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Mycophenolate mofetil plus prednisolone combination therapy for necrotising leukoencephalitis in a dog: long-term clinical observation, serial imaging analysis and histopathological findings

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Abstract: An 8-year-old intact male mixed-breed dog presented with tetraparesis, right side head tilt, and cluster seizure-like episodes. Based on the magnetic resonance imaging and cerebrospinal fluid analysis results, meningoencephalitis of an unknown aetiology was strongly suspected. The patient survived for 963 days under mycophenolate mofetil plus prednisolone therapy and was ultimately diagnosed with necrotising leukoencephalitis. This report describes the clinical findings, the serial magnetic resonance imaging characteristics, and the histopathologic features of a case of necrotising leukoencephalitis and the long-term survival after mycophenolate mofetil plus prednisolone therapy.

Keywords: autoimmune encephalitis; magnetic resonance imaging (MRI); meningoencephalitis of an unknown aetiology (MUE); necrotising meningoencephalitis (NME)

Necrotising meningoencephalitis (NME) is an idiopathic non-infectious inflammatory disease of the canine central nervous system (CNS) reported in diverse small breed dogs (Jung et al. 2007; Jung et al. 2012; Dewey 2016). The lesions of NME are multifocal and display asymmetrical necrosis in the cerebral cortex with leptomeninges, which leads to a loss of demarcation between the grey and white matters (Dewey 2016; Uchida et al. 2016). NME can be differentiated into cortex-dominant and white matter-dominant necrotising types; the latter, including the brain stem, is named necrotising leukoencephalitis (NLE) (Dewey 2016; Uchida et al. 2016). The clinical characteristics, breed predispositions, and prognosis of NLE are different from those of NME, but the pathological features sometimes overlap (Uchida et al. 2016).

NME and NLE in canines seem to result from autoimmune inflammation in the CNS, as several reports describe a beneficial efficacy in using immunosuppressants to treat NME and NLE (Jung et al. 2007; Jung et al. 2012; Jung et al. 2013; Dewey 2016). Recently, mycophenolate mofetil (MMF) has been shown to exert beneficial effects on autoimmune CNS inflammatory cases (Barnoon et al. 2016; Woolcock et al. 2016).

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This case report presents the clinical features, serial magnetic resonance imaging (MRI) findings, histopathological characteristics, and long-term management with mycophenolate mofetil (MMF) plus prednisolone in an NLE case.

Case description

An 8-year-old intact male mixed-breed dog (body weight: 6.5 kg) was referred with acute onset tetraparesis, right side head tilt, and cluster seizure-like episodes. The clinical signs were suddenly observed a day before the presentation. The neurological examination revealed that the postural reactions of all the limbs were reduced. The cranial nerve examination revealed bilateral miosis, exotropia, and spontaneous nystagmus with the fast phase toward the left side. The ophthalmologic examinations, including the fundus examination, were normal. The laboratory results, including the complete blood counts and serum chemistry, were not significant. The skull, thoracic, and abdominal radiography were not remarkable. We ruled out canine distemper virus infections and toxoplasmosis in the blood (Neodin Vetlab, Seoul, Republic of Korea).

Based on the physical and neurological examinations, we suspected intracranial lesions extending to the brain stem. Initially, we performed a brain computed tomography (Somatom Emotion Duo®, Siemens AG, Munich, Germany) examination and the results were unremarkable. We then performed a brain MRI examination (E-scan®, Esaote, Genova, Italy). T1- and T2-weighted images and postcontrast T1-weighted images were obtained. The MRI confirmed the presence of lesions in the midbrain and the interthalamic adhesion (Figure 1). Ill-defined hyperintense lesions were identified in the midbrain area and the interthalamic adhesion area on the T2-weighted images (Figure 1B). These lesions appeared isointense on the T1-weighted images (Figure 1A) and were not enhanced after a contrast study. The cerebrospinal fluid (CSF) was obtained from the atlanto-occipital cistern and analysed within 30 min for the gross appearance, total protein level and total nucleated cell counts and a cytological evaluation following cytocentrifugation. The results of the CSF analysis were normal. The volume of the CSF was not enough to perform other tests such as a bacterial culture and a neurologic PCR panel.

Based on all the examinations, we tentatively diagnosed the present case as meningoencephalitis of an unknown aetiology (MUE).

Management with prednisolone (Solondo®, Korea Pharma, Republic of Korea; 1 mg/kg, per oral (PO), bid) and mycophenolate mofetil (MMF; CellCept®, Roche, Switzerland; 20 mg/kg, PO, bid) was initiated, and the clinical signs improved gradually. Two weeks after therapy, the patient could stand and walk and the other clinical signs such as the seizures and head tilt disappeared. We regularly checked the patient's status every few months. Two months after the initial therapy, prednisolone was tapered to 0.7 mg/kg (PO, bid) and the initial MMF dosage was maintained. The neurological signs did not relapse after the first prednisolone tapering, therefore, the prednisolone dosage was maintained. Five, eleven, and thirteen months after the initial therapy, the prednisolone dosage was tapered to 0.5 mg/kg (PO, bid), 0.4 mg/kg (PO, bid), and 0.2 mg/kg (PO, bid), respectively. The MMF dosage was maintained and tapered about 20 months after the initial treatment (15 mg/kg, PO, bid). The MMF was tapered again at 27 months after the initial treatment (8 mg/kg, PO, bid). For the last 6 months, the prednisolone (0.2 mg/kg, PO, bid) and MMF (8 mg/kg, PO, bid) were maintained. About 30 months after the initial treatment, the neurological signs associated with painful reactions, ataxia, and the delayed postural reactions relapsed. The clinical signs did not improve by increasing the dosages of the drugs. Ultimately, the present patient expired suddenly 963 days after the initial treatment.

Following the initial MRI scan, we performed a second, third and fourth MRI scan at 5, 15, and 26 months, respectively (Figure 1). In the serial images, the lesions showed a hyperintense signal on the T2-weighted images and a hypointense signal on the T1-weighted images compared to the previous MRI scans. The isointense-signal midbrain lesion on the T1-weighted images from the first MRI scan changed to a hypointense-signal lesion on the second MRI scan. The third MRI scan also showed a hyperintense-signal lesion on the T2-weighted images, and a hypointense-lesion in the interthalamic adhesion and brainstem. The last conducted MRI scans revealed a mild deterioration of the lesion, especially in the interthalamic adhesion, on the T2-weighted images.

We performed a necropsy per the client's agreement. The necropsy findings revealed multifocal

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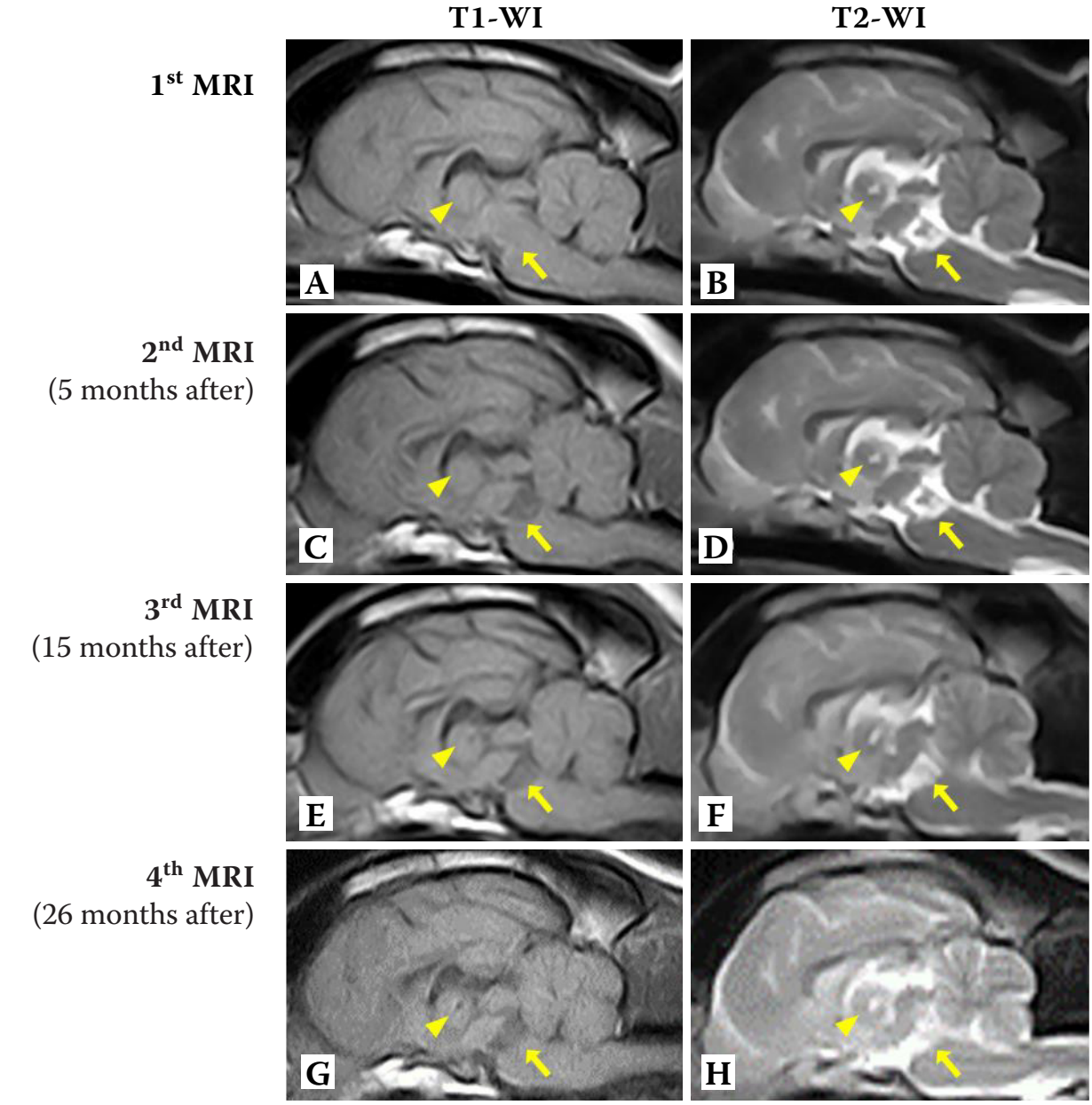

Figure 1. The serial MRI findings in time sequence. (**A**, **B**) Initial MRI findings. The hyperintense lesions were observed in the interthalamic adhesion (arrowhead) and the brainstem (arrows) on the T2-WI (B), which were isointense on the T1-WI (A). (**C**, **D**) The second MRI scan findings. The lesion in the brainstem was isointense and it became a hypointense-signal lesion on the T1-WI (C; arrow). The lesion in the interthalamic adhesion that had been hyperintense in the initial examination became an isointense-signal lesion on the T2-WI (D; arrowhead). (**E**, **F**) The third MRI scan findings. The lesion in the interthalamic adhesion became hypointense again (F; arrowhead). (**G**, **H**) The fourth MRI scan findings. Both the interthalamic adhesion (arrowhead) and brain stem (arrow) lesions mildly progressed on the T2-WI (H)

necrotic, inflammatory lesions in the brainstem and interthalamic adhesion (Figure 2), which was consistent with the MRI findings. Based on findings on the histopathological examinations, this case showed a neuronal necrosis, malacic change, demyelination, and microgliosis, which were prominent in the midbrain, thalamus, and basilar cortex at the piriform lobe (Figure 3). The lesions were diagnosed as NLE.

The patient was definitively diagnosed as NLE based on the serial MRI results and the results of the histopathologic findings.

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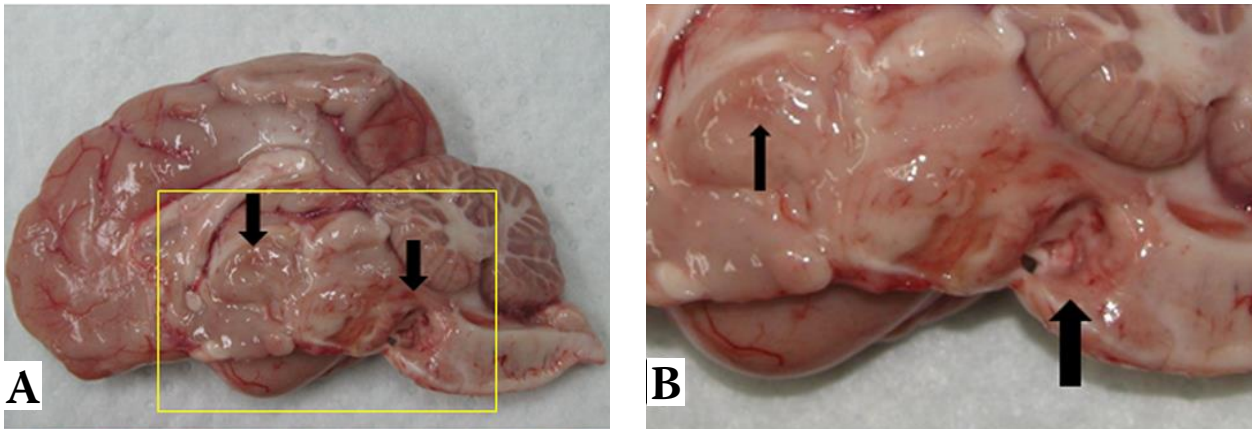

Figure 2. The macroscopic findings by necropsy (963 days after the presentation). The lesions are evident on the midline cut surface and are necrotised, liquefied, and discoloured to red. A malacic change of the interthalamic adhesion is observed (arrowhead) and a liquefied cavitation is identified in the pons and midbrain (arrow)

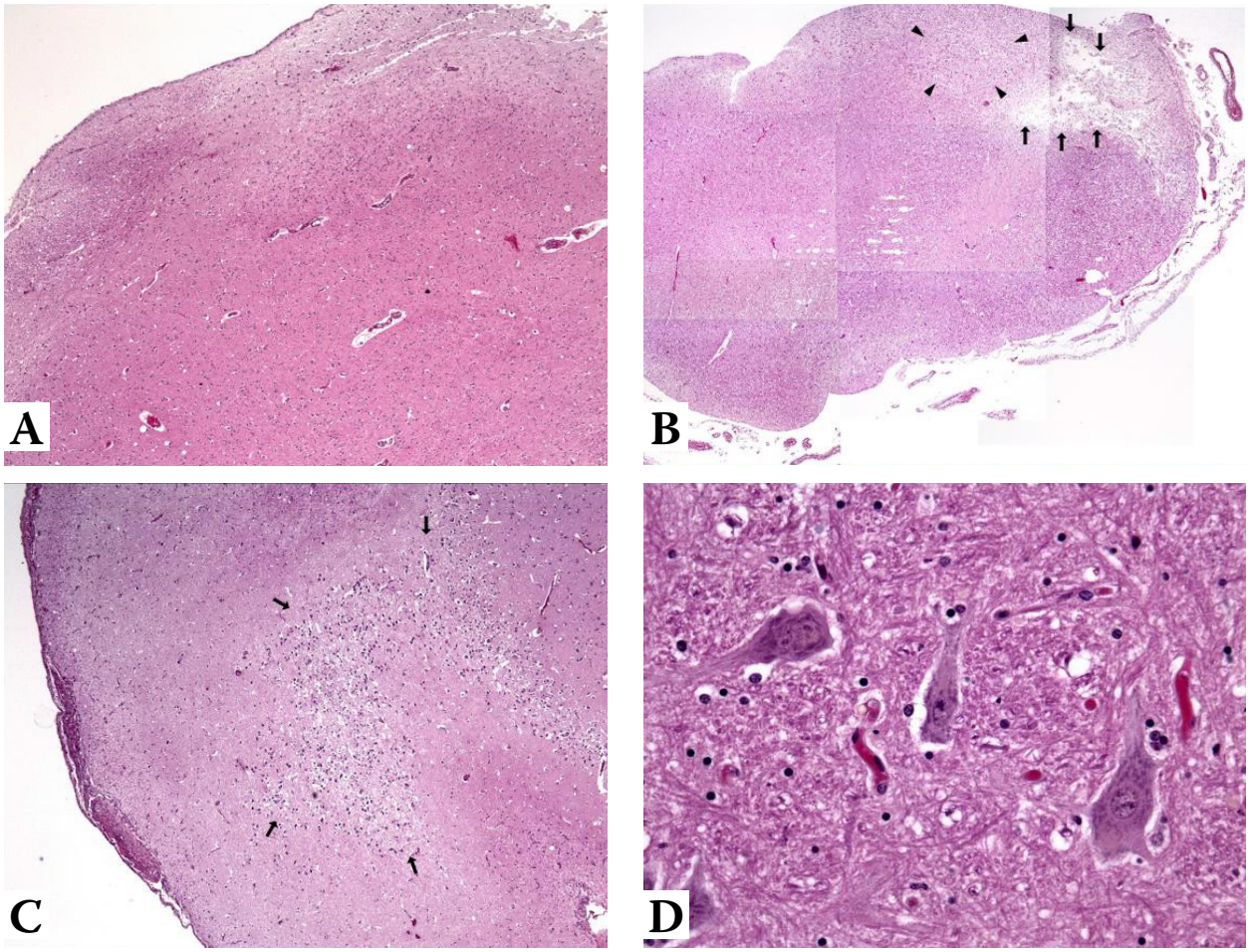

Figure 3. The histopathological findings of the patient. (**A**) Haematoxylin and eosin staining indicate severe neuronal necrosis in the thalamus (× 40). (**B**) The midbrain shows a severe demyelination (arrow) and necrosis of the neurons (arrowhead) located around the midline (× 20). (**C**) The basal cortex in the piriform lobe shows a neuronal necrosis and demyelination (× 40). (**D**) The brainstem shows damaged neurons surrounded by infiltrating microglial cells (× 400)

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DISCUSSION AND CONCLUSIONS

NME is recognised as a canine autoimmune CNS disease (Jung et al. 2007; Talarico and Schatzberg 2010; Jung et al. 2012; Jung et al. 2013; Barnoon et al. 2016; Dewey 2016; Uchida et al. 2016; Woolcock et al. 2016), which is characterised by progressive neurological abnormalities such as seizures, ataxia, and head tilt (Jung et al. 2007; Talarico and Schatzberg 2010; Barnoon et al. 2016). Since the brain lesions of the NLE cases often involve the brain stem with consistent white matter dominant type NME or those distinctively of the cortex and leptomeninges dominant type of NME, MRI is used to tentatively diagnose CNS inflammatory diseases (Thomas 1998; Dewey 2016). This present case was definitively diagnosed as NLE based on the *postmortem* histopathological findings. The histopathological results revealed the necrosis and liquefaction of the white matter, including the brain stem, which were compatible with the clinical signs and MRI findings.

Typical progressive NLE lesions are histopathologically characterised by extensive necrosis, infiltration of the moderate mononuclear cells, and mild macrophages in the white matter (Uchida et al. 2016). We performed the best management by reducing the immune reaction and minimising the side effects for a patient with NLE. Prednisolone is widely used for treating various canine neurological diseases because of its effect on reducing the inflammatory and immune reactions (Jung et al. 2007). However, long-term management with corticosteroids is difficult because of the drugs' side effects such as polyuria-polydipsia, hepatotoxicity, and iatrogenic hyperadrenocorticism (Jung et al. 2012).

Some reports (Talarico and Schatzberg 2010; Park et al. 2012) suggest that NME is associated with a T-cell mediated delayed type of hypersensitivity with an organ-specific autoimmune reaction. Accordingly, we expected lymphocyte-specific immunomodulatory effects of the MMF on these canine brain inflammatory lesions. MMF, a relatively new immunosuppressive drug, is a better target than other immunosuppressive drugs, with fewer side effects. Clinically, it is recommended for some autoimmune disorders (Gaubitz et al. 1999; Allison 2005). The drug inhibits the proliferation of the T- and B-lymphocytes in autoimmune diseases via lymphocyte hyperactivity and increased antibody production (Gaubitz et al. 1999; Allison 2005). In addition to the recruitment of lymphocytes, the induction of lymphocytic apoptosis results in the reduction of the antibody and cytokine production and prevention of tissue damage (Allison and Eugui 2000; Allison 2005). Since corticosteroids act immediately and initially improve the clinical signs until the MMF exerts its effect (Jung et al. 2007), we prescribed MMF combined with an immunosuppressive dose of prednisolone, which was tapered and maintained at a minimum dose until the patient expired.

The prognosis of MUE is generally considered to be "poor" to "grave" without aggressive immunosuppression (Talarico and Schatzberg 2010; Jung et al. 2013; Dewey 2016). The median survival time (MST) was reported to be 11.5 days in 36 dogs with NME (Jurney et al. 2007). The risk factors of the prognosis included the age at the onset, the duration, the severity of the clinical signs and the use of additional immunosuppressive treatments (Woolcock et al. 2016). In one study, the MST of dogs with MUE treated with MMF and corticosteroids was reported to be 731 days (Woolcock et al. 2016). The MST in another study was 250 days or longer, with 40% of the dogs still alive at the end of the study and an average follow-up time of 871 days (Barnoon et al. 2016). The patient in the present case had improved clinical signs after the acute onset, which was well controlled throughout the treatment. There are several reasons for the longer survival times in the present case. First, when the patient initially presented to us, we prescribed MMF at the recommended timing of the administration for the immunosuppressive drugs. Additionally, the relatively long survival times were reported in the dogs that presented with the onset of the clinical signs within a week, suggesting that an early diagnosis and therapy may extend the survival (Barnoon et al. 2016). Second, we presume that MMF, in combination with the prednisolone therapy, inhibited the inflammatory reactions, thereby reducing the pace of the disease's progression. The prognosis of NLE is related to the degree of the necrosis (Talarico and Schatzberg 2010). In this case, some areas of the brain exhibited necrosis without concurrent inflammation, additionally, we observed no infiltration of the inflammatory cells in the cortex and meninges. The necrotic parts may be the initial lesion (Thomas 1998), however, our serial MRI findings suggest that the MMF immunosuppressive therapy

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could not halt the progress of the necrotic brain lesions (Figure 1). We suppose that the long-term MMF therapy eliminates the inflammatory cells, with the rationale that MMF induces apoptosis in the activated T-lymphocytes (Allison and Eugui 2000; Allison 2005). Therefore, this present NLE case could have a comparatively long-term survival with a mild progression in the clinical signs.

Under the combined prednisolone-MMF therapy, the clinical signs improved and were comparatively well controlled.

In this case report, the therapy based on MMF plus prednisolone in a dog diagnosed with NLE was described. The combined use of MMF and prednisolone allowed for the good control of the brain lesions for 963 days followed-up with a minimal dose of an oral prednisolone. Thus, the combined prednisolone-MMF therapy can be an alternative treatment option to other immunosuppressive drugs for the long-term management in NLE cases.

REFERENCES

Allison AC (2005): Mechanisms of action of mycophenolate mofetil. Lupus 14, 2–8.

Allison AC, Eugui EM (2000): Mycophenolate mofetil and its mechanisms of action. Immunopharmacology 47, 85–118.

Barnoon I, Shamir MH, Aroch I, Bdolah-Abram T, Srugo I, Konstantin L, Chai O (2016): Retrospective evaluation of combined mycophenolate mofetil and prednisone treatment for meningoencephalomyelitis of unknown etiology in dogs: 25 cases (2005–2011). Journal of Veterinary Emergency and Critical Care 26, 116–124.

Dewey CW (2016): Encephalopathies: Disorders of the brain. In: Dewey CW, da Costa RC (eds): A Practical Guide to Canine and Feline Neurology. 3rd edn. Wiley-Blackwell, Ames. 141–236 p.

Gaubitz M, Schorat A, Schotte H, Kern P, Domschke W (1999): Mycophenolate mofetil for the treatment of systemic lupus erythematosus: an open pilot trial. Lupus 8, 731–736.

Jung DI, Kang BT, Park C, Yoo JH, Gu SH, Jeon HW, Kim JW, Heo RY, Sung HJ, Eom K D, Lee JH, Woo EJ, Park HM (2007): A comparison of combination therapy (cyclosporine plus prednisolone) with sole prednisolone therapy in 7 dogs with necrotizing meningoencephalitis. Journal of Veterinary Medical Science 69, 1303–1306.

Jung DI, Kim JW, Park HM (2012): Long-term immunosuppressive therapy with cyclosporine plus prednisolone for necrotizing meningoencephalitis in a Pekingese dog. Journal of Veterinary Medical Science 74, 765–769.

Jung DI, Lee HC, Ha J, Jung HW, Jeon JH, Moon JH, Lee JH, Kim NH, Sur JH, Kang BT, Cho KW (2013): Unsuccessful cyclosporine plus prednisolone therapy for autoimmune meningoencephalitis in three dogs. Journal of Veterinary Medical Science 75, 1661–1665.

Jurney CH, Van Winkle TJ, Shofer FS, Vite CH (2007): Necrotizing encephalitis: A retrospective study of 36 cases. Journal of Veterinary Internal Medicine 21. Abstract 253.

Park ES, Uchida K, Nakayama H (2012): Comprehensive immunohistochemical studies on canine necrotizing meningoencephalitis (NME), necrotizing leukoencephalitis (NLE), and granulomatous meningoencephalomyelitis (GME). Veterinary Pathology 49, 682–692.

Talarico LR, Schatzberg SJ (2010): Idiopathic granulomatous and necrotising inflammatory disorders of the canine central nervous system: a review and future perspectives. The Journal of Small Animal Practice 51, 138–149.

Thomas WB (1998): Inflammatory diseases of the central nervous system in dogs. Clinical Techniques in Small Animal Practice 13, 167–178.

Uchida K, Park E, Tsuboi M, Chambers JK, Nakayama H (2016): Pathological and immunological features of canine necrotising meningoencephalitis and granulomatous meningoencephalitis. Veterinary Journal 213, 72–77.

Woolcock AD, Wang A, Haley A, Kent M, Creevy KE, Platt SR (2016): Treatment of canine meningoencephalomyelitis of unknown aetiology with mycophenolate mofetil and corticosteroids: 25 cases (2007–2012). Veterinary Medicine and Science 2, 125–135.

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