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Clinicopathological findings in dogs with distemper encephalomyelitis presented without characteristic signs of the disease

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Abstract

The clinical diagnosis of distemper is difficult in dogs presented with nervous deficits in the absence of extraneural signs and myoclonus. The aim of this study is to verify how the clinicopathological findings may suggest distemper encephalomyelitis in such cases. We prospectively investigated 20 necropsied dogs presented with neurological signs without those characteristic signs of distemper at the time of hospital admission. Eight out of 20 dogs were diagnosed with distemper encephalomyelitis at *post mortem* by reverse transcription-polymerase chain reaction (RT-PCR) and histological examination. Cerebellar and/or vestibular signs progressing to tetraparesis/plegia were frequent neurological signs. Abnormalities in hematologic findings were non-specific, nevertheless the cerebrospinal fluid evaluation could suggest canine distemper virus (CDV) infection by a lymphocytic pleocytosis. At *post mortem* chronic CDV encephalomyelitis was predominant. Our clinical results, as well as the predominance of chronic encephalomyelitis, differ from other studies about CDV encephalomyelitis with naturally infected dogs presenting extraneural signs and myoclonus.

Keywords: Canine distemper virus; Encephalomyelitis; Hemogram; Cerebrospinal fluid; Histopathology; RT-PCR

1. Introduction

The canine distemper virus (CDV) is a non-segmented, negative sense single-stranded RNA virus, closely related to measles and rinderpest virus, two other members of the genus *Morbillivirus* of the *Paramyxoviridae* family (Fauquet et al., 2004). Gastrointestinal and/or respiratory signs (systemic signs), frequently with central nervous system (CNS) involvement, characterize the classical clinical presentation of distemper. Nevertheless, this conventional presentation is not the rule. Systemic findings, as well as myoclonus, a common and characteristic sign of nervous distemper (Koutinas et al., 2002), may be lacking at the moment of admission (Vandevelde and Cachin, 1993; Amude et al., 2006c). In such cases presented without these typical findings the clinical diagnosis of distemper is often difficult and may be a challenge for the veterinarian (Tipold et al., 1992; Amude et al., 2006b).

For the conclusive *ante mortem* diagnosis of distemper direct immunofluorescence test in scrapings and body fluids are routinely and widely used (Tipold et al., 1992). Unfortunately, this assay can confirm distemper only within 3 weeks after infection, because after this time the virus disappears from the epithelial cells. Thus, in the subacute or chronic forms of the disease, this technique gives false-negative results (Jóźwik and Frymus, 2005). Viral antigen may also be hard to find in the extraneural tissues in cases without systemic signs. Serological examination has not been very useful in the diagnosis of distemper, because a high titre of anti-CDV antibodies may be a result of prior vaccination, as well as previous subclinical or clinical infection. On the other hand, during severe distemper, the antibody titre may be low due to strong immunosuppressive properties of CDV. For *post mortem* diagnosis of CDV encephalomyelitis, detection of CDV antigen in CNS sections by immunohistochemistry has been a specific

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method. However, in chronic CDV encephalomyelitis, viral antigen may disappear from the inflammatory demyelinating lesions due to the antiviral immune responses (Muller et al., 1995), and false negative result has been reported with immunohistochemistry assay (Koutinas et al., 2002).

Considering the infectious potential of the disease, the diagnosis of distemper should be very important in order to prevent hospitalizing. Additionally, the diagnosis of CDV infection in animals with neurological disease is essential for the prognostic evaluation of affected individuals, and for ruling-out of differential diagnosis. Further the knowledge of possible clinicopathological presentations of CDV encephalomyelitis is important for distemper diagnosis, especially in cases in which the clinical diagnosis is difficult due to absence of myoclonus and systemic signs at the time of first examination. Subsequently, we attempted to verify how the clinicopathological findings might suggest the distemper diagnosis in such cases.

2. Materials and methods

2.1. Inclusion criteria

Dogs presented with neurological deficits without myoclonus and absence of systemic signs at the time of hospital admission, at which hematologic examination and CSF evaluation were performed *ante mortem*, were prospectively followed up. As systemic signs were considered fever, gastrointestinal and respiratory signs. Dogs in which systemic signs and/or myoclonus were observed after the admission were not excluded from this investigation. Animals whose myelography suggested compressive lesions, and where traumatic or toxic events were recognized, were excluded. Only dogs with CNS samples obtained at necropsy were included.

2.2. Animals and clinical samples

All dogs were followed up since the hospital admission to clinical outcome (euthanasia or natural death, despite medical treatment). All neurological data relative to evolution were recorded. The owner's consent was obtained. Animals were not submitted to any stressing or suffering procedure. The clinical decision, medical treatment, or other diagnostic procedures were performed by the attending veterinarian at the Veterinary Teaching Hospital. Blood and CSF were collected during the hospital routine procedures. CNS samples were obtained at necropsy.

2.3. Diagnostic criteria

The diagnosis of distemper encephalomyelitis was carried out at *post mortem*, through CDV detection from the fresh CNS fragments by RT-PCR, and by histological examination of the CNS. Dogs in which CDV induced lesions and/or virus RNA could not be detected at *post mortem* were grouped and used for comparative propose, since they were from the same hospital population and shared the same inclusion criteria.

2.4. RT-PCR

For CDV detection by RT-PCR assay, immediately after the CNS collection the RNA was extracted from an aliquot of 300 μl of fresh CNS pool (brain and spinal cord) suspension (10% w/v) in phosphate buffered saline (PBS), according to the silica/guanidine isothiocyanate method, described by Boom et al. (1990). Aliquots of ultrapure (MilliQ®) sterile water were included as negative control in all the RNA extractions. The cDNA was made using 100 U of M-MLV reverse transcriptase enzyme (Invitrogen™ Life Technology, USA).

RT-PCR was performed using the oligonucleotides primers CDV 1 (sense) and CDV2 (anti-sense), designed to amplify an amplicon of 287 bp length of the CDV nucleoprotein (NP) gene (Frisk et al., 1999). The reactions were performed as previously described (Amude et al., 2006a). All reactions were performed using clinical samples from a dog with a neurological disease caused by *Cryptococcus neoformans* as a negative control. CDV Rockborn strain infected MDCK (Madin Darby canine kidney) cells were used as CDV positive control.

The amplified products were analyzed by electrophoresis in 2% agarose gel with ethidium bromide (0.5 μg/ml) in TBE buffer pH 8.4 (89 mM Tris–HCl; 89 mM boric acid; 2 mM EDTA) in constant voltage (90 V) for approximately 45 min and visualized under UV light.

The identities of the RT-PCR amplicons were confirmed by restriction fragment length polymorphism (RFLP) with *Hinf* I enzyme (Invitrogen™ Life Technology, USA) digestion, and by nucleotide sequencing of the RT-PCR products from fresh CNS fragments. The CDV amplicon obtained from RT-PCR were sequenced in Mega Bace 1000/Automated 96 Capillary DNA Sequence (Amersham Biosciences, UK) using the anti-sense primer. The quality of each sequence obtained was analyzed with Phred/Phrap/Consed (http://www.phrap.org/) software and the sequence similarity was checked against sequences deposited in the GenBank using the Blast software (http://www.ncbi.nlm.nih.gov/BLAST).

2.5. Histopathology

Cerebellum sections were examined for CDV-induced lesions. The CNS fragments were fixed in 10% buffered neutral formalin, embedded in histological paraffin, sectioned at 5 μm and stained with hematoxylin and eosin (HE), following routine procedures.

2.6. Hematology and CSF analysis

The blood obtained was treated with 10% EDTA as anticoagulant. The hematologic parameters [hematocrit (Hct), red blood cell count (RBC), white blood cell count