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HISTOPATHOLOGICAL AND MOLECULAR STUDIES ON CANINE DISTEMPER IN A DOG

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ABSTRACT

The present study was aimed to detect canine distemper virus and study the histopathological changes in tissues of a dog affected with Canine Distemper. The tissue samples were confirmed for the presence of viral RNA with the partial L gene. The brain tissue revealed non suppurative encephalitis and necrosis of neurons. In spleen, lymphoid depletion and focal areas of necrosis in the white pulp were observed.

Keywords: Brain, Canine Distemper Virus, Lymphoid depletion, Perivascular cuffing

INTRODUCTION

Canine Distemper Virus is a single-stranded negative-sense, non segmented, enveloped RNA virus with a diameter of about 150 - 300nm (Murphy *et al.,* 1999). The genome of CDV consists of genes for one non-structural protein and six structural proteins: large protein (L), haemagglutinin (H), phosphoprotein (P), nucleocapsid protein (N), fusion protein (F) and matrix protein (M) (Lamb and Parks, 2013). The virus has a broad host range infecting different species of order carnivore such as dogs, wolves, foxes, jackals, ferrets, minks, skunks, weasels, badgers, raccoons, bears, civets, genets, lions, linsangs and hyenas (Beineke *et al.,* 2016). It causes generalised infection with prominent respiratory, gastrointestinal and nervous signs. Dogs with nervous signs usually die, but some might recover and may display lifelong residual signs such as a persistent myoclonus (Scagliarini *et al*., 2003) The variable neurological signs are chorea, paddling and cycling movement, muscle tremor, epileptic seizure with facial twitching (Buragohain *et al*., 2018). Now a day's regular vaccination against CD is almost customary to prevent the occurrence of the disease. However, the disease remains as a major problem in dogs even after regular vaccination (Temilade *et al*., 2015)

MATERIALS AND METHODS

In the present study, tissues were collected from a necropsied dog which had clinical neurological signs like chorea and epileptic seizures with facial twitching. Brain tissue was collected and preserved at -20 ^{0}C for molecular detection of CDV. Representative tissue samples for histopathology were fixed in 10 per cent formalin for routine tissue processing and sections of 4 to 5 µm thickness were cut and stained by Harris Hematoxylin and Eosin method (Luna, 1968).

For detection of CDV, the RNA was extracted from the brain sample using TRIZOL LS reagent as per manufacturer's instructions with minor modification in RNA pellet washing technique. For synthesis of cDNA, RNA template having absorbance ratio (260/280) between 1.9 and 2.0 were taken. The cDNA was obtained by using two-step GoScript Promega RT-PCR kit following manufacturer's instruction. The synthesized cDNA was stored at -80 °C for further use as template for PCR. For the amplification of L gene, PCR was carried out using previously published primers (Swati *et al*., 2016) with an initial denaturation at 94 ^{0}C for 10 minutes followed by -35 cycles of denaturation at 94^{0}C for one minute, annealing at 45 ^{0}C for 30 seconds, extension at 72 ^{0}C for one minute 30 seconds and final extension at 72 ^{0}C for 10 minutes.

RESULTS AND DISCUSSION

At necropsy of the dog, no significant gross lesions were observed except for congestion of the visceral organs. In the present study, CDV was detected in the brain tissue. The presence of virus in the brain tissue was confirmed using RT-PCR for L gene as reported earlier by Swati *et al*. (2016) and Pardo *et al*. (2005). The expected amplicon size of 268 bp for the partial L gene was observed when tested in 1.5 per cent agarose gel electrophoresis (Fig.1). In the present study, the dog had clinical signs such as chorea, epileptic seizures with facial twitching before death which was in accordance with the observations of Koutinas *et al*. (2002) . On microscopic examination of tissue sections, significant histological lesions were observed in brain and spleen. Sections of brain revealed non suppurative encephalitis characterized by perivascular cuffing of large number of lymphocytes (Fig. 2). The histological lesions such as non-suppurative encephalitis, neuronal degeneration and necrosis, lymphoid depletion in the spleen were in accordance with that described by Pardo *et al*. (2005). Spongiosis of brain due to demyelination as reported by Koutinas *et al*. (2002) was not observed in the present study. Neurons showed varying degrees of degeneration

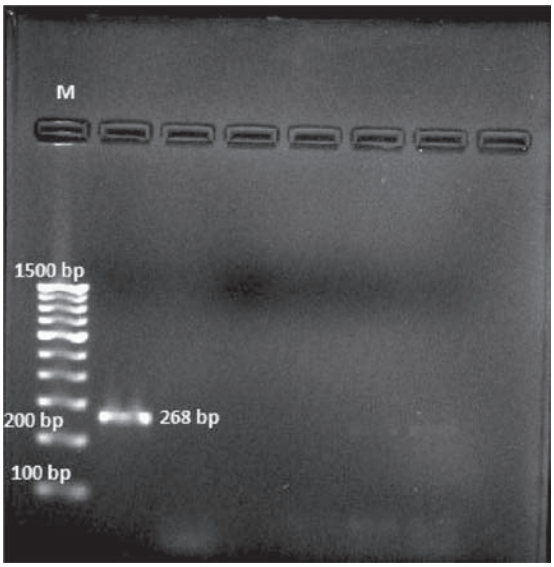

Fig. 1. RT-PCR detection of CDV partial L gene from brain tissue

ranging from cell swelling to chromatolysis and necrosis. The degenerating neurons were surrounded by oligodendroglia and microglia. Sections of spleen revealed severe lymphoid depletion and focal areas of necrosis in the white pulp (Fig. 3).

The study revealed that CDV infection in dogs is characterized by viral persistence in central nervous system and lymphoid tissue. In CNS it causes non suppurative encephalitis, neuronal degeneration and necrosis. In lymphoid tissue it causes lymphoid depletion. However, further studies are needed for analyzing CDV infection of the central nervous system subsequent to passing through the blood-cerebrospinal fluid barrier and infectious progression in the target cells in acute disease.

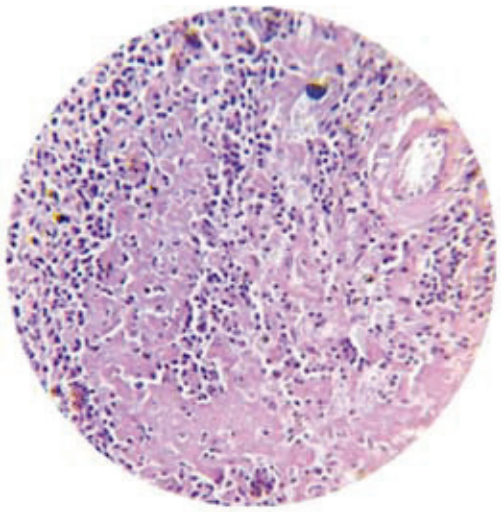

Fig. 2. Lymphoid depletion and focal areas of necrosis in the white pulp of spleen

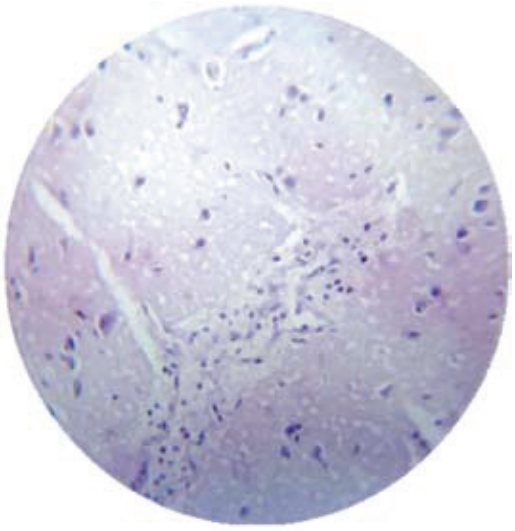

Fig. 3. Infiltration of neutrophills and lymphocytes, perivascular cuffing and necrosis of neuron in Brain tissue

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