International Journal of Advanced Biochemistry Research

ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2024; 8(6): 615-618
www.biochemjournal.com
Received: 01-03-2024
Accepted: 04-04-2024

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Adenovirus associated hepatitis in dog pups

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DOI: https://doi.org/10.33545/26174693.2024.v8.i6h.1388

Abstract
Infectious canine hepatitis is a serious liver illness that affects dogs, caused by canine adenovirus 1 (CAdV-1). The disease is now seen infrequently due to vaccination practices. In the present study, carcasses of two, one-month old male non-descript dog pups were presented to the Department of Veterinary Pathology, College of Veterinary Sciences, Hisar for necropsy. During necropsy, external examination revealed swelling around neck region and pale-white conjunctival mucous membranes. Internal examination revealed presence of serosanguineous fluid in thoracic and abdominal cavities. Liver was enlarged and showed haemorrhages and small multifocal areas of necrosis. Haemorrhages were noticed in lungs, spleen, kidneys, and brain (cerebellum). Kidneys, heart, and spleen revealed enlargement. Multiple necrotic foci were noticed in spleen. Histopathological examination of liver revealed diffuse fatty changes, focal necrosis, congestion, sinusoidal dilatation and haemorrhages. Hepatocytes also revealed characteristic basophilic intranuclear inclusion bodies surrounded by a clear zone. Sections of kidneys showed mild degenerative changes in the renal tubules, widespread congestion, and haemorrhages in the capsular region. Mesenteric lymph node showed severe congestion, mild lymphoid depletion, and infiltration of few neutrophils in medulla. Necrosis was observed in white pulp area of spleen. Lungs revealed congestion, haemorrhages, thickened interstitial septa and emphysema. The polymerase chain reaction (PCR) using hexon gene base primers, DNA of adenovirus was detected in liver tissue. In conclusion, pathological findings and positive PCR results for genus Adenovirus suggests that the dog puppies were affected by infectious canine hepatitis.

Keywords: Adenovirus, infectious canine hepatitis, intranuclear inclusion bodies, liver, pup

Introduction
Canine adenovirus 1 (CAdV-1) is non-enveloped, icosahedral, double stranded DNA virus in the genus Mastadenovirus in the family Adenoviridae which causes infectious canine hepatitis (ICH), also known as epizootic fox encephalitis or Rubarth's disease (De Jonge *et al.*, 2020; Manjunatha *et al.*, 2024) [1]. ICH is a systemic disease affecting mainly Canidae and Ursidae (Caudell *et al.*, 2005; Decaro *et al.*, 2007) [3, 4]. It is a highly infectious, acute and fatal disease of liver in dogs in which virus has a predilection for hepatocytes, mesothelium and vascular endothelium. Infection with CAdV-1 can lead to hepatic necrosis and widespread serosal haemorrhage of different organs (Zachary and McGavin, 2012; Caudell *et al.*, 2005) [5, 3] with a more severe clinical course in younger dogs than adults (Decaro *et al.*, 2007) [4]. In acute stage, virus spreads through all the secretions and excretions of infected animal. Virus can persist in the kidneys up to 9 months post infection which can cause shedding of the virus in urine even after recovery of infected one (Mosallanejad *et al.*, 2010; Jonge *et al.*, 2020) [6, 1]. Inappetence, dyspnoea, fever, abdominal pain, vomiting and diarrhoea are some clinical signs which are generally shown by animals. 1-3 weeks post recovery, animals may also develop corneal opacity which is also called as blue eye condition and interstitial nephritis as a result of deposition of circulating immune complexes (Decaro *et al.*, 2007) [4]. Circulation of CAdV-1 is diminished due to generalised use of vaccines in the canine population as a result reports of ICH cases are scanty (Decaro *et al.*, 2007; Mosallanejad *et al.*, 2010) [4, 6]. The present study is aimed to describe pathomorphological findings as well as molecular detection of adenovirus in unvaccinated pups and its association with hepatitis.

Materials and Methods

Carcasses of two, one-month old male non-descript dog pups were presented to the Department of Veterinary Pathology, College of Veterinary Sciences, Hisar for necropsy. Systemic necropsy was performed and appropriate tissue samples were collected in 10% neutral buffered formalin for histopathology. Liver tissue samples were also collected in sterile container for polymerase chain reaction (PCR) assay. After fixation, tissue processing was done using routine paraffin embedding technique (Luna, 1968) [7].

Tissue sections were cut into 4 µm thickness using rotary microtome (Yorco YSI 060 semi-automatic rotary microtome) and stained with haematoxylin and eosin (H&E) stain (Luna, 1968) [7]. For PCR, genomic DNA was extracted from the liver tissue samples using the QIAamp DNA Mini Kit (Qiagen) as per the manufacturer's protocol. The PCR was carried out on extracted DNA samples to amplify the hexone gene fragment specific to genus adenovirus using the primer set H1f, 5′-TGGACATGGGGGCGACCTA-3′ and H1r, 5′ AAGGGATTGACGTTGTCCA-3′ as per the following protocol: 94ºC for 5 min followed by 30 cycles of denaturation at 94ºC for 1 min, annealing at 55ºC for 1 min, and extension at 72ºC for 1 min with a final extension at 72ºC for 10 min (Cui *et al.*, 2020) [8]. The amplified PCR products were analysed by agarose gel electrophoresis using 1.5% agarose containing 0.5 µg/ml ethidium bromide in Tris-acetate EDTA buffer and visualized under UV transilluminator (Sambrook *et al.*, 1989) [9]. DNA extracted from fowl adenovirus (accession no. MT708597/10AD/India/FAdV-8a) was used as positive control in the present study.

Results

During necropsy, external examination revealed the presence of swelling in neck region and pale-white conjunctival mucous membranes. Internal examination revealed presence of serosanguineous fluid in both thoracic as well as abdominal cavity (Fig. 1). Liver was enlarged with rounding of edges and showed presence of haemorrhages and small multifocal necrotic foci (Fig. 2). Haemorrhages were also noticed in lungs (Fig. 1 A), spleen (Fig. 1 B), kidneys, and brain (cerebellum). Kidneys, heart, and spleen were also enlarged. Multiple necrotic foci were noticed in spleen (Fig. 1 B).

Histopathological examination of liver revealed diffuse fatty changes, focal necrosis, congestion, sinusoidal dilatation and haemorrhages. Hepatocytes also revealed characteristic basophilic intranuclear inclusion bodies surrounded by a clear zone (Fig. 3). Sections of kidneys showed mild degenerative changes in the renal tubules, widespread congestion, and haemorrhages in the capsular region (Fig. 4 A). Mesenteric lymph node showed severe congestion, mild lymphoid depletion, and infiltration of few neutrophils in medulla (Fig. 4 B). Necrosis was observed in white pulp area of spleen (Fig. 5 A).

Lungs revealed congestion, haemorrhages, thickened interstitial septa and emphysema (Fig. 5 B). Molecular detection of adenovirus genus in liver tissue was carried out by PCR using hexon gene-based primers and revealed the product size of 690 bp (Fig. 6). The findings from the gross, histopathological, and molecular examination suggest that both puppies were suffering from infectious canine hepatitis caused by adenovirus.

Discussion

ICH is caused by canine adenovirus 1 (CAdV-1) which is asymptomatic to fatal disease and is transmitted by the oro-nasal route and the infected animal remains carrier for up to 6 months by excreting virus in urine. Virus can gain entry into the host via direct contact of contaminated saliva, urine and faeces (Agnihotri *et al.*, 2019) [10]. In the present study the affected dogs were one month old without any history of vaccination. It has been reported that ICH is seen mostly in young dogs of age less than one year. However, dogs of all ages can be affected which are generally unvaccinated (Mosallanejad *et al.*, 2010) [6]. The post mortem findings revealed enlarged liver with necrotic foci and hemorrhages. Other visceral organs also revealed hemorrhages such as lungs, spleen, kidneys and cerebellum. Histopathologically, liver showed the necrosis with scattered basophilic intranuclear viral inclusion bodies in numerous hepatocytes. Other organs also showed haemorrhages and necrotic areas. Similar kind of pathological findings were also reported in earlier studies (Mosallanejad *et al.*, 2010; Jonge *et al.*, 2020; Manjunatha *et al.*, 2024) [6, 1, 2]. Virus causes hepatic necrosis, as it has affinity for vascular endothelium and hepatocytes (Caudell *et al.*, 2005) [3]. Liver tissue samples were positive for molecular detection of adenovirus by using hexon gene base primers. However, to confirm CAdV-1 infection in the pups, this study requires a diagnostic confirmation using PCR with a species-specific primer targeting CAdV-1.

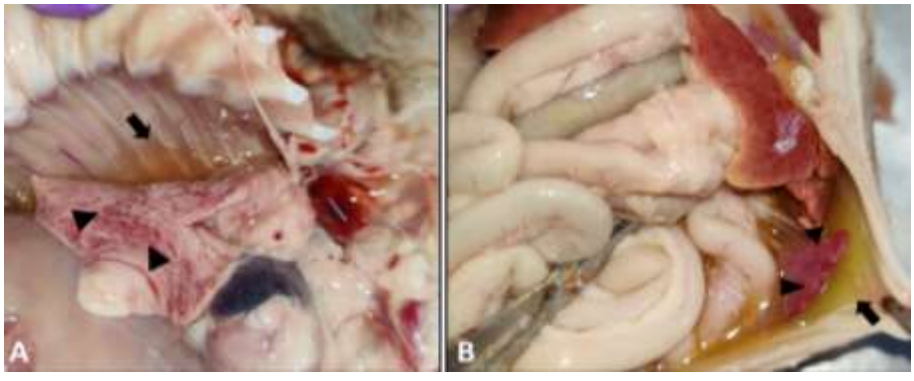

Fig 1: A) Photograph showing presence of straw-coloured fluid (arrow) in the thoracic cavity and widespread haemorrhages on the lung parenchyma (arrow heads). B) Photograph showing presence of straw-coloured fluid (arrow) in the abdominal cavity and multiple necrotic foci on spleen (arrow heads).

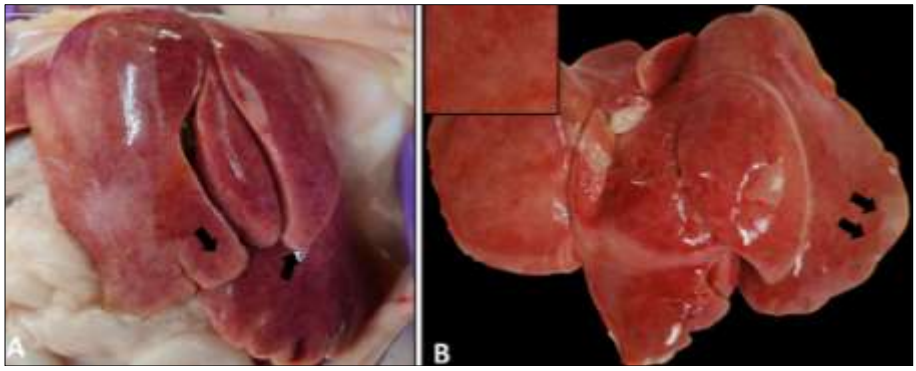

Fig 2: A) Photograph showing presence of multiple necrotic foci (arrows) on liver (Dorsal surface). B) Photograph showing fibrin deposition (arrows) on liver surface (inset: Petechial haemorrhages).

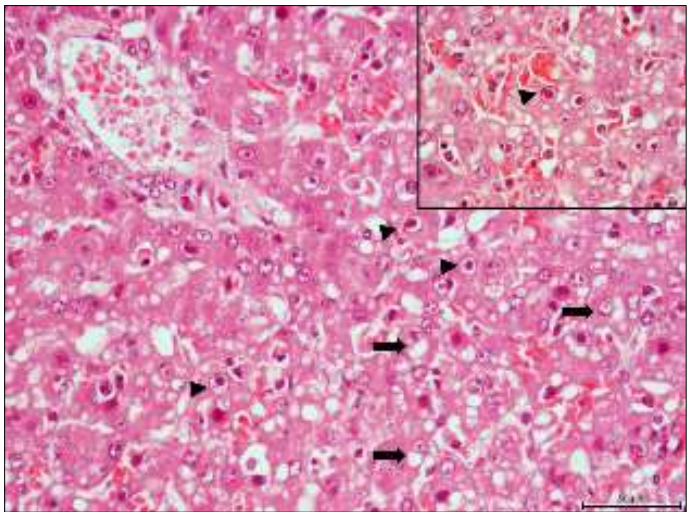

Fig 3: Photomicrograph of liver showing characteristic basophilic intranuclear inclusion bodies surrounded by a clear zone (arrow heads) along with fatty changes (arrows) and sinusoidal dilatation (Inset: Hepatocytes showing basophilic intranuclear inclusions (arrow head) under higher magnification). H&E×400

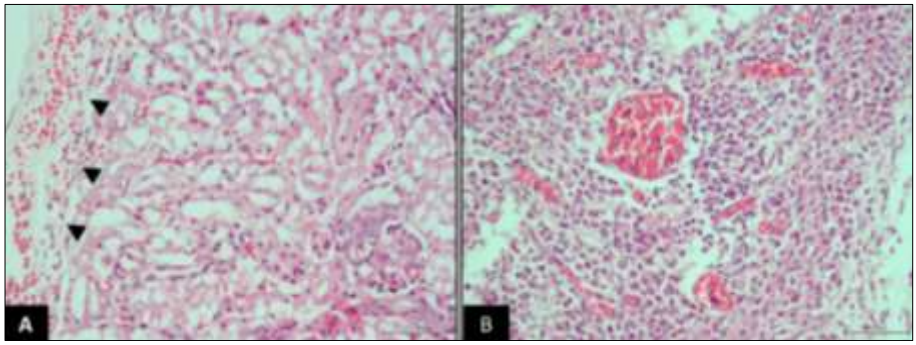

Fig 4: A) Kidney section showing capsular haemorrhages (arrow heads) and degeneration in tubular epithelial cells. H&E×400 B) Section of lymph node showing severe congestion and lymphoid depletion. H&E×400

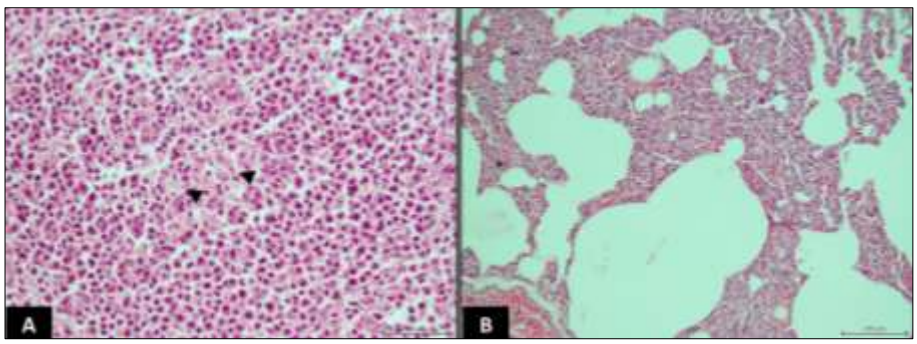

Fig 5: A) Photomicrograph of spleen showing necrotic foci. H&E×400 B) Section of lung showing congestion, haemorrhages and emphysema. H&E×200

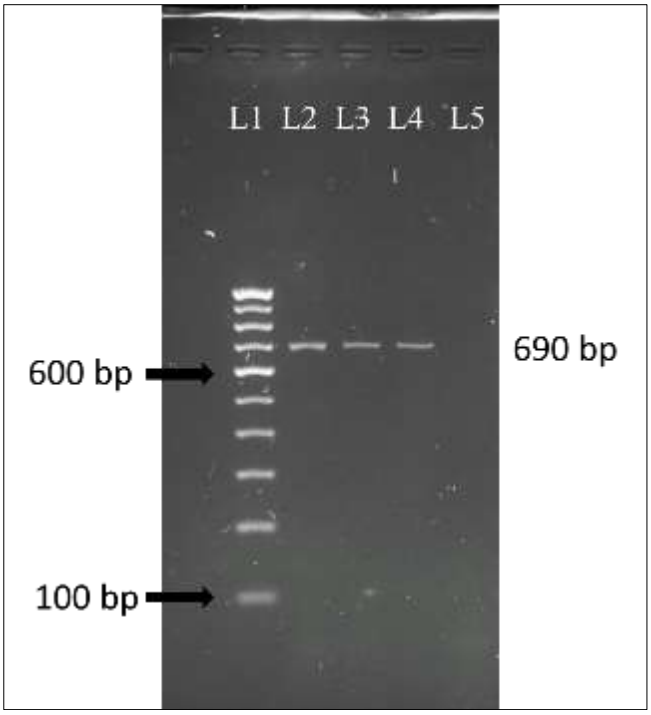

Fig 6: PCR products in ethidium bromide-stained 1.5% agarose gel electrophoresis with 100 bp ladder (L) and positive samples having band size of 690 bp for Adenovirus (Lane 3 & 4 having samples), L2- positive control; L5- no templet control.

Conclusion

In conclusion, liver tissue samples positive for adenovirus along with pathological findings are suggestive of infectious canine hepatitis in pups. Molecular detection of canine adenovirus can suggest the presence of adenovirus in the unvaccinated dog populations. These infected animals can also serve as a source of infection. However, additional techniques are required for definitive diagnosis.

Acknowledgments

The authors are grateful to the administration of LUVAS, Hisar and Head, Department of Veterinary Pathology, LUVAS, Hisar, for providing necessary facilities to carry out the investigation.

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