

The role of viruses in encephalitides of unknown origin in dogs

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Idiopathic inflammatory diseases or inflammatory disturbances of unknown etiology are involved in the incidence of brain diseases in dogs. Breed-specific necrotizing encephalitis (NE), such as necrotizing meningoencephalitis and necrotizing leukoencephalitis, has been recognized as an important neuropathological identity in different small-sized dog breeds. Granulomatous meningoencephalomyelitis (GME) is also a type of encephalitis that has been commonly identified in dogs. Other non-suppurative meningoencephalitides of unknown etiology have also been recorded in dogs. Viral etiology has been suspected in such cases of meningoencephalitides of unknown etiology. Different viral pathogens have been identified in encephalitides of unknown etiology in dogs with the use of broadly reactive PCR assays and sequence analysis, as well as the wide use of immunohistochemical assays. Evidence of viral infections is conspicuous even in instances where a virus has not yet been identified. In this mini-review the main encephalitides of unknown etiology will be addressed and the features that might suggest viral infection will be discussed.

Keywords dog; virus; encephalitis of unknown etiology; non-suppurative encephalitis; necrotizing encephalitis; necrotizing meningoencephalitis; necrotizing leukoencephalitis; granulomatous meningoencephalitis

1. Introduction: encephalitis in dogs

Degenerative, metabolic, autoimmune, nutritional, inflammatory, toxic, and vascular conditions can cause neurological disturbances that may affect the brain and lead to encephalopathies in dogs [1-3]. Inflammatory brain conditions are especially important and have been implicated in the incidence of encephalopathies in dogs in the practice of veterinary neurology [4-7]. In 2006, Platt [5] referred to inflammatory diseases of the central nervous system (CNS) as a common quandary.

The signalment, historical findings, extraneural and neurological signs, and the lesion site may contribute marginally to a specific diagnosis of encephalitis [4, 8]. Multifocal signs have been traditionally expected in inflammatory brain diseases [1]; however, they have only been noticed in one third of dogs with inflammatory brain conditions [4].

Cerebrospinal fluid examinations including immunoglobulin G index and cytology have been useful for separating meningoencephalitis from other brain diseases; nevertheless, it is often difficult to diagnose the etiology of encephalitis and distinguish infectious from non-infectious varieties, even when using techniques for detection of infectious agents [5, 9, 10].

Ante mortem diagnosis of the specific etiology of brain inflammation remains very difficult if not impossible, even when extensive diagnostic testing (ancillary diagnostic aids) is performed [4, 8]. The precise etiological diagnosis of encephalitis may be a challenge, even with the use of advanced techniques such as computed tomography, magnetic resonance imaging, and techniques for *post mortem* detection of infectious agents such as PCR and immunohistochemical assays [4-6, 8, 10]. The methods available for *ante mortem* etiological diagnosis of encephalitis to date are of limited value [5, 10, 11]. Thus, in many cases the definitive diagnosis of encephalitis in dogs is only possible *post mortem*, and histological examination of the brain by routine methods (H&E stain) is essential.

Post mortem neuropathological investigations have clustered the encephalitides in two distinct groups: infectious and “pathogen-free” encephalitis [6, 12, 13]. Important causes of infectious diseases with neuroparenchymal inflammation include viral, fungal, protozoal, and bacterial meningoencephalitis [4, 6, 8, 12-14]. “Pathogen-free” encephalitides have also been known as non-infectious idiopathic encephalitides or encephalitides of unknown etiology [6, 13, 15].

The main “pathogen-free” encephalitides recognized are breed-specific conditions, which are often characterized by non-suppurative inflammation. In such breed-related encephalitides, there are specific patterns for distribution of non-suppurative inflammatory lesions within the CNS [16-20].

Besides the breed-specific encephalitides, different non-suppurative meningoencephalitides without breed predisposition have been observed in which extensive *post mortem* studies fail to detect a precise pathogen [6]. Viral infection has been suggested to play a role in these non-suppurative encephalitides of unknown origin in dogs [13, 15], and some viruses have already identified in encephalitides of unknown origin [6, 21]. Evidence of viral infection is conspicuous even in instances where the virus is not yet identified [15, 17, 22, 23].

2. “Pathogen-free” or encephalitides of unknown etiology

Idiopathic encephalitides or inflammatory brain disturbances of unknown etiology have been implicated in the incidence of nervous disease in dogs [6]. The major neuropathological entities classified within the “pathogen-free” encephalitides are breed-specific encephalitides such as necrotizing encephalitis (NE) [necrotizing meningoencephalitis (Pug-type NE) and necrotizing leukoencephalitis (Yorkshire-type NE)], and granulomatous meningoencephalomyelitis (GME) [18-20, 24-27].

Non-suppurative meningoencephalitis of unknown etiology was also reported to affect Greyhounds [17; 18]. Other meningoencephalitides without breed predisposition have also been classified as non-suppurative inflammation of unknown etiology [6].

2.1 Necrotizing encephalitis

Breed-specific NE, such as necrotizing meningoencephalitis (NME) and necrotizing leukoencephalitis (NLE), are unique pathogen-free canine disorders frequently described in different breeds of dogs. Although a Golden retriever dog with NME was reported [25], NE has been recognized as an important brain inflammatory condition of small-sized dogs, particularly toy breeds [19, 27].

Initially NE was recognized in Pug and Yorkshire terrier dogs; however, different breeds have been affected by this condition, including Pekingese, Shih Tzu, Maltese, Papillon, French bulldog, and Chihuahua dogs [18-20, 24, 25, 27].

2.1.1 Necrotizing meningoencephalitis

The first brief account of this disease was given in 1983 [29]; however, NME was only characterized as a neuropathological identity in 1989 when it was described in 17 adolescent to mature (6 months to 7 years) Pug dogs [22]. Because of its high incidence in Pug dogs, this disease was initially called Pug encephalitis. In 1987, a one-year-old female Maltese terrier was diagnosed with necrotizing meningoencephalitis of unknown etiology with lesions similar to those of Pug encephalitis [30]; nevertheless, NME was only recognized as a disease of Maltese dogs in 1995, when it was described and characterized in 5 young to mature (9 month to 4 years) Maltese dogs [31].

Currently, NME is known to affect small dog breeds other than Pug and Maltese, such as Pekingese, Papillon, West Highland white terrier, and Chihuahua dogs [18-20, 23, 24, 27, 32]. In Brazil, the authors already diagnosed NME in three mature (one to six years) Miniature Pinscher and one (8 months) Shi Tzu dog (unpublished data). Although NME is classically a disease of small toy breeds, a Golden Retriever dog with NME has been reported [25]. Different dog breeds were already reported with this disease, and other small dog breeds may also be affected; however, there is no doubt that young to mature Pugs are the main breed affected by NME [33, 34].

NME is characterized by parenchymal necrosis with or without cyst-like formation located predominantly within the gray and white matter of the forebrain (cerebrum) with sparing of the hindbrain (cerebellum and caudal brainstem) [12, 16, 22, 31, 32]. Two forms of the disease have been observed: an acute and a chronic form [22, 25, 31]. At necropsy, the cerebrum is often asymmetrically swollen in acute cases, with a loss of distinction between the gray and white matter and mild to moderate asymmetrical dilation of the lateral ventricles. In chronic cases, marked atrophy of the affected cerebral cortex may be observed, often also with asymmetrical dilation of the lateral ventricles [22, 27, 31].

Histopathological evaluation of the brain is characterized by multifocal areas of malacia with Gitter cell infiltration and cribiform to cystic lesions within both gray and white matter of the forebrain with sparing of the hindbrain; non-suppurative inflammatory response and marked glial reaction might also be observed surrounding the necrotic neuropil mainly in chronic cases [22, 27, 31]. Necrotic and non-suppurative inflammatory lesions of the cerebral gray and white matter may also be observed overlying the meninges and adjacent thalamus and hippocampus [31, 32].

Two types of inflammatory reactions have been described in NME: an acute type with discrete to mild inflammatory response and a chronic type with a marked mononuclear inflammatory reaction [25]. In addition, in severe chronic cases (longest duration of clinical signs, often months) the neuroparenchymal lesions may be characterized by little inflammation but extensive atrophy of affected areas, with severe astrocytosis and astrogliosis and a marked gemistocytic response [22, 31].

2.1.2 Necrotizing leukoencephalitis

NLE was firstly reported in 1993 in six mature (one to five years) Yorkshire terrier dogs [23]. Since that initial report, the disease has been reported in Yorkshire dogs of both sexes. The age of the affected dogs varied from one to 10 years. To the authors' knowledge until the time of writing, there have been 16 cases of Yorkshire dogs officially described with the disease [19, 23, 35, 36]. In Brazil, the authors diagnosed NLE in a Yorkshire terrier dog in 2003 by *post mortem* neuropathological exam (unpublished data). In 2007, the disease was described for the first time in a non-Yorkshire terrier dog; the affected dog was a 20-month-old female French bulldog [20]. It can be expected that this disease will be recognized in other small dog breeds in the future [20].

The findings of NLE are unique and characterized by extensive and multifocal neuroparenchymal necrosis with cyst formation in the deep white matter of the cerebrum (telencephalon and diencephalon) and/or brainstem with sparing of the gray matter. Affected white matter includes the corona radiata, centrum semiovale, internal capsule, thalamus, mesencephalon, pons, and medulla [12, 19, 20, 23, 36].

Acute and chronic forms of the disease were already reported; however, the chronic presentation is frequently observed. At necropsy, the chronic cases usually present with multifocal cystic necrotic lesions in the deep white matter of the brain [19, 20, 23, 36]. In acute cases, focal greyish discoloration of the affected white matter may be recorded at necropsy in absence of cyst-like formation [23]. In both acute and chronic NLE, mild to moderate asymmetrical dilation of the ventricles may also be observed, and curiously, the lesions occur in the hindbrain, sparing the cerebellum [20, 23, 36].

Although the deep white matter of both forebrain and hindbrain may be affected (with sparing of the cerebellum), often the lesions prevail in one neuroanatomic compartment (cerebrum or brainstem) and the gross lesions are typically unilateral, even in cases with multifocal lesions [23].

Histological findings of NLE are unique, and interestingly the cerebellum and meningeal surface are usually not affected [19, 20, 23, 36]. In acute cases, the typical cyst-like formations are not evident and the injured white matter shows a characteristic structure with a clear center surrounded by a dense ring. The center consists of infiltrating lymphohistiocytic cells and occasional macrophages (Gitter cells) as well as a striking proliferation of fibrillary and gemistocytic astrocytes. The dense ring surrounding the gliotic center consists of vascular proliferation, mononuclear perivascular cuffing, intense microglial proliferation and varying amounts of fibrotic tissue [20, 23].

The typical chronic cases are characterized by extensive multifocal cavitation necrosis, often associated with a strong non-suppurative inflammatory reaction composed of perivascular cuffing with lymphocytes, plasma cells and macrophages [19, 23, 35, 36]. In addition, sclerosis with intense reactive changes of glial cells (astrogliosis and astrocytosis with gemistocytic reaction) may be observed surrounding the necrotic parenchymal lesions [12, 20, 23].

Acute and chronic cases are not different variants of the disease; the chronic lesions are probably due to lesion progression. With the increase of sclerosis during disease progression, the tissue becomes less compact and in chronic cases, microcavitation occurs, characterized by typical cystic lesions that are surrounded by glial scar tissue [23].

In contrast to NME, in which the non-suppurative inflammatory reaction may be absent in acute cases [25], moderate to severe inflammation is always observed in NLE, even in acute cases [20, 23]. Cases of necrotizing encephalopathy (Leigh syndrome-like) were already reported in the Yorkshire Terrier [37], Alaskan Husky [38], and Australian cattle dog [39], with major necrosis and little or no inflammatory reaction. However, such cases of brain necrosis without inflammatory reaction in the Yorkshire Terrier may represent a mitochondrial encephalopathy rather than NLE.

2.2 Granulomatous meningoencephalitis

Granulomatous meningoencephalomyelitis (GME) is a well-recognized disease entity that affects dogs. The disease was recognized as early as 1971 or 1972 [40, 41] and has been reported world-wide [12, 42]. Initially the disease was termed reticulosis [40, 41]; however, after extensive studies about histiocytic proliferative disorders of the CNS, this inflammatory non-neoplastic condition was correctly named GME [12, 25, 43].

This special form of non-suppurative encephalitis has been estimated to comprise 5–25% of all central nervous diseases of dogs. GME may comprise almost 80% of the inflammatory brain conditions of dogs [42, 44, 45]. It is the second most common inflammatory disease of the canine CNS after canine distemper virus (CDV) encephalitis [46, 47]. The incidence of GME is greatest among female toy and small-breed dogs. Terrier breeds also tend to be at increased risk; however, dogs of any breed can be affected, even large dog breeds [42–45, 47]. It is often a disease of young to middle-aged dogs, but it can occur at any age [42–45, 47].

The neuropathological lesions are localized predominantly in the white matter of the brain and sporadically in the spinal cord. Histological changes are characterized by perivascular cuffing and granulomas in the neuropil; the perivascular cuffing is composed of lymphocytes, varying numbers of macrophages and plasma cells. Macrophages may show little mitosis and differentiate into epithelioid cells [12, 25, 26, 42, 43, 47]. Malacia was described with GME [44]; however, it has not been often observed [12, 25, 26, 42]. Malacia is a typical feature of NE and other necrotic conditions of the CNS, but it is not a frequent finding in GME [25, 26].

Typically, the lesions of GME tend to be of an angiocentric nature; the brain has numerous vessels surrounded by thick cuffs of epithelioid histiocytic cells and several lymphocytes [12, 25, 26, 44, 45]. Cases with prolonged duration show confluence of the lesions, proliferation of blood vessels, and reparative changes [46]. Often, the inflammatory changes of GME spread to the leptomeninges. Mononuclear infiltration of the meninges may vary from mild and multifocal to locally extensive, and the infiltration is more pronounced in the depths of the sulci [48].

Neuroparenchymal lesions at various sites in the CNS may be classified as mild, moderate or severe [44]. Mild lesions consist of one-to-two-cell-thick perivascular cuffs or mild glial reactivity and incipient granuloma formation within the parenchyma. Moderate lesions are classified by the presence of two-to-eight-cell-thick perivascular cuffs as well as small granulomas and astrocytosis. Severe lesions are composed of large cuffs more than eight cells thick with large granulomas and astrocytosis in associated white matter. Meningeal infiltrations are classified similarly to perivascular cuffs [44].

GME has been classified into 3 different forms: disseminated, focal, and ocular. This classification was made based on the nature and degree of histological lesions found in different neuroanatomical sites of the CNS [12, 44, 45]. In the disseminated form, lesions are usually distributed widely throughout the CNS but are primarily located within the white matter of the cerebrum, brain stem, cerebellum, and cervical spinal cord. Comparable lesions may also be found in the grey matter and in the leptomeningeal and choroid plexus vasculature [12, 43, 44].

Focal GME is characterized by focal granulomas, often with the appearance of tumor-like masses (space-occupying lesions) that develop when cells from a large number of perivascular lesions coalesce. Focal lesions most commonly occur in the brainstem and cerebral white matter but may occur elsewhere in the CNS. Focal disease was already described in the cerebellum [49] and in the cervical spinal cord. Because focal GME behaves like a tumor, it is especially important to distinguish GME from tumors and other types of focal space-occupying lesions of the brain or elsewhere in the CNS [49].

The ocular form of GME may occur with lesions involving the optic nerves and eventually the retina [40, 50]. The ocular form is the slowest to progress and may occur alone or accompanied by the focal or disseminated disease [51].

At necropsy, the brain and meningeal vessels may be diffusely congested and occasionally hemorrhagic [48]. In the disseminated form, gross findings may be absent, and in the focal disease, a tumor-like mass may be observed [25]. The optic nerve may be thickened in the ocular disease [40, 50].

Typically GME is a disease that primarily affects the CNS; however, in 2006 a case of GME with widespread peripheral nervous system (PNS) involvement was described [47]. To the authors' knowledge, this was the first detection of MEG affecting the PNS.

2.3 Greyhound meningoencephalitis

Non-suppurative meningoencephalitis of unknown etiology was reported in fourteen 4- to 18-month-old vaccinated Greyhounds (10 males, 4 females) from three kennels [17].

In Greyhounds, gross meningoencephalitis lesions may be not evident but microscopic examination often reveals important lesions in the forebrain, mainly in the caudate nucleus and cortical gray matter of the cerebrum as well as the periventricular gray matter of the anterior brainstem [17].

The predominant forebrain lesions are characterized by severe diffuse and focal/nodular gliosis and gemistocytosis accompanied by mononuclear cell perivascular cuffing. The diffuse and nodular gliosis is due to prominent microglial and astrocytic cells. The perivascular cuffing is composed of lymphocytes and plasma cells, but in some instances perivascular infiltrates also contain aggregations of histiocytes [17].

In addition to the forebrain lesions, milder lesions may also be noted in the caudal brainstem, cranial spinal cord, and the molecular layer of the cerebellum [17].

The inflammatory and gliotic lesions are often accompanied by lymphocyte and plasma cell infiltration of the cerebral and cerebellar meninges [17]. Demyelination, neuropil necrosis, neuronophagia, and vasculitis are not often observed; inclusion bodies, protozoal cysts, or fungal agents have also not been observed on routine neurohistological examination [17].

3. Pathogenesis of non-infectious encephalitides and the possible role of viral infection

The major encephalitides of unknown origin have been traditionally classified by neuropathological evaluation as non-suppurative encephalitides [12, 17, 22, 23]. Although there are some differences among the encephalitides of unknown origin, these brain inflammatory conditions of dogs share similar histological changes when assessed by routine methods (i.e., H&E stain); such changes include: meningitis, perivascular cuffing of mononuclear cells, inflammatory mononuclear infiltration into the neuropil, and reactive changes within the nervous parenchyma characterized by glial reaction (astrocytosis and astrogliosis, with gemistocytic astrocytes, as well as microgliosis) [17, 19, 20, 25, 26, 46]. The non-suppurative inflammation is typically characterized by influx of lymphocytes, plasmocytes and monocyte/histiocyte-lineage cells [17, 19, 25, 26]. The nature of the perivascular cuffing reaction often provides clues to the possible etiologic agent, with nonsuppurative reactions rich in lymphocytes and plasma cells being more typical of viral infection in the first instance [11, 12, 15, 17].

Strong similarities exist between NME and NLE lesions, including chronic progressive course, strong microglial proliferation in active lesions, occurrence of necrotizing lesions in the brain parenchyma, and generalized non-suppurative inflammation [16, 18-20, 22, 23, 27]. However, the distribution pattern of NME and NLE lesions is different. Whether the differences in the pattern of lesion distribution represent breed variations of a single disease process or individual breed-specific encephalitis variants remains to be determined [18, 23].

It has been postulated that viruses might be involved in the pathogenesis of encephalitides of unknown origin [6, 13, 15, 22, 23, 25, 26]. Canine distemper virus (CDV) was already speculated to play a role in the pathogenesis of such encephalitides; however, diagnostic techniques have failed to detect CDV within the CNS of most affected dogs [11, 17, 23, 25, 26]. Additionally, PCR amplification had not detected herpesvirus, adenovirus, or canine parvovirus nucleic acids in brain specimens from dogs with NME, NLE, and GME [52]. Nucleic acid sequences for Borna disease virus or

louping ill, tick-borne encephalitis, West Nile, and other flaviviruses in addition to those of bacterial organisms were also not detected in Greyhound meningoencephalitis [28]. In addition, CDV, herpesvirus, and protozoal nucleic acids were also absent in most of the Greyhound cases [17, 28].

Different findings suggest that immunological mechanisms may play an important role in non-suppurative encephalitides of unknown etiology in dogs. Immunohistochemical characterization of GME and NE lesions revealed suggestive findings of an immunological mechanism in the pathogenesis of such encephalitides [19, 25, 26, 46]. The observations of anti-glomerular basement membrane glomerulonephritis in NE may also be an important step in the identification of an immune-mediated mechanism for NE [32]. The clinical response of both NE and GME to immunosuppressive therapies including corticosteroids, cyclosporine and cytosine arabinoside is also supportive of an immune-mediated pathogenesis [51, 53-55]. Additionally, anti-astrocyte autoantibodies have been detected in CSF from animals with GME and NE [56-59], although it is unknown if such antibodies are the cause or consequence of inflammation [57].

Although immune-mediated pathogenesis has been suggested, infectious agents, especially viruses, are suspected to be the cause or trigger for immune-mediated events in the brain of the affected dogs [15, 25, 26]. A yet-unrecognized virus might infect the CNS, trigger immune-mediated mechanisms, and be cleared from the lesions by the effective immune response at the moment of the diagnosis of such encephalitides. Many viral infections generate not only direct inflammatory lesions but also subsequent autoimmune responses in multiple organs including the brain [32, 60]. Multiple sclerosis is a demyelinating encephalitis that affects the human brain and observations strongly suggest that the disease is caused by a virus that induces an immune-mediated disease within the brain [61, 62].

Immunohistochemical detection of CDV nucleoprotein revealed non-specific immunolabeling in the cytoplasm of most cortical neurons and in some nuclei of the brainstem closely associated with lesions of NE in a Yorkshire Terrier dog [19]; similar non-specific immunolabeling was also observed in some neurons of a dog with MEG [19]. Suzuki et al. [25] also found non-specific labeling for CDV antigen that could be misdiagnosed as the presence of intra-neuronal CDV antigens in both GME and NE. However, studies on a molecular level that employed virus protein gene-specific probes for RT-PCR failed to detect CDV RNA, calling into question the specificity of the antibody [19].

The "non-specific" CDV immunolabeling in both NE and GME could be a non-specific cross-reaction [19, 25]. The monoclonal antibodies (anti-CDV nucleoprotein) might recognize an epitope of a yet-unrecognized morbillivirus present in the CNS that might be implicated in the pathogenesis of such conditions (NE and GME) because the sequence of the CDV nucleoprotein gene has regions of high homology (77% of nucleotides and 88% of encoded amino acids) with other morbilliviruses [63]. Additional studies should be performed to determine why some cases of NE and GME have immunoreactivity to CDV antigens even without CDV RNA detection by RT-PCR.

The authors have studied both NE and GME, and RT-PCR assays for CDV detection have been carried out in nervous tissue to exclude the presence of CDV. The RT-PCR assay was carried out using set of primers designed especially for detection of the CDV nucleoprotein gene [64]. Occasionally, a non-specific RNA was amplified by RT-PCR from fresh brain tissue of animals with both NE and GME. This molecular finding was not misdiagnosed as CDV infection because the non-specific amplified RT-PCR product (unique and strongly stained amplicon of approximately 500-bp size) had a different bp length from the expected CDV amplicon (287-bp size). Such a non-specific molecular result using primers targeting the CDV nucleoprotein gene might suggest the presence of a morbillivirus-like agent in the brain of dogs with GME and NE. The set of primers used was designed using a region of the nucleoprotein gene with high sequence homology within the morbillivirus genus [64, 65]. Previously, the nucleoprotein gene from other morbilliviruses such as measles virus was amplified using the same set of primers [66]. Alternatively, this non-specific RNA might code a protein up-regulated during brain inflammation. Prospective studies have been designed to sequence such non-specific amplicons obtained from the brains of these dogs; however, the results were not available at the time of writing. Such molecular results are thus far inconclusive, and additional studies should be carried out.

The role of a possible yet-unrecognized morbillivirus has been speculated in multiple sclerosis (MS). Brain samples from patients with MS were examined by immunohistochemistry using a panel of measles virus (MV) and CDV monoclonal antibodies [67]. All antibodies were negative except for one anti-MV antibody that bound to 8/9 MS plaques but not six control samples or four samples from patients with ischemic stroke. However, no evidence for the presence of MV in MS plaques was obtained by RT-PCR, calling into question the specificity of the antibody. Geeraedts et al. [67] suggested that the monoclonal antibody may recognize an epitope on an as-yet unrecognized morbillivirus present in the human CNS that might be implicated in MS pathogenesis. Alternatively, the positive staining might also represent a protein that is up-regulated during inflammation [67].

The breed predilection often observed in the encephalitides of unknown etiology may suggest a possible genetic link to predisposition. Outbreaks involving a single breed and variable numbers of siblings within one litter, such as observed in NE, GME, and Greyhound meningoencephalitis, may suggest of a genetically linked pathology [17, 18]. In NME of Pug dogs, a hereditary character was already demonstrated [33, 34]. Speculatively, some breeds may have a breed-specific tissue antigen that might act as a receptor for a virus or a breed-specific composition of immune response genes, leading to an atypical immunological reaction toward an unknown pathogen [15]. In addition, the possibility that viruses may cause different lesion patterns depending on the breed is not unreasonable [23], as genetic factors determine the clinical and morphological expression of experimental CNS infections [68].

The authors surveyed records of the past 50 years and noted that NME, NLE, and Greyhound meningoencephalitis were never observed before 1983, 1986, and 2002, respectively. In addition, before 1971, GME was not registered as often as it is nowadays. Therefore, these observations suggest that such encephalitides might be caused by a relatively new infectious agent or a new strain of a known virus. The histopathological findings in such non-suppurative encephalitides suggest a viral etiology; however, the precise pathogenesis remains uncertain. GME was already reported to be associated with CDV antigens and rabies-like inclusions in two occasions [69, 70].

An intriguing finding that also raises the question of the role of viruses in such idiopathic encephalitides is the immunoreactivity for Mx protein in the brains of dogs with non-suppurative encephalitides of unknown origin. Immunoreactivity for the Mx protein is consistent in most cases of idiopathic encephalitis, including GME and NE of Pug dogs, Yorkshire terrier and Maltese breeds [13]. Mx proteins are a group of interferon-induced GTPases whose expression has been demonstrated in a number of human viral infections and in some idiopathic inflammatory diseases. It has been suggested that MxA expression in humans is a potential diagnostic marker of viral infection [13].

4. Viruses already reported to play a role in the development of encephalitides of unknown origin in dogs

In an immunohistochemical study, 53 dogs and 33 cats with non-suppurative meningoencephalitis of unknown etiology were examined for 18 different infectious agents, including viruses, bacteria and prion protein^{Sc} [6]. In 14 (26%) of the dogs and 13 (39%) of the cats, an infectious causative agent was identified in the CNS. Two dogs and one cat were simultaneously positive for two infectious agents. The study revealed that encephalitis of unknown origin in dogs can be linked with known viral agents such as porcine herpesvirus 1, and canine parainfluenza virus. Curiously, previously unknown viral agents of encephalitis in dogs were also identified such as the West Nile virus (WNV) and encephalomyocarditis virus (EMCV). The significance of the detection of WNV and EMCV antigens requires further study; however, such results suggest that infections with hitherto unrecognized agents cannot be ruled out as causative agents for encephalitis of unknown etiology in dogs.

A new disease pattern of parvovirus infection in the CNS of dogs and cats was identified in 2007 through the studies of encephalitides of unknown origin [6]. In the new neuropathological pattern, the traditionally expected features for CNS infection by parvovirus such as cerebellar hypoplasia or necrotic Purkinje cells were not observed. Histopathologically, all affected dogs showed mild to moderate lymphohistiocytic meningitis or leukoencephalitis (or both), as well as mild to moderate, and in one case severe, vacuolation in the white matter of cerebrum and cerebellum. Previously, leukoencephalomalacia in absence of cerebellar hypoplasia and necrotic Purkinje cells was reported in 1999 with parvovirus infection [71].

West Nile virus (WNV) appeared in recent decades, causing illness, encephalomyelitis, and death in birds, horses, and humans. Different animals have been infected by WNV, but the horse is the most affected [72]. However, the virus was recognized in a case of non-suppurative encephalitis in a dog in 2005 [73], and in 2007, WNV was identified in encephalitides of unknown origin in both dogs and cats [6]. Histopathologically, the lesions in the brain are characterized by moderate to severe meningoencephalitis affecting the grey and white matter; the inflammatory involvement can be granulomatous, pyogranulomatous, lymphohistiocytic or fibrinopurulent and is occasionally associated with neuronal necrosis or malacia [6]. Inflammation was also already observed to be restricted to the grey matter of the cerebrum and cerebellum and to be associated with severe malacia in the hippocampus [6].

CDV is an important pathogen for encephalomyelitis in dogs and might be involved in encephalitides of unknown origin; such speculation appears justified because of the great variety of CNS lesions known to be caused by this agent. CDV may be detected within the white and gray matter of the CNS, and different kinds of neuropathological lesions have been reported. CDV neuropathology is diverse [11], and distemper encephalomyelitis may even mimic other inflammatory diseases of the CNS [11, 42, 46]. The neuropathological manifestations of nervous distemper may also present in ways never previously reported, making the neuropathological diagnosis of nervous distemper difficult [11, 21, 70]. In such situations, it is not unusual to see CDV encephalitis classified as encephalitis of unknown origin [21]. CDV was demonstrated in case of non-suppurative meningoencephalitis of unknown etiology, which had never been associated with CDV before; in this case, CDV was detected by advanced molecular techniques such as broadly reactive PCR and sequence analysis [21].

The authors have diagnosed CDV encephalitis in absence of typical/conventional neuropathological features expected for nervous distemper. CDV has been diagnosed in encephalitides that were not previously reported to occur with CDV infection [11]. The virus was detected in an instance in which the severe infiltration of lymphocytes and monocyte/histiocyte-lineages concentrically through the white matter of the hindbrain mimicked GME [11]. Additionally, non-conventional necrotizing encephalopathies affecting the forebrain have been observed in dogs with CDV infection; the CDV-related necrotic changes mimicked the gross and microscopic features of the necrotizing encephalitis of Pug, Yorkshire, and Maltese dogs [11]. In such cases, CDV was characterized within the CNS lesions by a combination of immunohistochemistry and RT-PCR [11].

5. Final considerations

In summary, the broad spectrum of neuropathological lesions, even arising from known viral pathogens, may lead to non-conventional lesions in the brain. In such situations, the diagnosis of encephalitis of unknown origin may be carried out by routine histological evaluation (i.e., H&E). Thus, infections with known viral pathogens and even hitherto unrecognized viral agents cannot be ruled out as causative agents for encephalitides of unknown etiology in dogs.

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