THE NERVOUS FORM OF CANINE DISTEMPER

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ABSTRACT

Canine distemper is a systemic infection, frequently lethal in dogs. The canine distemper virus (CDV) causes a persistent infection within the central nervous system resulting in a progressive, multifocal demyelinating disease. In dogs, CDV infection may lead to gastrointestinal and/or respiratory signs, frequently with central nervous system involvement. Myoclonus has been a common and characteristic sign observed in dogs with distemper encephalomyelitis. However, the nervous form of distemper may occur in the absence of myoclonus and systemic involvement. This review will point the clinical course and the neurological signs of nervous distemper, as well the clinical syndromes of CDV infection, neuropathology of acute and chronic demyelination, and diagnostic aids of CDV encephalomyelitis.

Key words: dog, canine distemper virus, nervous distemper, encephalomyelitis.

A FORMA NEUROLÓGICA DA CINOMOSE CANINA

RESUMO

A cinomose canina é uma infecção sistêmica, freqüentemente letal em cães. O vírus da cinomose canina (*canine distemper virus* - CDV) causa infecção persistente do sistema nervoso central, resultando em uma doença desmielinizante, progressiva e multifocal. Em cães, a infecção pelo CDV pode resultar em sinais gastrintestinais e/ou respiratórios, freqüentemente associados com sinais neurológicos. Mioclonia é um sinal comum e característico observado em cães com encefalomielite pela cinomose. No entanto, a forma nervosa da cinomose pode ocorrer na ausência de mioclonia e sinais sistêmicos. Esta revisão focará o curso clínico e os sinais neurológicos da cinomose nervosa, assim como as síndromes clínicas da infecção pelo CDV, a neuropatologia da desmielinização aguda e crônica, e os recursos diagnósticos da encefalomielite pelo CDV.

Palavras-chave: cão, vírus da cinomose canina, cinomose nervosa, encefalomielite.

LA FASE NERVIOSA DEL MOQUILLO CANINO

RESUMEN

El moquillo canino es una infección sistémica, frecuentemente letal en perros. El virus del moquillo canino (*canine distemper virus* - CDV) causa una infección persistente del sistema nervioso central, resultando en una enfermedad desmielinizante, progresiva y multifocal. En

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perros, la infección por CDV puede resultar en señales gastrointestinales y/o respiratorios, frecuentemente asociados con señales neurológicos. La mioclonia es un señal común y característica observada en perros con encefalomielitis derivada de moquillo canino. Sin embargo, la enfermedad nerviosa del moquillo puede ocurrir en ausencia de mioclonia y otros señales. Esta revisión enfocará en el curso clínico y los señales neurológicos de la cinomosis nerviosa, así como las síndromes clínicos de la infección por el CDV, la neuropatología de la desmielinización aguda y crónica, y los recursos diagnósticos de la encefalomielitis por CDV.

Palabras-claves: perro, virus del moquillo canino, moquillo nervioso, encefalomielitis.

INTRODUÇÃO

Canine distemper is a systemic disease caused by canine distemper virus (CDV), frequently lethal in dogs, other non-domestic carnivores and some marine mammals. The CDV is a non-segmented, negative single-stranded RNA virus, closely related to measles virus and rinderpest virus, two other members of the genus Morbillivirus of the *Paramyxoviridae* family. The virion is relatively large (150-240 nm), surrounded by a lipoprotein envelope derived from virus glycoproteins incorporated into the cell membrane (FAUQUET et al., 2004). The virus leads a multisystemic infection, which is often associated with viral spread to the CNS, resulting in a progressive, multifocal demyelinating disease (MULLER et al., 1995; MEERTENS et al., 2003).

CDV is generally transmitted as an aerosol infection to the upper respiratory tract. The primary virus replication takes place in the lymphoid tissues. Infection of these tissues is associated with severe long lasting immunossupression. At about 10 days post infection, CDV starts to spread from the sites of primary replication to various epithelial tissues and the CNS (VANDEVELDE & ZURBRIGGEN, 1995). During viraemia CDV infects the brain in the majority, if not all cases, regardless of the clinical signs (SUMMER et al., 1995).

In dogs, CDV may lead to a subclinical infection, or clinical disease characterized by gastrointestinal and/or respiratory signs, the systemic signs of distemper, frequently with CNS involvement. However nervous signs may also occur without other signs (TIPOLD et al., 1992). In patients with only neurological signs, the CDV infection is only one possible differential diagnosis (MORITZ et al., 2000). Nevertheless, in areas where CDV is endemic, the virus should be always considerate as an important differential diagnosis in dogs with progressive and multifocal neurological disease, even when the typical systemic signs of CDV infection (vomiting, diarrhea, respiratory signs) and myoclonus are not present (AMUDE et al., 2006d).

Despite the relative large literature about neuropathology, immunology and laboratorial findings on nervous distemper, there are only a few indepth studies on the clinical neurology of distemper involving the neurological signs (AMUDE et al., 2006d).

CLINICAL COURSE AND NEUROLOGICAL SIGNS

The neurological manifestation of distemper may occur simultaneously to the systemic signs or begin 1 to 3 weeks after recovery from systemic illness (GREENE & APPEL, 1998). The systemic signs include decreased appetite, fever, serous oculonasal discharge, coughing, dyspnea, vomiting and diarrhea. These signs can occur in various combinations. Nervous signs may also occur without any preceding or concomitant systemic signs. The clinical signs, course of the disease, and the type of nervous system involvement vary depending on virulence of the virus strain, environmental conditions, and the host age and immune status.

On clinical examination about 2/3 of the animals are presented with extraneural signs, including conjunctivitis and fever, respiratory signs, gastrointestinal involvement, tonsillitis, and cachexia. In a 1/3 of the dogs no extraneural signs may be found on the physical examination (TIPOLD et al., 1992).

The type of the neurological signs depends on the CDV distribution in the CNS and localization of the lesions, nevertheless a clinicopathological correlation is often lacking (VANDEVELDE & CACHIN, 1993; KOUTINAS et al., 2002). CDV affects both white and gray matter into the CNS. Thus a variety of neurological signs may be observed, including behavioral changes, seizures, cerebellar (head and truncal ataxia, intentional tremor, hypermetria) and vestibular signs (head tilt, falling, circling, nystagmus), visual deficits, paresis, paralysis, limb weakness, and myoclonus (TIPOLD et al., 1992; VANDEVELDE & CACHIN, 1993; AMUDE et al., 2007). Although the clinical signs of seizures depend on which part of the cerebrum is affected, many seizures are described as "chewing-gum fits" (SHELL, 1990; AMUDE et al., 2006c). Signs of leptomeningitis, such as cervical rigidity and generalized hyperesthesia, may also occur (GREENE & APPEL, 1998; KOUTINAS et al., 2002). Neurological signs may be acute or chronic, however are typically progressive (VANDEVELDE & CACHIN, 1993; GREENE & APPEL, 1998; AMUDE et al., 2007).

Generalized or localized myoclonus has been shown a common and characteristic sign observed in dogs with distemper encephalitis (KOUTINAS et al, 2002). It is characterized as a rhythmic jerking of single muscles or muscle groups. Experimental studies have shown that focal spinal cord lesion may be responsible for this sign. It is speculated that the site of damage is the lower motor neurons of the spinal cord or the cranial nerve nuclei. It is also possible that a basal nuclei lesion may initiate myoclonus by establishing a "pacemarker" in the cord or brainstem. The mechanism of myoclonus in distemper is not well understood. In a clinicopathological study 5 out of 13 spontaneous distemper cases with myoclonus, no lesions were found in the neural gray column of the relevant spinal cord segments to account for the presence of myoclonus (KOUTINAS et al, 2002).

In several researches with naturally-occurring nervous distemper, systemic signs simultaneously to the neurological disease have been the most common clinical course, as well seizures and myoclonus have been found as the most frequent neurological findings (HEADLEY & GRAÇA, 2000; KOUTINAS et al., 2002; SAITO et al., 2006a/b). Nevertheless, these clinical findings may result from a bias of selection, since the authors considered as inclusion criteria classical clinical findings of distemper. The classical distemper presentation is not the rule. Authors about nervous distemper had been claiming that the typical systemic findings are absence in about half of the cases (VANDEVELDE & CACHIN, 1993), and myoclonus may be absent in more than half of the cases (TIPOLD et al., 1992). Seizure is a cortical and subcortical sign often observed in immature dogs infected with CDV, however it is not an usual finding of mature dogs suffering from CDV encephalomyelitis (SHELL, 1990; BRAUND, 1994; AMUDE et al., 2007).

Distemper encephalomyelitis usually is a multifocal neurological disease, and the clinical findings reflect the wide virus and lesions dissemination in the CNS (SHELL, 1990; KOUTINAS et al., 2002). Sometimes neurological signs in distemper dogs, suggest a restricted localization of the lesion within the CNS (TIPOLD et al., 1992; KOUTINAS et al., 2002; AMUDE et al., 2006b) with a single distinct neurological syndrome (BRAUND, 1994).

It had been claimed that neurological signs of CDV might occur much later (months or years) after the systemic infection, as a "post infectious encephalitis". Nevertheless, there is no experimental or natural evidence at all to support this notion (VANDEVELDE & ZURBRIGGEN, 1995).

Several clinical syndromes associated with distemper have been recognized in dogs (SHELL, 1990; BRAUND, 1994, BRAUND, 2001), like as: canine distemper

encephalomyelitis in immature dogs; multifocal distemper encephalomyelitis in mature dogs; old dog encephalitis and, post-vaccinal canine distemper encephalitis.

Canine Distemper Encephalomyelitis in Immature Dogs

This is the most common form of CDV infection and is initially characterized by systemic evidence of gastrointestinal and respiratory disturbances in the most of the cases (BRAUND, 1994). In some situation the nervous disease may takes place in the absence of the systemic involvement (BRAUND, 2001; AMUDE et al., 2006c). Hyperkeratosis of the footpad may be seen. Additionally many animals have conjunctivitis and chorioretinitis. Neurological signs are quite varied, often asymmetrical, and usually suggest a multifocal distribuition of lesions (SHELL, 1990; BRAUND, 2001). Signs of localization in cortical and subcortical (seizures, behavioral changes) areas, brainstem (cranial nerves deficits), and cerebellum (hypermetria, head and truncal ataxia) are often observed, while spinal cord (paresis/paraplegia) signs are observed occasionally (SHELL, 1990). Myoclonus may be a characteristic sign. CDV is probably the most common cause of seizures in dogs younger than 6 month of age (BRAUND, 1994).

Multifocal Distemper Encephalomyelitis in Mature Dogs

In mature dogs between the age of 4 and 8 years, CDV can produce a type of multifocal encephalomyelitis that is characterized by a chronic course (BRAUND, 2001). Vaccinated animals may be affected. This neurological manifestation is not preceded by, nor is it coincident with, the systemic signs (SHELL, 1990). The initial neurological presentation consists of signs of weakness, generalized incoordenation, and occasional falling. Usually these signs progress to tetraplegia (AMUDE et al., 2006b). During the course of the disease signs of localization in cerebellum, brainstem, and spinal cord are common. Cortical and subcortical signs are not features of this disease and affected animals maintain a normal mental status (BRAUND, 1994; AMUDE et al., 2006b).

Old Dog Encephalitis

Old dog encephalitis (ODE) is a rare subacute or chronic progressive panencephalitis with very low incidence around the world that is believed to be caused by CDV infection (SHELL, 1990; BRAUND, 1994). There was some speculations that this form of distemper no longer existed since no spontaneous cases had been observed at several institutes over the past decade (BRAUND, 2001). Affected dogs are usually older than 6-years-old and there are no related systemic signs (BRAUND, 2001). The only clinical signs are related to the cortical and subcortical lesions (VANDEVELDE et al., 1980) such as visual deficits, depression, compulsive circling, and head-pressing against objects (SHELL, 1990). In contrast to the signs associated with acute or chronic encephalomyelitis in immature dogs, signs of brainstem, and spinal cord disease are usually absent in cases of ODE (SHELL, 1990; BRAUND, 2001). There is also a relative sparing of the cerebellar signs (VANDEVELDE et al., 1980). Histopathological changes are similar to those seen in multiple sclerosis, a demyelinating disease that takes place in human being.

Post-vaccinal Canine Distemper Encephalitis

Post-vaccinal canine distemper encephalitis is a rare condition that occurs in young animals, especially those less than six months of age (SHELL, 1990; BRAUND, 2001). It is

believed to be associated with vaccination using live CDV strains. It can occur one to two weeks after the animal vaccination. The pathogenesis of this disease is unclear. It may result from: i) insufficient attenuation of the virus vaccine which causes subsequent infection of the CNS; ii) the triggering of a latent distemper infection by vaccination; or iii) an enhanced susceptibility of the animal. Other vaccine components may also be involved.

NEUROPATHOLOGY OF CDV INFECTION

The exact mechanism of CDV entry into the CNS has not been entirely clarified. The frequent occurrence of periventricular and subpial lesions and the fact that CDV can easily be found in chroid plexus cells and ependyma, suggest entry of the virus into the brain tissue by cerebrospinal fluid (CSF) pathways, presumably by infected immune cells (VANDEVELDE & ZURBRIGGEN, 1995).

The spectrum of the lesions appears to be wide, however the neuropathology of spontaneous distemper is remarkably constant. The variability of the neuropathology is largely due to the lesions evolution when the disease progress (VANDEVELDE & ZURBRIGGEN, 1995; SCHOBESBERGER et al., 2002). Some variability may be due to virus strain differences, although there is little concrete evidence that these play a role in natural disease in dogs (VANDEVELDE & ZURBRIGGEN, 1995).

The CDV causes multifocal lesions in the gray and white matter of the CNS. Generally, white matter lesions prevail, and gray matter lesions may be lacking. In the gray matter, CDV infects neurons which can lead to neuronal necrosis and even polioencephalomalacia. Nevertheless there are possibilities that the malacia lesions are relationship to seizure-induced hypoxia-ischemia (BRAUND, 2001). It has been known that the white matter lesions in distemper are characterized by selective loss of myelin sheaths. The demyelinating lesions are not the only responsible for severe neurological signs but are also thought to be a model for human demyelinating conditions such as multiple sclerosis. Because of these features the white matter pathology has been the focus of recent studies (SCHOBESBERGER et al., 2002; MORO et al., 2003; STEIN et al., 2004).

The histopathological findings of distemper encephalomyelitis usually reflect a multifocal disease with the wide virus dissemination in the CNS (SHELL, 1990). Experimental and natural-occurring studies have shown a very high incidence of subclinical lesions with the absence of clinicopathological correlation in neurological distemper (VANDEVELDE & CACHIN, 1993; KOUTINAS et al., 2002).

Studies have to consider an acute and a chronic stage in the development of CDV-induced demyelination. Different lesion stages can occur within the CNS of the same animal. The temporal course of the lesions development after CDV infection is reflected by grouping distemper lesions into three different categories (acute, subacute and chronic) according to the degree of demyelination and inflammation within the white matter. In addition to these three categories, there are very early findings in which no demyelination is noted, although the CDV infection may be identified. Recently, Schobesberger et al. (2002) introduced the term of peracute lesions to consider this last category.

The pathogenesis of acute demyelination

The initial myelin lesions develop during a period of severe immunosuppression and are not inflammatory. This mechanism of demyelination have been examined and it was shown that the initial lesions is directly virus-induced, since there is a clear correlation between the occurrence of demyelination and the CDV replication in the cells of the white matter (VANDEVELDE & ZURBRIGGEN, 1995).

The obvious explanation for the phenomenon of demyelination would be the oligodendroglial infection. Segmental demyelination and degenerative oligodendroglial changes in acute foci are strongly suggestive of a primary oligodendroglial lesion. The effect of CDV on oligodendrocytes and other glial cells was studied extensively in primary dog brain cell cultures (DBCC) (ZURBRIGGEN & VANDEVELDE, 1993). DBCC contain numerous astrocytes and oligodendrocytes, which can be unequivocally identified with antibodies against cell specific markers. Despite considerable efforts using immunocytochemical and ultrastructural techniques, CDV proteins or viral nucleocapsids were only very rarely found in oligodendrocytes, in contrast to astrocytes and microglial cells which easily support CDV infection (VANDEVELDE & ZURBRIGGEN, 1995). Using *in situ* hybridization technique it was found that oligodendrocytes, in CDV-infected brain cultures, contain CDV mRNA corresponding to all viral genes, despite the fact that these cells do not produce viral proteins (ZURBRIGGEN et al., 1993). This *in vitro* found suggest that the oligodendrocytes degeneration may be probably result from a restricted CDV infection without viral protein production. Using a combination of immunocytochemistry and *in situ* hybridization Graber et al. (1995), demonstrate the transcription of the entire virus genome in oligodendrocytes in CDV-infected brain cell cultures. Graber et al. (1995) also show that a restricted infection of oligodendrocytes down-regulates the transcription of the major myelin genes coding for proteolipid protein, myelin basic protein and myelin-associated glycoprotein in similar way. Consequently, the infected cells are no longer able to synthesize all the membrane compounds, which are necessary for maintaining their structure integrity. Zurbriggen et al. (1998), confirmed that restriction infection of oligodendrocytes also occurs *in vivo.* They reported that CDV infection *in vivo* leads to massive down-regulation of myelin gene expression in demyelination lesions, and that this effect correlates in part with restricted infection of oligodendrocytes. However the number of CDV mRNA-expressing oligodendrocytes found in infected but not yet demyelinated areas seems rather small to explain subsequent demyelination (ZURBRIGGEN et al., 1998). The majority of infected cells in the CNS are astrocytes (Schobesberger et al., 2002), which are important in maintaining the tissue homeostasis. The alteration of the astrocytes population may contribute to the development of demyelination, and an indirect mechanism could play a role in oligondendrocytes dysfunction.

Schobesberger et al. (1999) have addressed the question of apoptosis or necrosis importance in the pathogenesis of oligodendroglial degeneration in distemper. However, in dog brain tissue sections, was found no obvious morphological or biochemical evidence for oligodendroglial apoptosis in the acute initial demyelinating lesions (SCHOBESBERGER et al., 1999; SCHOBESBERGER et al., 2002). However, apoptosis can not be totally excluded from playing a role in the disappearance of oligodendrocytes from CDV-induced demyelinating plaques. Lesion development in distemper is a slow process lasting over weeks. Apoptosis on the other hand is a fast event and apoptotic cell fragments are quickly phagocytosed (SCHOBESBERGER et al., 2002).

The contribution of the immune response to early acute lesions development is not clear. Despite severe immunosupression and lack of perivascular cuffing, numerous $CD8^+$ cells are found in acute demyelinating lesions and also diffusely distributed in the brain parenchyma, roughly corresponding with areas of viral infection (TIPOLD et al., 1999).

Findings in acute non-inflammatory demyelination included viral replication in astrocytes, restricted infection of low numbers of oligogendrocytes and diffuse invasion with $CD8^+$ cells, none of which have by themselves provided a satisfactory explanation for myelin destruction in distemper. Diffuse upregulation of MHC II in the white matter in the early acute state of distemper (ALLDINGER et al., 1996), upregulation of metallo-proteinases (MAIO et al., 2003), and immunocytochemical evidence of microglial hyperplasia in initial

demyelinating lesions (TIPOLD et al., 1999), suggest that microglia could be involved in the pathogenesis of early demyelination. Stein et al. (2004) showed a clear correlation between upregulation of various microglial functions and the presence of demyelination.

The mechanism of acute demyelination in distemper is not yet completely understood, despite the extensive studies.

The pathogenesis of chronic demyelination

The chronic lesions are characterized by influx of inflammatory cells, mostly mononuclear, and coincide with the recovery of the immune system (VANDEVELDE & ZURBRIGGEN, 1995). The inflammatory cells accumulate around blood vessels forming partly multi-layered perivascular cuffs, and also invade the parenchyma (SCHOBESBERGER et al., 1999, 2002). The inflamatory reaction in the demyelinating lesions can lead to progression of the tissue damage. There is often necrosis of the tissue in such lesions (SCHOBESBERGER et al., 2002; GEBARA et al., 2004a).

Evidence of autoimmunity is not unusual in virus infection in different organs system, including the brain. Anti-myelin antibodies was already found in serum and in CSF of dogs with distemper. One mechanism by which anti-myelin antibodies could induce demyelination would be antibody-depending cytotoxicity. However the autoimmune reaction in distemper are probably epiphenomena which are not primary involved in the chronic demyelinating process (VANDEVELDE & ZURBRIGGEN, 1995).

The chronic stage of the disease is characterized by immunopathological complication. Tissue damage and demyelination may result from the innocent bystander effects of infiltrating virus-specific T and B cells and their cytokine products (VANDEVELDE & ZURBRIGGEN, 1995). The inflammation is also associated with intrathecal immunoglobulin synthesis. It has been known for a long time that antiviral antibodies play a dominant role in immunity against CDV (VANDEVELDE & ZURBRIGGEN, 1995). The occurrence of anti-CDV antibodies in the CSF coincided with clearance of CDV and CDV containing cells from the inflammatory lesions. Since oligodendrocytes do not express viral proteins, progression of demyelination could hardly be explained by an antiviral cytotoxic reaction killing infected oligodendrocytes. Other types of antiviral immune response could be responsible for the inflammatory tissue damage seen in distemper. Macrophages, which are very numerous in distemper lesions, would play an important role. It was shown that antiviral antibodies bound to the surface of CDV infected cells interacted with the Fc receptors of neighbouring macrophages by way of their Fc portions (VANDEVELDE & ZURBRIGGEN, 1995). This interaction resulted in a respiratory burst of the macrophages with release of reactive oxygen radicals, which can be harmful to oligodendrocytes. So the humoral antiviral immune response could also leads to destruction of oligodendrocytes as innocent bystander cells.

The inflammatory phenomenon is related to virus clearance from CNS lesions. However it has been shown that CDV can persist in white matter areas outside of the inflammatory demyelinating lesions or over even in the immediate periphery of such lesions (SCHOBESBERGER et al., 1999; 2002). The virus persistence is the key to the pathogenesis of the chronic lesions, and seems to contribute to lesion maintenance and progression. The mechanism of persistence of CDV is not yet understood. Have been demonstrated, by *in vitro* assay, that CDV virulent strains which are able to persist in the CNS have a noncytolytic selective virus spread, and a different way of virus release so that very little virus is released outside the nervous cells (MEERTENS et al., 2003). It also has been shown that these virus strains have an impaired and limited budding. It leads a very limited release of cell debris and virus particles in the extracellular space. As a result, macrophages stimulation attracting the antiviral immune response in the areas of active viral replication is avoided. This particular

type of spread is related to differences in viral assembly as compared to attenuated distemper viruses. Sequencing studies have shown differences between virulent and attenuated CDV at the level of the nucleoprotein gene (FRISK et al., 1999; SCAGLIARINE et al., 2003). Its difference has been incriminated as a molecular determinant of persistence.

DIAGNOSIS AIDS

The clinical diagnosis of distemper encephalomyelitis is often hard when the typical signs, such as systemic involvement and myoclonus, are absent. CSF examination in such animals may reveal the presence of inflammatory involvement suggesting the possibility of CDV infection. CSF analysis may suggest the diagnosis of CDV infection if a lymphocytic pleocytosis (greater than 5 leukocytes/µl) is present (SHELL, 1990). A CSF pleocytosis that is more than 60% lymphocytes has been typically associated with viral encephalitis, but has also been reported with granulomatous meningoencephalitis (GME) and bacterial infection especially after antibiotic therapy. In the inflammatory distemper the cell count rarely exceeds 30 cell/µL (VANDEVELDE & CACHIN, 1993). However, Amude et al. (2006c) reported a severe lymphocytic pleocytosis (> 500 cell/ µL) in a distemper case. By the other hand, sometimes there are no abnormalities in the CSF from distemper dogs with neurological disease (SHELL, 1990). In 8 out of 19 dogs with distemper encephalomyelitis the CSF was normal (KOUTINAS et al., 2002).

In experimentally infected animals frequent hematological findings is lymphopenia, sometimes combined with leucopenia or leucocytosis with left shift, anemia, and rarely thrombocytopenia. Several hematological abnormalities have been reported with distemper in natural occurring disease (MORITZ et al., 2000). Tudury et al. (1997) reported that anemia and lymphopenia were frequent hematological changes found in distemper dogs. However the previous studies were descriptive studies, and a cause-consequence relationship could not be assured. According Shell (1990), the hematological changes are frequently absent or non-specific in naturally infected distemper dogs. When the hematological findings from distemper dogs were compared with data from non-distemper dogs, both from the same hospital population and sharing resembling clinical conditions, no significant statistic difference was found (GEBARA et al., 2004b).

Serological examination is not very useful in the diagnosis of distemper, because a high titers of anti-CDV antibodies may be a result of prior vaccination, as well as of previous subclinical or clinical infection. On the other hand, during severe distemper, the antibody titers may be low because of the strong immunosuppressive properties of CDV. Detection of neutralizing antibodies did not correlate with the form of distemper, antigen distribution, or RT-PCR results, indicating the noncontributory role of neutralizing antibody titers for the etiological diagnosis of distemper (FRISK et al., 1999). The anti-CDV antibodies detection in CSF could be a good option to diagnose distemper in dogs with neurological disease. However dogs can die from distemper without anti-CDV neutralizing antibodies titers in the CSF in the acute state of the disease (SHELL, 1990). The virus neutralizing antibody titers in CSF samples was detected in only 2 out of 10 dogs with confirmed CDV infection (FRISK et al., 1999).

A final diagnosis is based on the demonstration of viral antigens in scrapings and body fluids such as conjunctival smears, tracheal washing, and urine sediment (TIPOLD et al., 1992). Direct immunofluorescence test are routinely and widely used with this propose. However, in the subacute or chronic forms of the disease this test gives false-negative results. In 12 dogs suffering from natural distemper confirmed by N-PCR, Józwik and Frymus (2005) diagnosed CDV infection by direct immunofluorescent test in only 6 dogs. No CDV antigen could be detected in the peripheral blood of 6 out of 7 distemper dogs with only

systemic signs, 5 out of 13 distemper dogs with systemic and neurological signs, and all (7/7) distemper dogs with only nervous signs (MORITZ et al., 2000). Viral antigen may be hard to find in the extraneural tissues in cases of distemper encephalomyelitis without systemic involvement (TIPOLD et al., 1992). The methods available for *ante mortem* distemper etiologically diagnosis to date are of limited value, and in the majority of cases a definitive diagnosis is only possible at *post mortem*.

Recently the RT-PCR has been introduced as a useful, fast, sensitive, and specific method to diagnose CDV infection in dogs. Urine, serum, whole blood, and CSF are body fluids that have been used for CDV detection by RT-PCR in dogs with characteristics and commons signs of the disease (FRISK et al., 1999; MORITZ et al., 2000; GEBARA et al., 2004 a/b; SAITO et al., 2006 a/b; NEGRÃO et al., 2006). The sensitivity of the RT-PCR varies with selected primers, RNA extraction methods, and clinical sample analyzed. Frisk et al. (1999) had a sensitivity of 86% (25/29) and 88% (14/16) when serum and whole blood, respectively, were used as biological sample in RT-PCR for distemper diagnosis in naturally-occurring classical disease. Gebara et al. (2004 a/b) and Saito et al. (2006b) used urine, from dogs with characteristics clinical findings of distemper, in order to detect CDV by RT-PCR in naturally-occurring disease. We recently observed that urine might also be a good biological sample for *ante mortem* CDV detection by RT-PCR in dogs with distemper encephalomyelitis presented without myoclonus and in the absence of typical systemic signs (AMUDE et al., 2006a). By RT-PCR, CDV could be detected in 4 out of 5 urine samples from dogs with distemper encephalomyelitis, however the virus only could be detected in one blood sample and none of the serum samples from the same distemper dogs. Curiously the CDV only could be detected by RT-PCR in only 2 out of 5 CSF samples, being the urine the biological sample more sensitive (4/5). The CSF RT-PCR results probably were false negative results that might be due to a complete lack or the presence of only low levels of CDV RNA in the CSF. Pre-analytical errors, such as the low cellularity of the CSF, as well the pathogenesis of the virulent CDV strains over the nervous parenchyma, may contribute to RT-PCR false negative results found on CSF samples. The use of RT-PCR with two differents body fluid (urine and CSF) can increase the technique sensitivity for *ante mortem* diagnosis of distemper in dogs with the clinical presentation restricted to neurological disease without myoclonus (AMUDE et al., 2006a). Nested-PCR has also been used to increase the sensitivity of the RT-PCR assay (JÓZWIK and FRYMUS, 2005).

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