RESEARCH ARTICLE

Molecular detection of Infectious canine hepatitis in a dog

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Abstract

A 7 year old male Labrador breed of dog was presented at Veterinary Clinical Complex, SOA, (DU) with a history of inappetance, vomition, black stools and tick manifestation with no history of vaccination. Clinical examination revealed yellowish mucous membrane with dark yellow urination. Based on the clinical report there was higher level of liver enzyme ie. ALT (Alanine Aminotransferase) 125 µ/L, ALP (Alkaline phosphatase) 435 µ/L. During treatment the animal collapsed and died and the carcass was brought to the Department of Veterinary Pathology, IVS&AH for postmortem examination. On necropsy, external examination revealed icteric mucous membrane. Liver was enlarged, hemorrhagic with mottled appearance. Gall bladder was thickened and distended. There was shrinkage on both sides of the kidney and the surface was rough. Histopathological examination of the liver revealed hepatocellular necrosis with presence of intranuclear inclusion bodies. Molecular detection was carried out by PCR using gene based primers and revealed the product size of 508 bp. The findings from molecular detection confirmed that the dog was suffering from infectious canine hepatitis.

Key words: ICH, gross, histopathology, PCR

Ind J Vet Sci and Biotech (2025): 10.48165/ijvsbt.21.6.42

Introduction

Canine adenovirus (CAdV) (genus *Mastadenovirus*, family *Adenoviridae*) is an important pathogens of dogs worldwide and exists in two distinct types. CAdV-1 a non-enveloped, icosahedral, double-stranded DNA virus causing infectious canine hepatitis (ICH) while CAdV-2 is responsible for mild respiratory illness. CAdV-1 primarily infects vascular endothelial and hepatic parenchymal cells resulting in acute necrohemorrhagic hepatitis (Ditchfield *et al*., 1962; Greene, 2005). Although their pathogenic effects differ both viruses are antigenically related and share approximately 75% genetic identity. ICH also known as Rubarth's disease named after Carl Sven Rubarth who first described it in the late 1940's (Rubarth, 1947). Transmission occurs via direct contact between dogs or through contaminated fomites such as hands, utensils and clothing with ectoparasites like fleas and ticks serving as possible mechanical vectors (Cabasso, 1962). The disease is most frequently seen in dogs less than one year of age though cases in adults were common prior to the introduction of widespread ICH vaccination. The present study report on the occurrence of detection of infectious canine hepatitis in unvaccinated male dog in Bhubaneswar.

Materials and Methods

Collection of samples

A detail postmortem examination was conducted at the Department of Veterinary Pathology, IVS & AH, SOA. The liver, lungs and kidney were collected in 10% neutral buffered formalin for histopathological examination. For the molecular detection, liver and spleen tissue samples was collected and stored at -20°C.

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How to cite this article: Lalrinkima, W., Wani, M. A., Moqbool, Y., Lalawmpuii, K., Shah, Z. A., Mohanty, R. N., & Patro, D. (2025). Molecular detection of infectious canine hepatitis in a dog. Ind J Vet Sci and Biotech, 21(6), 207-210.

Source of support: Nil

Conflict of interest: None

Submitted 14/08/2025 **Accepted** 15/09/2025 **Published** 10/11/2025

Histopathological studies

The liver, lungs and kidney fixed tissues in 10% neutral buffered formalin were processed as per the standard protocol and stained with haematoxylin and eosin stain (Suvarna *et al*., 2018).

Polymerase chain reaction

For molecular confirmation, total DNA was extracted from tissue samples (ie. liver and spleen) using the Wizard® Genomic DNA Purification Kit (A1120, Promega) following the manufacturer's instructions. Initial screening was carried out using PCR to amplify a 508 bp fragment corresponding to the E3 gene as described by Kiss *et al.* (1996) and Hu *et al.* (2001). The PCR assay was performed in a total volume of 25

µl, containing 2 µl of template DNA, 12.5 µl of 2X Go Green Master Mix, 0.5 µl each of forward and reverse primers and nuclease-free water to make up the final volume. The specific primers (Forward-5′ CGCGCTGAACATTACTACCTTGTC 3′ and Reverse-5′ CCTAGAGCACTTCGTGTCCGCTT 3′) and thermal cycling conditions used to target the E3 gene followed the protocol of Chander et al. (2013). Amplified products were separated on a 1% agarose gel containing ethidium bromide and visualized under UV illumination using a gel documentation system (Vilber, Eppendorf).

Phylogenetics

The E3 gene sequences of the isolated ICH strains were analyzed with available GenBank sequences using MEGA 11 software as previously reported by Kiss *et al.* (1996) and Hu *et al.* (2001). Phylogenetic analysis was achieved for the nucleotide sequence of the E3 gene of the present isolates with eight E3 gene sequences of the other CAdV-1 isolates retrieved from the NCBI GenBank nucleotide database. The phylogenetic tree was constructed with the MEGA 11 software using the Neighbour joining method and a distance matrix was generated using Kimura 2 parameter method (Tamura *et al*., 2021).

Result and Discussion

In the present study, based on the clinical examination the dog show clinical signs of inappetance, vomition, black stools with tick manifestation. The animal was icteric and liver enzymes were high (ALT 125 **µ**/L, ALP 435 **µ**/L). Syamily *et al*. (2023) reported the incidence of higher level of liver enzyme in dog (i.e. ALT and ALP) affected with infectious canine hepatitis.

On necropsy, external examination revealed icteric mucous membrane. Internal examination revealed presence of yellowish discoloration of subcutaneous tissue (Figure 1). Heart was flabby and congested. Diffuse white necrotic patches were observed on left side of the lungs. Liver was enlarged, hemorrhagic with mottled appearance (Figure 2). Gall bladder was thickened and distended. Chouinard *et al.* (1998) reported that most significant gross lesion observed in dog affected with infectious canine hepatitis was necrohemorrhagic hepatitis along with hepatomegaly which supports our findings. There was shrinkage on both the side of the kidney and surface was rough with capsule difficult to peel off (Figure 3), cut surface of cortex and medulla was pale. The findings are in accordance with the earlier work of Majunatha *et al.* (2024) who reported pale medulla with capsule difficult to peel off in Dhole (*Cuon alpines*) pup infected with canine adenovirus type-1. In intestine there were areas of hemorrhages on the mucosal surface of small intestine. Pancreas showed multiple white nodules. Spleen show enlargement with sub-capsular hemorrhages.

Histopathological examination of the liver revealed hepatocellular necrosis with congestion of central vein and presence of intranuclear inclusion bodies (Figure 4). The findings are in accordance with the earlier work of Cheema *et. al.* (2011) and Syamily *et al.* (2023) who reported intranuclear inclusion in hepatocytes and it is the most significant histopathological lesion observed in dog affected with infectious canine hepatitis. Lungs showed emphysema with haemorrhagic areas. Kidney show tubular hemorrhages.

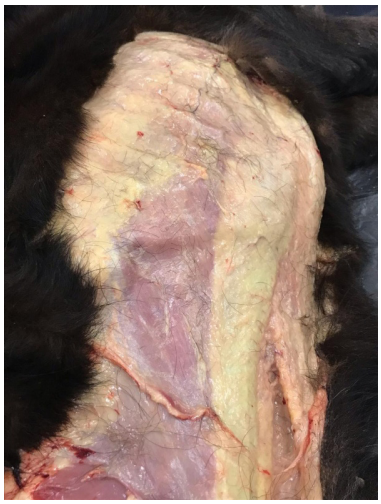

Fig. 1: Yellowish subcutaneous tissue

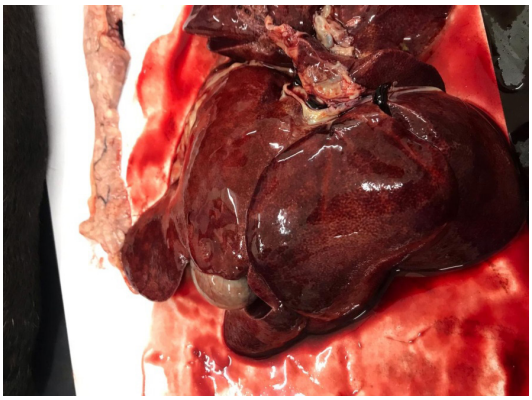

Fig. 2: Liver: Enlarged, hemorrhagic with mottled appearance

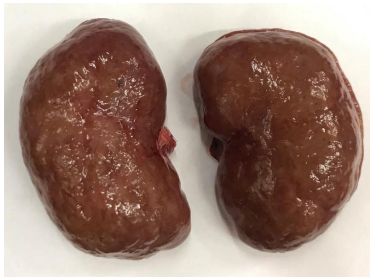

Fig. 3: Kidney: Shrinkage with rough surface

Molecular detection of canine adenovirus-1 (CAV-1) in liver and spleen tissue was carried out by PCR using gene based primers and revealed the product size of 508 bp targeting E3 gene (Figure 5). The findings from molecular detection confirmed that the animal was suffering from infectious canine hepatitis and the obtained sequences were analyzed using Finch TV (website), revealing fragment length of 500bp for CAdV 1 E3 gene fragment (Table.1).

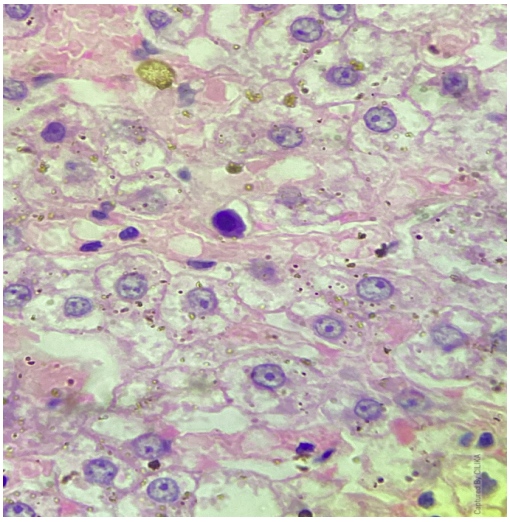

Fig. 4: Liver: Intranuclear inclusion bodies. H & E, 1000x

Gene	Partial nucleotide sequence
E3 gene Cav-1 SOA ODISHA/ IND	CGCTGAACATTACTACCTTGTCTATATTTATGAGAACTGC-CACCAGATGCCGTATGATTCCCCACGGCAC ACCGGCCACAAAGGGACCTCATTCAACTGGTCCATGGGAC-TATGGCTGGTAAAATGCAGCCATAACAAAA CTTTCTTTTTGCCATTTGTTCTAGACTCTGCAAAAAGT-GCTCCCATTATTATGACTGAGACTGCTATAAC TATATACATTTCCATGATATTTTTAATTGTAAGCCTGCTAAC-CTTTCTAAATGTTTTAATAACGCTAAAC AATAAATATAAACACTATGGAGTTTAAAAATAAACTTAC-CTAATTTTTGTCAAGACTTCTGGGTCCTGTG TCTCTATGTCCACAAGGGCCCCCTCTTCCCAACTTTGA-TACTTCCACTTGTGTGTGCGAGCCAACTTGCG CAAATGCTTAAAAGACAATGTGGTCTCTCCCGACAGCTTC-CCGTTCACCATCACCAAAGCCATGAAGCGG ACACGAAGTG

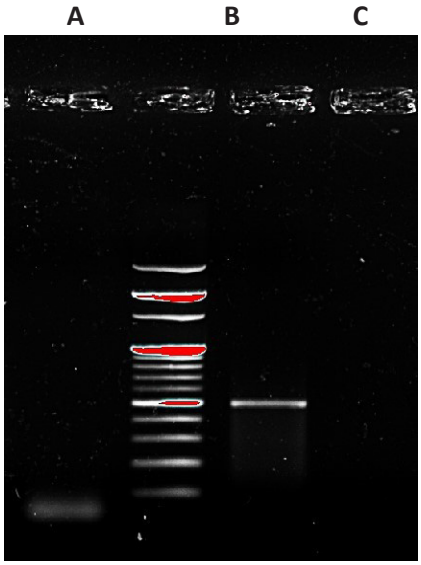

Fig. 5: PCR amplification of E3 gene segment of Infectious canine hepatitis
A – Negative control
B – 100 bp ladder
C – Positive (508bp)

Sequencing and nucleotide sequence analysis

The phylogenetic analyses of the CAdV-1, based on the sequence of the E3 gene, revealed a formation of cluster (subgroups) with other CAdV-1 strains in Indian subcontinent subgroups (Figure 6).

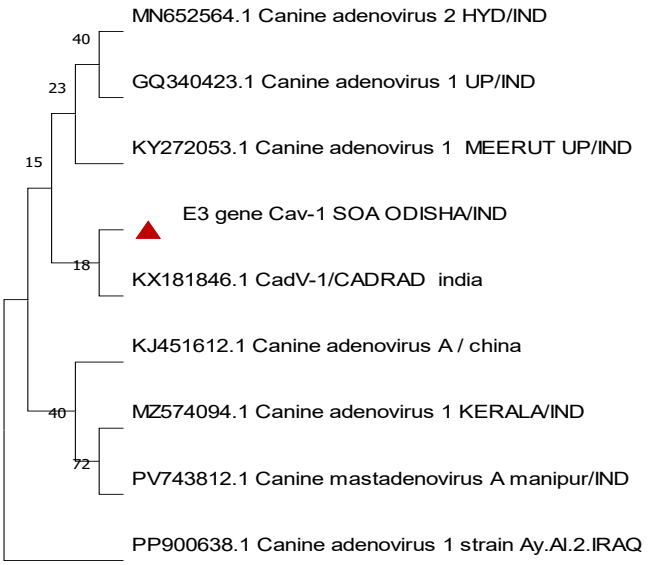

Fig. 6: Phylogenetic analysis

Sequence homology assessed using the BLASTn algorithm in the NCBI database revealed 100% identity with Canine adenovirus 1 isolate (KX181846.1) and 99.80% identity with the Canine mastadenovirus A isolate (PV743814.1) the phylogenetic analysis of CAV-1 isolate is close to other isolates of CAV-1 isolated from Hyderabad, Uttar Pradesh, Kerala, Manipur India.

Nucleotide BLAST analysis of nucleotide sequence generated in this report ascertained the presence of CAdV-1-specific nucleic acid in the spleen thereby confirming the identity of the virus.

Conclusion

The study reported on the significant characteristic gross and histopathological lesion of Infectious canine hepatitis in dog and which was confirmed by PCR. The findings reported the presence of diseases within the Bhubaneswar.

References

Cabasso, V.J (1962). Infectious canine hepatitis virus. Ann. N.Y. Acad. Sci., 101:498-514.

Chander, V., Nandi, S and Verma, R. (2013).Detection of infectious canine hepatitis virus in vaccines by PCR. Adv. Anim. Vet. Sci., 1:191-193.

Cheema, A.H., Ahmed, I., Mustafa, G. and Aslam, A. (2011). Peracute infectious canine hepatitis. Vet J., 32(2):277–279.

Chouinard, L., Martineau, D., Forget, C. and Girard, C. (1998). Use of polymerase chain reaction and histochemistry for detection of canine adenovirus type 1 in formalin-fixed, paraffin-embedded liver of dogs with chronic hepatitis or cirrhosis. J. Vet. Diagn. Invest., 10: 320.

Ditchfield, J., Macpherson, L.W. and Zbitnew, A. (1962). Association of canine adenovirus (Toronto A 26/61) with an outbreak of laryngotracheitis ("kennel cough"): a preliminary report. Can. Vet. J., 3:238.

Greene, C.E. (2005). Infectious Diseases of the Dog and Cat. 3rd edn. Elsevier, St. Louis, USA.

Hu, R.L., Huang, G., Qiu, W., Zhong, Z.H., Xia, X.Z. and Yin, Z. (2001). Detection and differentiation of CAV-1 and CAV-2 by polymerase chain reaction. Vet. Res. Commun., 25: 77-84.

Kiss, I., Matiz, K., Bajmoci, E., Rusvai, M. and Harrach, B. (1996). Infectious canine hepatitis: detection of canine adenovirus type 1 by polymerase chain reaction. Acta Vet. Hung., 44: 253-258.

Manjunatha, V., Rout, M., Srinivas, U.K. and Kshamaa, L.M. (2024). Fatal Canine Adenovirus Type 1 Infection in Dhole (Cuonalpinus) Pups. Indian J. Anim. Res., 1: 1–8.

Rubarth, S. (1947). An acute virus disease with liver lesions in dogs (Hepatitis contagiosacanis): a pathologico-anatomical and etiological investigation. Acta Pathol. Microbiol. Scand. Suppl., 69: 1–222.

Suvarna, K.S., Layton, C. and Bancroft, J.D. (2018). Bancroft's theory and practice of histological techniques. 8th edn. Philadelphia: Elsevier Health Sciences, Philadelphia, USA.

Syamily, S., Rajasekhar, R., Anoopraj, R., Ravishankar, C. and Jishnu, H.P. (2023). Detection and molecular characterization of canine adenovirus type 1 from a fatal case of infectious canine hepatitis from India. Vet. Rec. Case Rep., 11(4): e698.

Tamura, K., Stecher, G. and Kumar, S. (2021). MEGA11: molecular evolutionary genetics analysis version 11. Mol. Biol. Evol., 38 (7): 3022-3027.