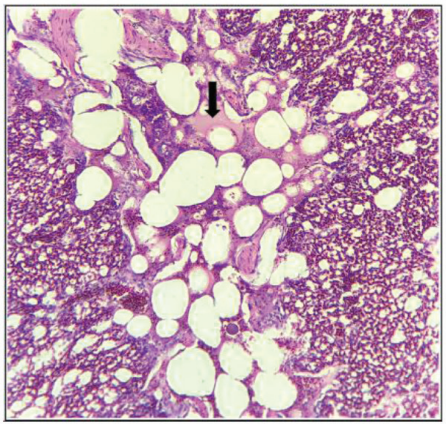

Fig. 2(l). Histopathology of lung showing oedema (black arrow) between the parabronchi (H & E, 200X).

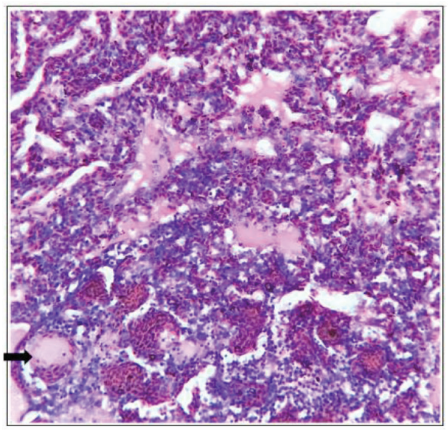

Fig. 2(m). Histopathology of lung showing plenty of haemorrhagic exudate (black arrow) in parabronchi and air capillaries (H & E, 400X).

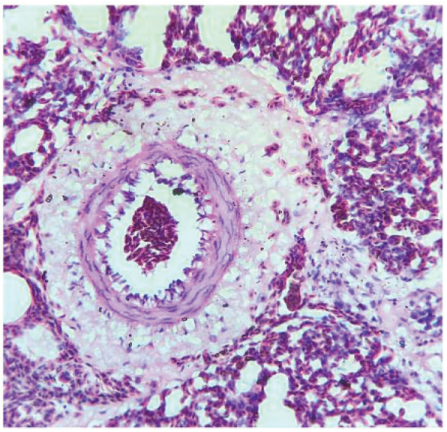

Fig. 2(n) Histopathology lung showing congestion and haemorrhage (H & E, 400X).

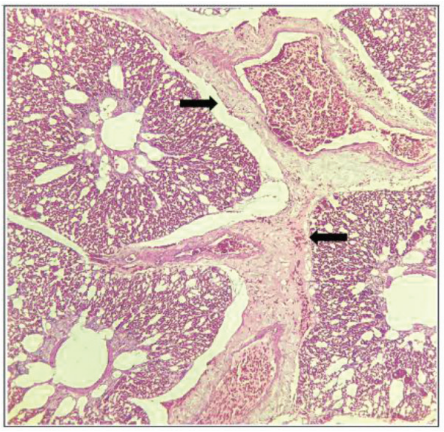

Fig. 2(o). Histopathology of lung section showing the marked thickening of interlobular septa (black arrow) (H & E, 100X).

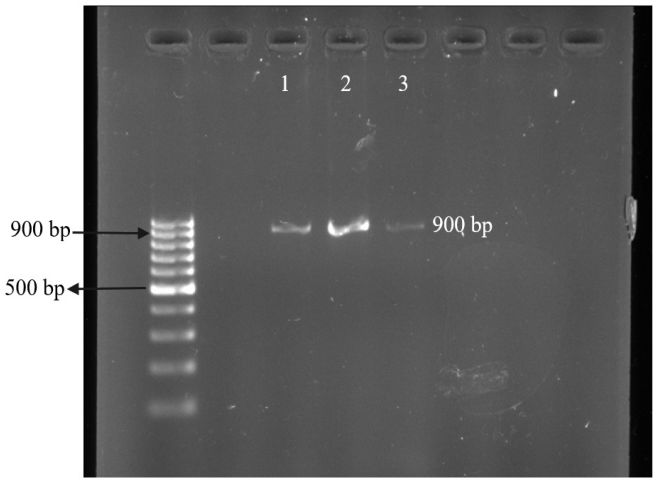

Fig. 3. Well 1, 2 and 3 showing PCR amplified product (900 bp) of virus isolate in agarose gel electrophoresis. The size of ladder 100 bp.

by hepatocyte enlargement, sometimes accompanied by partial cytoplasmic clearing or cell membrane rupture; in certain regions, hepatocytes appeared shrunken with pyknotic nuclei.

Heart

The transparent viscous fluid was observed in the pericardial sac and it was a hallmark of the IBH-HPS illness. The fluid's characteristics in the sac ranged from watery and crystal clear to gel-like. Fig. 1g showing severe pathological alterations on the heart. Histopathological changes in heart of broiler chicken infected with IBH-HPS virus are shown in Fig. 2g. Histopathological examination of heart revealed mononuclear cell infiltration, degeneration of myocardial fibres and necrosis.

Kidney

The kidneys of IBH-HPS positive broiler chicken showing congestion (Fig. 1h). Histopathological changes in kidney of broiler chicken infected with IBH-HPS virus are shown in Fig. 2h and 2i. The histopathological changes observed mild interstitial congestion and tubular degeneration. At subsequent stages of the disease, intensity was more pronounced during later stage. The tubules appeared markedly distended in some of the birds.

Lung

Gross lesion of the lung does not showing appreciable lesion. Figures 2i to 2o depict the histopathological alterations in the lungs of broiler chicken infected with the IBH-HPS virus. The interlobular septae were noticeably thicker as a result of oedema and lymphocyte and heterophil infiltration. The pulmonary blood arteries displayed significant disruptive alterations in their wall, appeared dilated, and were highly crowded. There was occasionally a lot of hemorrhagic exudate seen in the air capillaries and parabronchi.

Spleen

Birds died due to IBH-HPS, showing the specific gross pathological changes in spleen like congestion shown in Fig. 1i. Histopathological changes in spleen of broiler chicken infected with IBH-HPS virus are shown in Fig. 2j and 2k. There was oedema, depletion of white pulp with associated with haemorrhage in splenic parenchyma.

Multiple studies have documented multisystem lesions in poultry affected by IBH-HPS, with the liver being the primary target. Researchers such as Anjum (1990), Nakamura et al. (1999) and Philippe *et al*. (2005) have reported pale, enlarged livers with necrotic foci, hemorrhages, and both acidophilic and basophilic inclusion bodies in hepatocytes. These hepatic lesions not only serve as key diagnostic markers but also impair protein synthesis, reducing plasma colloidal osmotic pressure and leading to fluid accumulation in the pericardial sac [22, 23, 24]. Cardiac pathology is also prominent, with observations including fluid accumulation, malformed or flabby hearts, and myocardial degeneration. Liver dysfunction contributes to cardiac complications through reduced osmotic pressure and increased workload on the heart [3, 28, 29]. Although less extensive, the kidneys show notable alterations such as tubular necrosis, degenerative epithelial changes, and urate deposits. Additionally, lymphoid depletion in the spleen and various lung lesions indicate that IBH-HPS is a truly systemic disorder [30, 31, 32].

PCR

The amplified PCR, which was examined using agarose gel electrophoresis, included a DNA fragment of about 900 bp, as anticipated by primers HexonA (F) and HexonB (R) (Fig. 3). Comparable PCR findings have been reported across multiple studies using various primer sets. Some scientists also observed that while common primers yielded expected amplicons, cells infected with the group III adenovirus (EDS strain A127) showed no product [9]. Steer et al. (2009) detected consistent amplification with Hexon A (F) and Hexon B (R) primers in 11 of 12 reference serotypes, except for FAdV-5, which produced minimal results. Additionally, the hexon gene was fragmented into ~191 bp and ~590 bp amplicons using the Hex-S and Hex L1 primers [30, 35]. Moreover, Thakor *et al*. [27] detected an ~890 bp fragment in liver samples from IBH-HPS-affected chickens using HexonA F and HexonB R primers [27].

CONCLUSION

Collectively, these findings underscore the need for integrated diagnostic approaches—combining gross pathology, histopathology, and modern molecular methods—to effectively understand and manage IBH-HPS in poultry.

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