Possible Participation of Canine Distemper Virus in the Development of Neuromuscular Disease in an Adult Dog

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ABSTRACT
This report investigated the possible participation of canine distemper virus (CDV) in an eight-year-old, male, Akita dog with neuromuscular disease. Clinically, there was tetraparesis, muscular atrophy, generalized weakness, intolerance to exercise, and diminished or absent spinal reflexes. The dog was serologically negative for Toxoplasma gondii. Necropsy confirmed generalized muscular atrophy. Histopathology revealed white matter demyelinating encephalitis, generalized atrophy and fibrosis of skeletal muscle fibers, myocardial atrophy and fibrosis, loss and demyelination of peripheral nerve fibers, axonal degeneration, endoneurial fibrosis, and interstitial pneumonia. Immunohistochemistry identified CDV antigens within the cerebellum, spinal cord, skeletal muscle, lungs, and spleen. RT-PCR and direct sequencing amplified the CDV nucleoprotein gene from the cerebellum and sciatic nerves. Collectively, these findings suggest that this dog demonstrated systemic canine distemper that also affected the muscular system and probably triggered the manifestations of the neuromuscular disease observed in this case.

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INTRODUCTION

Neuromuscular diseases (NMD) are disorders that affect the peripheral nerves, neuromuscular junctions or muscles, and are characterized by an insidious onset of generalized weakness and slow progression over several months, with variable muscle atrophy and neurological deficits (Lorenz and Kornegay, 2004). These manifestations are predo-minantly due to axonal degeneration, loss of large caliber nerve fibers and neurogenic atrophy (Thieman et al., 2010).

NMD in dogs have been induced by infectious diseases (toxoplasmosis and neosporosis), toxic agents, inflammatory conditions (immune-mediated myasthenia gravis), congenital myopathies, and inherited disease including muscular dystrophies (Shelton, 2010). In addition, acute canine polyradiculoneuritis (ACP), being a consequence of a previous infection, has been associated with T. gondii (Holt et al., 2011). Pathological alterations observed in ACP include leukocytic infiltration, paranodal and segmental demyelination, as well as concomitant degeneration of axons and myelin (Summers et al., 1995).

Canine distemper virus (CDV) infects a wide range of mammalian hosts, produces demyelinating encephalomyelitis in dogs, and is the major cause of canine mortality in Brazil (Headley et al., 2012). Moreover, CDV is a pantropic virus that produces several clinical manifest-ations in dogs including old dog encephalitis (Headley et al., 2009), and the recently coined necrotizing encephalitis in young puppies (Amude et al., 2011). However, no data was found in major databases associating CDV with NMD in dogs. With the exception of Neospora caninum and Toxoplasma gondii (Holt et al., 2011), other infectious agents that affect peripheral nerves and muscles of companion animals have not been described. This study investigated the possible participation of CDV in the pathological findings of NMD in a dog.

Clinical history: An eight-year-old, male, Akita dog, with a history of limited mobility of the hind limbs, muscular weakness, and pain at both hip joints was attended. The dog was routinely immunized with a commercial vaccine, and received a booster approximately one year before the onset...
of clinical disease. The dog was initially treated with non-steroidal anti-inflammatory drugs because of hip dysplasia and was serologically negative to *T. gondii*. However, 30 days thereafter, the animal returned to the hospital due to the worsening of clinical signs. At this time, he was alert, with normal body temperature (38.9°C) and hydrated mucous membranes. Neurological examination revealed tetraparesis, severe muscular atrophy of all limbs, generalized weakness, with the absence of patellar reflexes and exercise intolerance of the hind limbs. Two hematological and serum biochemical analyses were done in intervals of 30 days; however, marked alterations to physiological values were observed during the second visit and included lymphopenia (440 x10^9/μL) and an elevated increase in creatine kinase activity (909 U/L).

At the second visit, the dog was hospitalized and medicated with prednisone due to a suspected autoimmune disease causing myopathy and polynuropathy. However, clinical improvement was not observed after 44 days of medication. The general clinical condition of the dog deteriorated rapidly with worsening of muscular atrophy and tetraparesis and the owners requested euthanasia.

**Necropsy and histopathology:** A routine necropsy revealed severe and generalized muscle atrophy, particularly affecting the temporal, triceps, quadriceps, and gastrocnemius muscles, and manifestations of interstitial pneumonia. Tissue fragments of the affected muscles, peripheral nerves (sciatic, tibia, and radial), cerebrum, cerebellum, spinal cord, lungs, myocardium, intestine, liver, spleen, and kidneys were routinely processed for histopathological evaluations. Duplicate tissue fragments of the lumbar spinal cord and nerve roots were stained with the Masson histochemical method.

CDV was suspected due to the interstitial pneumonia and the multifocal demyelinating leukoencephalitis observed within the brain. The participation of this multi-systemic infectious agent was investigated by a combination of immunohistochemistry (IHC) and molecular biology techniques.

**Immunohistochemistry and molecular identification of canine distemper virus:** Selected formalin-fixed paraffin embedded (FFPE) tissue sections of the cerebellum, muscles (gastrocnemius, quadriceps, and triceps), nerves (sciatic and radial), cerebral, cerebellum, spinal cord, lungs, myocardium, intestine, liver, spleen, and kidneys were processed for IHC and used to detect the antigen of the CDV nucleoprotein (N/NP) gene (Headley et al., 2009), by using the monoclonal antibody anti-CDV-NP (VMRD, Pullman, WA, USA).

The molecular investigation of the presence of CDV was done to amplify a 287 base pair (bp) fragment of the CDV N gene in a RT-PCR assay (Headley et al., 2009), using duplicates of frozen cerebellar fragments and FFPE tissue sections of the skeletal muscle, spinal cord, and peripheral nerves. Positive controls for both assays consisted of tissue fragments and viral RNA from a previous case of systemic canine distemper, CD (Headley and Sukura, 2009); negative controls consisted of the diluent of the primary antibody during IHC and nuclease-free water in the RT-PCR assay. The amplified PCR products were then purified and submitted for direct sequencing using the forward and reverse primers.

The partial nucleotide sequences obtained were compared by the BLAST program with similar sequences deposited in GenBank. Sequence alignment and phylogenetic tree were created using MEGA 6; sequence identity was determined by using BioEdit.

**Pathological findings:** Histopathology revealed discrete interstitial pneumonia due to the moderate influx of mononuclear inflammatory cells within the alveoli and hyperplasia of type II pneumocytes; lymphoid depletion was observed at the spleen. Histopathological evaluation of the cerebrum, cerebellum, brainstem, and spinal cord revealed multifocal demyelinating (Fig. 1A) white matter encephalomalyltis; demyelination was characterized by discrete astrogliosis and discrete accumulation of glial cells close to the areas of demyelination associated with rare eosinophilic intranuclear inclusion bodies within astrocytes. The myocardium and skeletal muscles demonstrated marked atrophy due to the reduction in the diameter of muscle fibers that contrasted with normal myocardial and skeletal muscle tissues, interstitial fibrosis (Fig. 1B-D), fatty infiltration, and lymphocytic infiltration. Concurrent with this pattern of muscle fiber atrophy were severe loss of nerve fibers, axonal degeneration, and endoneurial fibrosis. The Masson stain revealed multifocal fibrosis within several areas of the roots of the spinal nerves and muscle fibers (Fig. 1E). Loss of large-caliber nerve fibers and axonal degeneration were identified at several peripheral nerves. The axonal degeneration and demyelination (Fig. 1F) associated with muscular atrophy and loss of nerve fibers suggested a possible NMD; nerve fiber demyelination demonstrated similar histopathological features as observed within white matter demyelinating encephalitis of the cerebellum; protozoan cysts were not observed.

**Immunohistochemistry and RT-PCR:** Positive immunoreactivity to the anti-CDV antibody occurred at the cerebellum, brainstem (Fig. 1G), spinal cord, and quadriceps muscle (Fig. 1H). The RT-PCR assay amplified the CDV N gene from the cerebellum and sciatic nerve; the partial sequences have been deposited in GenBank (Accession # KJ933692). BLAST analysis revealed that the sequences derived from this investigation were similar to other isolates of CDV deposited in GenBank; phylogenetic evaluation (Fig. 2) confirmed the results of the BLAST analysis and demonstrated that the strain from this study had 96-99% similarity with other strains of CDV.

**DISCUSSION**

The results of the histopathological findings (multifocal demyelinating leukoencephalitis and interstitial pneumonia) associated with those of the IHC and RT-PCR investigations confirmed the participation of CDV, particularly with the clinical manifestations and cerebellar disease, and to some extent with the muscular lesions observed in this dog. Demyelinating encephalitis is the hallmark of CDV-induced encephalitis and a diagnostic future of CD (Headley et al., 2012). The positive immunoreactivity to CDV antigen within several tissues of this dog suggest the systemic manifestation of CD with neurological and muscular involvement; antigens of CDV were identified in the stratum spinosum and granulosum of dogs with clinical
Fig. 1: Pathological findings of canine distemper virus in neuromuscular disease. Observe white matter demyelination of the cerebellum (A), interstitial fibrosis of the myocardium (B), interstitial fibrosis and inflammation of the brachial muscle (C), and atrophy of the quadriceps muscle (D). There is interstitial fibrosis of the myocardial muscle (E), multifocal demyelination of peripheral nerve (F), which is better observed at the insert. Immunohistochemical detection of CDV at the brainstem; observe positive immunoreactivity to CDV antigen within the neuron (G) and quadriceps muscle (H). A–D, F; Hematoxylin and eosin stain. Bar; A and D 100 µm; B – C, F 50µm. E, Masson stain, Bar, 50µm. Immunoperoxidase; H–G, Bar, 10µm.

Fig. 2: The phylogenetic relationship of selected strains of canine distemper virus based on the nucleoprotein gene. The evolutionary history was inferred by using the Maximum Likelihood method based on the Jukes–Cantor model. The isolate from this study is highlighted (box); the GenBank accession numbers of the CDV strains and the country of origin are given. Phocine distemper virus (PDV) was used as the out-group.


Other common infectious causes of polyradiculoneuritis were discarded. Clinical signs of canine toxoplasmosis or neosporosis were not observed; the dog was serologically negative for T. gondii, and tissue cysts were not observed. Peripheral neuropathy due to diabetes was not considered since this dog was not hyperglycemic during hospitalization, and glucose levels were within physiological limits.

REFERENCES