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SHORT COMMUNICATION

Pathology of infectious canine hepatitis in captive Indian wolves (*Canis lupus pallipes*): An outbreak study

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ABSTRACT

This study reports an outbreak of infectious canine hepatitis (ICH) in a captive pack of four sub-adult Indian wolves *(Canis lupus pallipes)*. The first wolf showed signs of lethargy, loss of appetite and fever before dying suddenly. Despite symptomatic treatment, the remaining three wolves also died within 24 hours, showing similar symptoms. Necropsy examination of all wolves revealed a soft, enlarged and discoloured liver with widespread haemorrhages, which were also present in other internal organs. Histopathology of the liver showed severely engorged and dilated vasculature and sinusoids, areas of haemorrhages, degeneration and necrosis of hepatocytes and basophilic intranuclear inclusion bodies in hepatocytes and Kupffer cells. Similarly, vascular changes were also seen in the lungs, kidneys, spleen, heart and intestines. Immunohistochemistry confirmed Canine Adenovirus-1 (CAdV-1) antigen in hepatocytes, endothelium and Kupffer cells, while PCR detected CAdV-1 in liver. This study highlights the significance of ICH in Indian wolves and its implications for their conservation.

Keywords: Canine Adenovirus-1, indian wolf, liver, pathology, PCR

The Indian wolf *(Canis lupus pallipes)*, classified as endangered and listed under Schedule I of the Wildlife Protection Act (1972) in India, faces serious conservation threats due to habitat loss, fragmentation and emerging infectious diseases[1,2]. Canine adenoviruses, CAdV-1 and CAdV-2, belong to the family *Adenoviridae* and genus *Mastadenovirus*[3]. CAdV-1 is the causative agent of infectious canine hepatitis (ICH), a highly contagious disease affecting primarily canids, as well as ursids and mustelids worldwide[4,5]. CAdV-1 mainly targets hepatocytes and endothelial cells, causing systemic illness and widespread tissue damage[5,6]. In contrast, CAdV-2 primarily infects the respiratory tract, leading to infectious canine tracheobronchitis (kennel cough)[4]. CAdV-1 is environmentally stable, capable of surviving for extended periods and spreads through direct contact, contaminated fomites or urine from infected animals[7]. While vaccination with modified live CAdV-2 vaccines provides cross-protection and has reduced the prevalence of CAdV-1 in domestic dogs in developed regions[6], unvaccinated dogs and wildlife reservoirs such as foxes, wolves and bears continue to support viral circulation and pose a risk of re-emergence[7]. In India, ICH has been reported in unvaccinated domestic dogs and occasionally in wild canids, including dholes (Asiatic wild dogs) and foxes[8]. The present study documents an outbreak of ICH in captive Indian wolves, highlighting the ongoing threat posed by CAdV-1 to wildlife conservation.

A sudden death in a captive pack of four sub adult Indian wolves two male and female each occurred at the Nawab Wajid Ali Shah Zoological Garden, Lucknow, Uttar Pradesh. Initially, one wolf showed signs of anorexia, lethargy, high fever (104.2^{O}F) and bloody diarrhoea, leading to sudden death (Fig. 1a). The remaining three wolves died within 24 hours, despite receiving symptomatic treatment. Blood and serum samples were collected prior to death for laboratory analysis. A systematic necropsy was performed and tissue samples from major organs were collected in 10% neutral-buffered formalin and on ice. Formalin-fixed tissues were processed and stained with Haematoxylin and Eosin for histopathological evaluation. Indirect immunohistochemistry (IHC) was conducted on liver sections using mouse anti-CAdV-1 monoclonal antibody (VMRD, USA)[9]. DNA was extracted from liver tissues and PCR targeting the E3 gene was performed following established protocols[10].

Serum biochemical analysis revealed elevated liver enzymes (ALT: 418±52 IU/L; ALP: 332±34 IU/L) indicating hepatic damage. Major gross lesions included congested or icteric conjunctival

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Fig. 1. Pathological findings of ICH in Indian wolves. **a.** Clinically affected wolf showing dull and recumbent in position. **b.** Weak and dehydrated carcass of wolf died due to ICH. **c.** Congested discoloured and enlarged liver with widespread haemorrhages and distended gall bladder. **d.** Bloody contents in the lumen of small intestine due to ICH.

membranes, dehydrated and weak carcasses, an enlarged, soft, severely congested, discoloured and haemorrhagic liver with rounded borders and distended gall bladder (Fig. 1b & c). Haemorrhages were also seen throughout the gastrointestinal tract, which contained bloody contents (Fig. 1d). The congestion and haemorrhages were also observed in lungs, spleen, heart and kidneys. Histopathological examination of the liver showed diffuse engorgement and dilatation of vasculature including sinusoids, areas of haemorrhages, hepatocytic degeneration and necrosis, increased Kupffer cell activity and basophilic intranuclear inclusion bodies in hepatocytes, Kupffer cells and endothelial cells (Fig. 2b & c). Severe vascular lesions like highly engorged blood

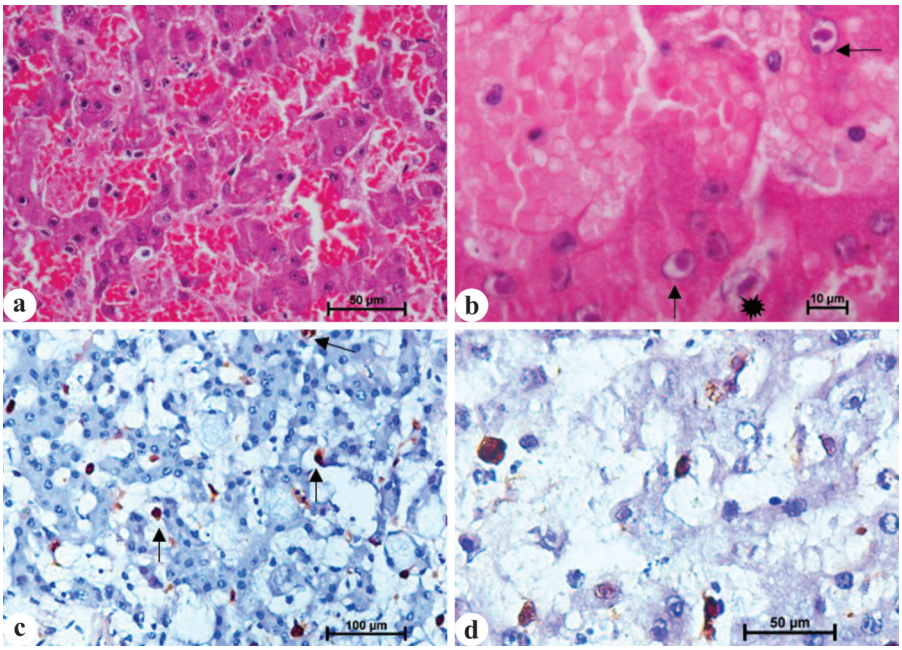

Fig. 2. Microscopic findings of ICH in Indian wolves. **a.** The liver section showing severely dilated and engorged sinusoids with degeneration and necrosis of hepatocytes (H&E x400). **b.** Basophilic intranuclear inclusion bodies in the hepatocytes (arrow) and endothelial cells (star) (H&E x1000). **c & d.** Liver section showing positive immune staining in hepatocytes, endothelial cells and Kupffer cells to CAdV-1 antigen (arrow). (IHC DAB x200, x400).

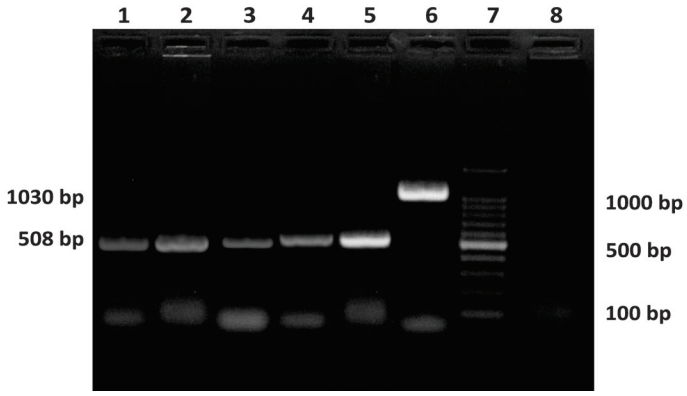

Fig. 3. Detection of Canine adenovirus virus in Indian wolves by conventional PCR assay. Lane 1-4: Liver samples positive for CAdV-1 (508 bp), Lane 5: Positive control CAdV-1 (508 bp), Lane 6: Positive control CAdV-2 (1030 bp), Lane 7: 100 bp ladder, Lane 8: Negative control.

vessels with haemorrhages were also observed in the lungs, kidneys, spleen, intestine and heart. Immunohistochemistry showed positive labelling for CAdV-1 antigen in hepatocytes, Kupffer cells as well as endothelium (Fig. 2d & e). PCR confirmed the presence of CAdV-1, yielding a 508 bp product specific to the E3 gene (Fig. 3).

Necro-haemorrhagic hepatitis accompanied by hepatomegaly is commonly reported in CAdV-1 infection among both domestic and wild canids, which is consistent with the findings of this study[5,6]. CAdV-1 infection in foxes has occasionally been referred to as 'fox encephalitis' due to the presence of associated neurological signs[11]. However, no neurological symptoms were observed in the present outbreak. In India, data on the prevalence of CAdV-1 in wild canids remain limited, yet the virus poses a significant threat to species like the Indian wolf, which shares close genetic ties with domestic dogs[8]. These wolves often inhabit areas that overlap with domestic dogs, jackals *(Canis aureus)* and other wildlife, increasing the risk of interspecies transmission[7]. The absence of widespread vaccination in rural areas, combined with overlapping territories of domestic and wild canids, may facilitate the continued circulation of CAdV-1[6,7]. The detection of CAdV-1 in captive Indian wolves underscores its potential threat to other vulnerable wildlife species. Its presence in a zoological setting raises concerns about spillover to susceptible animals such as foxes, jackals and bears. These findings highlight the urgent need for regular health monitoring, active surveillance and vaccination of captive wild canids to prevent and control the spread of CAdV-1.

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