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Short Communication

Atypical necrotizing encephalitis associated with systemic canine distemper virus infection in pups

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This report describes the naturally occurring atypical neuropathological manifestation of systemic canine distemper virus (CDV) infection in two 16-day-old Pit Bull pups. CDV-induced changes affected the gray and white matter of the forebrain while sparing the hindbrain. Histologically, there was necrosis with destruction of the nervous parenchyma due to an influx of inflammatory and reactive cells associated with eosinophilic intranuclear inclusion bodies within glial cells. Positive immunoreactivity against CDV antigens was predominantly observed within astrocytes and neurons. RT-PCR was used to amplify CDV-specific amplicons from brain fragments. These findings suggest the participation of CDV in the etiopathogenesis of these lesions.

Keywords: canine distemper virus, immunohistochemistry, neuropathology, RT-PCR

The neuropathological manifestations of canine distemper virus (CDV) are diverse; however, acute to chronic demyelinating encephalomyelitis are the most prevalent forms under natural conditions [19,21]. Although CDV infection might occur within both the gray and white matter, CDV-induced lesions in the white matter are the predominant and most prominent changes in classical demyelinating cases [20,21]. CDV-induced neuroparenchymal necrotic lesions with an associated influx of Gitter cells are observed predominantly within the injured white matter at the cerebellopontine angle (hindbrain) of dogs with acute to chronic distemper demyelinating encephalomyelitis [4,10,20,21]. This report describes the atypical necrotizing manifestation of canine distemper encephalitis (CDE) that affected both the gray and white matter of the cerebrum (forebrain) while sparing the cerebellopontine angle (hindbrain) in two canine pups.

Two 16-day-old Pit Bull pups from the same litter were examined at Southern Brazil with a 1-day history of acute seizure-like episodes (generalized tonic-clonic movement of all skeletal muscles). Furthermore, bilateral mucopurulent ocular discharge, increased lower respiratory sounds, and focal seizures were observed. Gastrointestinal and dermatological (impetigo and hyperkeratosis) signs were absent. Impairment of postural reactions (proprioception and hopping), normal spinal reflexes in both forelimbs (flexor) and hindlimbs (patellar and flexor), and decreased levels of consciousness (stupor) without cranial nerves deficits led to the neuroanatomical diagnosis of bilateral forebrain dysfunction.

Due to the severity of the seizures, the owner requested that the dogs be euthanized and routine necropsies were performed. Tissue fragments of the brain, lungs, stomach, intestinal segments, kidneys, urinary bladder, and spleen were fixed in a 10% buffered formalin solution and routinely processed for histopathological evaluation. Special staining procedures were also performed on forebrain sections to identify the presence of possible bacterial (Giemsa and Gram staining) and fungal organisms [periodic acid-Schiff (PAS), and Grocott staining]. Selected paraffin-embedded tissue fragments from the brain were prepared for immunohistochemical

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(IHC) detection of CDV antigens (Serotec, UK), *Toxoplasma gondii* P30 antigen (Leica Biosystems Newcastle Ltd., UK), and the glial fibrillary acidic protein (GFAP; Serotec, UK) as previously described [9]. Fragments from the spleen, lungs, and brain were submitted to an RT-PCR assay to detect CDV RNA as previously described [3,5]. In addition, duplicate sections of formalin-fixed paraffin-embedded tissues sections of the brain from both dogs were submitted to the Diagnostic Center for Population and Animal Health, Michigan State University, USA, to examine the possible involvement of canine herpesvirus-1 (CHV-1) using PCR.

Grossly, the vessels of the meninges were congested without any apparent change to the coronal sections; the lungs were heavy, wet, and mildly congested. Histopathological findings were similar in both pups; all lesions were restricted to the lungs (interstitial pneumonia), spleen (marked depletion of the germinal centers), and the brain. The neuroparenchyma was greatly altered throughout both the gray and white matter of the forebrain (telencephalon and diencephalon) in both dogs, but this was not observed within the hindbrain (cerebellum, cerebellar peduncles, pons, and medulla oblongata) of either animal. Within the forebrain there was moderate/severe asymmetrical necrosis with intranuclear and intracytoplasmic eosinophilic inclusion bodies in the glial cells. Necrosis was characterized by the complete loss of nervous parenchyma due to a large influx of monocytic and histiocytic cells along with a small to moderate influx of neutrophils within the neuroparenchymal lesions. Additionally, a mild to moderate influx of fibrillary, gemistocytic astrocytes, Gitter and syncytial cells were observed. Perivascular cuffs were not observed, but there was discrete to moderate small vessel proliferation. At the cerebral cortex, there were also foci of discrete to moderate neuronal degeneration and necrosis with satellitosis and neuronophagia. Special stains did not reveal the presence of bacterial, fungal, or protozoal organisms within any samples of the evaluated tissue.

Within the necrotic areas, IHC detected weak immunoreactivity against CDV antigens predominantly within the astrocytes, and sporadically in the gemistocytes, macrophages, and Gitter cells. In the surrounding neuropil, there was strong immunoreactivity against CDV antigens within normal and degenerated neurons in the cerebral cortex and a few astrocytes within all the neuroanatomical sites that were evaluated (cerebrum, mesencephalon, cerebellum, pons, and medulla oblongata). At the cerebrum, plump GFAP+ gemistocytes were observed within the areas of severe parenchymal destruction. Positive immunoreactivity against toxoplasma antigen was not observed with IHC.

RT-PCR of samples from all anatomic sections of the brain (cerebrum, mesencephalon, cerebellum, pons, and medulla oblongata), as well as the visceral organs (spleen and lungs) produced amplicons of 287 bp that were cleaved by Hinf I, yielding fragments of 60 and 227 bp as expected for CDV. Additionally, nucleotide sequencing of the RT-PCR amplicons was performed [5] and confirmed that the 287 bp amplicons were CDV-specific. PCR did not produce any evidence of CHV-1 within the brain samples.

The importance of these cases was the association of a systemic distemper infection with an unusual necrotizing manifestation of nervous distemper. Protozoan, bacterial, and mycotic agents were excluded as being responsible for the necrotizing encephalitis due to the negative histochemical staining results. However, detection of characteristic CDV inclusion bodies in addition to the immunohistochemical and RT-PCR results suggest the participation of CDV in the development of the necrotizing lesions observed in these animals.

Notwithstanding the unique neuropathological characteristics of these cases, the lesions observed in this should be differentiated from those associated with CHV-1 encephalitis in neonates and young pups. However, the negative PCR results of the brain samples from both pups excluded the participation of CHV-1. Furthermore, the exclusion of CHV-1 from these lesions was based on the absence of morphological characteristics of this multisystemic necrohemorrhagic disease [2,15,18].

Excitotoxic insults to neurons via glutaminergic signaling has been reported to induce massive necrosis of nervous cells in infected and non-infected cells in CDV infection [7]. This might have affected the development of the necrotizing lesions observed in these dogs. Additionally, the characteristics of the lesions might be a direct result of the CDV strain circulating in Southern Brazil, since recent studies have demonstrated that different distinct cluster of CDV might be present in the geographical area where these dogs were maintained [16].Variation in the CDV strains is a hallmark of CDE [22].

To the best of our knowledge, necrotizing encephalitis affecting the forebrain while sparing of the hindbrain has not been previously associated with classical CDE. Other neuropathological manifestations of CDV are acute encephalitis [1], polioencephalomalacia [13], inclusion body polioencephalitis [17], and old dog encephalitis [9]. However, the neuropathological lesions of previously reported CDE are very different from the lesions described in these two pups.

The neuroparenchymal lesions examined in these 16-day-old pups might represent an unusual neuropathological manifestation of CDV rather than pathological variants of previously described CDE [9,13,17]. Similar manifestations of necrotizing distemper encephalitis have not been described [8,21]. Necrotizing lesions similar to these findings were not described in a recent study of 620 cases

of spontaneous CDE in Southern Brazil [19]. Furthermore, necrotizing encephalitis has not been observed during experimentally induced distemper encephalitis in newborn gnotobiotic dogs [11,12].

The cortical splenic lymphoid tissue was severely depleted in these animals, probably resulting in the destruction of cells that express the human equivalent of CD 150, a signaling lymphocytic activation molecule (SLAM). This is a hallmark of immunosuppression associated with CDE [6,14]. SLAM is expressed by thymocytes, activated macrophages, and dendritic cells [14], and is up-regulated in the lymphoid cells of dogs infected with CDV [6].

The manifestation of the neuropathological effects of CDV is widespread [9,13,17]. Since distemper is endemic in Brazil [4,19,20], retrospective and prospective investigations are being carried out by our group to study the unusual manifestations of CDV in dogs from South America.

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