Clinical presentation and magnetic resonance imaging findings in 11 dogs with eosinophilic meningoencephalitis of unknown origin

Summary

Objectives: To describe the clinical presentation, MRI findings and outcome in dogs with eosinophilic meningoencephalitis of unknown origin.

Methods: A retrospective study was performed. Dogs were included if they had: complete medical records, complete neurological examination, MR imaging, cerebellomedullary cerebrospinal fluid sample consistent with eosinophilic pleocytosis and negative infectious disease testing.

Results: 11 dogs were included with a median age of 22.0 months (range 7.6–92.0 months). Nine breeds were represented. Neurological abnormalities included obtundation (n=10), menace response deficits (n=9) proprioceptive deficits (n=7), ataxia (n=7) and seizures (n=2). Neuroanatomical localisation was multifocal (n=4), central vestibular system (n=4), diffuse forebrain (n=2), or left trigeminal/facial nerves (n=1). Seven dogs had a peripheral eosinophilia and all had an eosinophilic pleocytosis. Ten dogs had bilateral symmetrical lesions affecting the cortical gray matter that were hyperintense on T2-weighted and FLAIR images, iso- to hypointense on T1-weighted images with associated meningeal contrast enhancement. MRI findings were consistent with diffuse meningitis and atrophy or necrosis of cortical grey matter. One dog had increased contrast uptake of the left trigeminal nerve. Ten dogs receiving corticosteroids survived to discharge with 7 receiving additional cytarabine arabinoside. Median survival time was 762 days.

Clinical significance: eosinophilic meningoencephalitis of unknown origin affects younger larger breed dogs with the majority having a suspected diffuse cerebrocortical meningitis and cortical (polio)encephalitis which can be identified on MRI. Response to immunosuppressive treatment is good in the medium to long term although further studies are required in this area.

Key words: eosinophilic, meningoencephalitis, MRI, cortical atrophy, dog

Introduction

Eosinophilic meningoencephalitis is diagnosed in humans and animals when neurological disease is associated with an eosinophilic pleocytosis (Williams 2008). In humans, an eosinophilia of greater than 10% of the total white blood cell count in the CSF is often used as a criterion for the diagnosis of eosinophilic meningoencephalitis (Kuberski 1981, Graeff-Teixeira *et al.* 2009). Eosinophilic meningoencephalitis in dogs can be associated with infectious and non-infectious causes. Infectious causes in dogs include *Neospora caninum* and less often *Toxoplasma gondii* (Bennett *et al.* 1997, Smith-Maxie *et al.* 1989). Other infectious causes include *Angiostrongylus vasorum*, *Prototheca spp*. *Crytococcus spp*., canine distemper virus, rabies and bacterial encephalitis (Smith-Maxie *et al.* 2008; Windsor *et al.* 2009). Non-infectious causes of eosinophilic meningoencephalitis include neoplasia, infarction, shunt placement, and trauma (Smith-Maxie *et al.* 2008; Windsor *et al.* 2009).

Where no infectious or systemic causes have been identified, the term eosinophilic meningoencephalitis of unknown origin (eosinophilic MUO) is used. Eosinophilic MUO is considered a rare condition with only 30 canine cases reported to date and is now considered part of the spectrum of the canine non-infectious meningoencephalitides (Bennett *et al.* 1997; Salvadori *et al.* 2007; Smith-Maxie *et al.* 2008; Williams *et al.* 2008; Henke *et al.* 2009; Windsor *et al.* 2009; Granger *et al.* 2010; Olivier *et al.* 2010; Lowrie *et al.* 2013). Eosinophilic MUO affects multiple breeds and is suggested to be more common in young, male, large breed dogs. Reported clinical signs are variable and include mentation changes, ataxia, visual deficits, cervical hyperaesthesia and seizures (Bennett *et al.* 1997; Salvadori *et al.* 2007; Smith-Maxie *et al.* 2008; Williams *et al.* 2008; Henke *et al.*

2009; Windsor *et al.* 2009; Granger *et al.* 2010; Olivier *et al.* 2010). Descriptions of pathological findings and magnetic resonance imaging (MRI) characteristics of eosinophilic MUO are limited and range from a severe and diffuse eosinophilic meningitis with infiltration of the superficial cortex to multifocal granulomatous intra-axial mass lesions associated with eosinophilic infiltration of the parenchyma (Bennett *et al.* 1997; Salvadori *et al.* 2007; Henke *et al.* 2009; Olivier *et al.* 2010). Eosinophilic MUO is hypothesised to be an immune-mediated condition, although the pathogenesis remains unknown (Dorta-Contreras & Reiber 1998; Williams *et al.* 2008). Treatment typically consists of immunosuppressive doses of corticosteroids, medication for potential infectious causes and supportive care. Reports of response to immunosuppressive therapy are limited, with some dogs experiencing resolution of clinical signs with corticosteroid treatment whilst others rapidly succumb despite aggressive treatment and supportive care (Bennett *et al.* 1997; Salvadori *et al.* 2007; Smith-Maxie *et al.* 2008; Williams *et al.* 2008; Henke *et al.* 2009; Windsor *et al.* 2009; Granger *et al.* 2010; Olivier *et al.* 2010). In 15-20% of reported cases diagnosed with eosinophilic meningoencephalitis, the clinical presentation, imaging findings and outcomes were potentially influenced by a possible infectious aetiology meaning these cases may not have been truly 'idiopathic' (Smith-Maxie *et al.* 2008; Williams *et al.* 2008; Henke *et al.* 2009). Given the diverse clinical and imaging findings, the aims of this study were to better describe the clinical presentation, diagnostic test results, imaging findings and treatment outcomes in dogs with eosinophilic MUO without evidence of infectious disease.

Materials and methods

Case selection and medical records review— The electronic medical records of the University of London Royal Veterinary College (RVC) Small Animal Referral Hospital and Ghent University (GU) were searched to identify dogs with suspected eosinophilic MUO that were diagnosed between January 1st 2000, and April 1st 2017. Search terms used included 'eosinophilic meningoencephalitis', 'eosinophilic meningitis', 'eosinophilic pleocytosis', 'eosinophilia', 'MUA', 'MUO', 'meningoencephalitis of unknown aetiology/etiology/origin' and various combinations of these terms. Dogs were included if they had: 1) complete medical records available, 2) a complete neurological examination performed leading to a focal or multifocal intracranial neuroanatomical localization, 3) an eosinophilic pleocytosis on cerebrospinal fluid (CSF) analysis (Total nucleated cell count >5 cells/mm^3 of CSF, >10% eosinophils on a 100 cell differential count), 4) and MRI of the brain (Granger *et al.* 2010). Dogs were excluded if: 1) clinical records or imaging studies were incomplete or not available for review, 2) meningomyelitis without clinical signs of intracranial involvement was diagnosed or 3) if no eosinophilic pleocytosis was found on CSF analysis (Bosch & Oehmichen 1978; Windsor *et al.* 2009). Information obtained from medical records included signalment, duration of clinical signs prior to diagnosis (time to presentation), treatment received prior to referral, general physical examination and neurological examination findings, neuroanatomical localisation and results of diagnostic tests including complete blood count (CBC), serum biochemistry profile, ancilliary tests for infectious agents and results of cisternal CSF analysis. Dogs were classified as small (<10 kg), medium (10–30 kg) or large (>30 kg) breeds based on body weight (Cardy *et al.* 2015). Possible neuroanatomical localisations included diffuse forebrain, multifocal brain, central vestibular system or cranial nerves. The study was approved by the Royal Veterinary College Ethics and Welfare committee (protocol number URN 2017 1684-3).

84

85 **Ancillary diagnostic tests**

86 For cisternal CSF analysis total nucleated cell count (TNCC), total protein (TP) concentration and nucleated cell

87 differential count were recorded. TNCC was considered normal if the TNCC was less than 5 cells/mm^3 (Di

88 Terlizzi & Platt 2009, Dewey & da Costa 2016). Total protein concentration was considered normal for cisternal

89 collection if <0.25 g/l (Di Terlizzi & Platt 2009, Dewey & da Costa 2016). Infectious disease testing on serum,

90 CSF, or both was performed on a case-by-case basis and included: *Cryptococcus* latex agglutination Cryptococcal

91 antigen test, *Toxoplasma gondii* IgG and IgM antibody, *Neospora caninum* immunofluorescent antibody test

92 (IFA), PCR on CSF for Canine Distemper Virus, PCR on CSF for *Toxoplasma gondii*, PCR on CSF for *Neospora*

93 *caninum*, SNAP 4Dx ELISA™ (Idexx) for *Angiostrongylus vasorum*, *Ehrlichia canis, Borrelia burgdorferi,*

94 *Anaplasma phagocytophilum* and *Anaplasma platys*, Canine Distemper Virus IgG and IgM antibody, Angio

95 Detect™ ELISA (Idexx) for *Angiostrongylus vasorum*. Faecal analysis was performed on three dogs for

96 *Angiostrongylus vasorum*. All dogs had two or more tests for infectious agents performed.

97

98 **Diagnostic imaging**

99 MRI was performed under general anaesthesia with a permanent 1.5 T magnet (Intera, Philips Medical Systems,

100 Eindhoven, the Netherlands) or a permanent 0.2 T magnet (Airis Mate, Hitachi Ltd, Tokyo, Japan). All images

101 were reviewed by a board-certified radiologist using Osirix DICOM viewer (Osirix Foundation, V.5.5.2 Geneva,

102 Switzerland). Studies included a minimum of T2-weighted (T2W) (repetition time (ms) (TR)/echo time (ms) (TE),

103 3000/120), T1-weighted (T1W) (TR/TE, 400/8) and fluid attenuating inversion recovery (FLAIR) images of the

entire brain in a sagittal, transverse and dorsal plane. The T1W images were acquired before and after intravenous administration of paramagnetic contrast medium (0.1 mg/kg, gadoterate meglumine, Dotarem, Guerbet, Milton Keynes, UK). Variables recorded were lesion localisation and distribution, presence of parenchymal or meningeal contrast enhancement, presence of mass effect (brain herniation, midline shift, flattening of gyri/sulci), T1W/T2W/FLAIR signal intensity of the cerebral grey matter and size of the cerebral sulci/subarachnoid spaces.

Treatment and follow-up

The specific treatment protocol was recorded for all dogs (corticosteroids with or without cytosine arabinoside). Following admission, all dogs underwent at least one daily general physical examination and a complete neurological examination by a board-certified neurologist or a neurology resident during hospitalisation. Neurological examination results and response to treatment (improvement, deterioration, or static) were recorded in the medical records until discharge. Following discharge medical records were searched for the presence of a reexamination or owner/vet communication to confirm the dog was alive or dead and the current treatment as of April 1st 2017. For those dogs managed at their referring practices, the veterinary surgery was contacted directly (Thomas Cardy) by telephone for a verbal update on neurological status and current treatment.

Statistical analysis

Data analysis was performed using a standard statistical software package (SPSS: Statistical Package for the Social Sciences 22.0.1, SPSS). Non-parametric data were described using median and range. Comparisons between gender and neuter status were performed using a Chi-Squared test. Values of $P<0{\cdot}05$ were considered

significant. The relationship between variables was investigated using Pearson correlation coefficient. Preliminary analyses were performed to ensure no violation of the assumptions of normality, linearity and homoscedasticity. Survival analysis was performed using both a Log-rank (Mantel-Cox) and Gehan-Breslow-Wilcoxon test, resulting in median survival time (MST) calculation and a Kaplan-Meier survival curve. Survival was defined as time from diagnosis to death or euthanasia because of disease progression, or time from diagnosis to last follow-up for dogs that were alive at time of data capture.

Results

Signalment

Initial database searches identified 27 dogs from the RVC and 4 dogs from GU. Eleven dogs met the inclusion criteria (9 from the RVC and 2 from GU). Four dogs (36.4%) were small breed, 4 dogs (36.4%) were medium sized and 3 dogs (27.2%) were large breed. Median bodyweight at presentation was 18.0 kg (Ranging from: 4.9 kg–36.0 kg) and median age at presentation was 22.0 months (7.6–92.0 months). Six dogs (50%) were male, of which 2 were neutered, compared to 5 females (50%), of which 1 was neutered. No significant variation was found in either gender or neuter status. There were 9 breeds represented including 2 Flat-Coated Retriever and 2 Welsh Terriers (Table 1). The 11 dogs included in the current study were predominantly younger, entire, large breed dogs as previously reported. (Bennett *et al.* 1997; Salvadori *et al.* 2007; Smith-Maxie *et al.* 2008; Williams *et al.* 2008; Henke *et al.* 2009; Windsor *et al.* 2009; Granger *et al.* 2010; Olivier *et al.* 2010).

Clinical signs

Median duration of clinical signs prior to diagnosis was 24 days (Ranging from: 2-78 days). The most common reasons for presentation included one or more of the following neurological signs: behavior changes (10 dogs, 90.9%), ataxia (6 dogs, 54.5%), seizures (2 dogs, 18%), suspected blindness (3 dogs, 27.3%) and head tilt (2 dogs, 18.2%). A single dog (9.1%) was referred for masticatory muscle atrophy and an inability to blink with the left eye. Three dogs (27.3%) were treated with oral prednisolone (0.35 - 1.8mg/kg/day) prior to referral for suspected otitis media/interna or suspected MUO. Median duration of corticosteroid treatment was 16.5 days (Ranging from: 1–90 days) (Table 1).

151

152 *Neurological examination*

153 Mentation was classified as abnormal in 10 dogs (90.9%) with all being described as mild to moderately obtunded.
154 Seven dogs (63.6%) had ataxia affecting all limbs, 7 dogs (63.6%) had proprioceptive deficits (Table 1). Nine
155 dogs (81.8%) had a reduced or absent menace response and 2 dogs (18.2%) had a reduced or absent pupillary
156 light reflex (PLR) (Table 1). Two dogs (18.2%) presented with neurological signs consistent with a diffuse
157 forebrain localisation, 4 dogs (36.4%) with a multifocal localisation, 4 dogs (36.4%) with a central vestibular
158 system localisation and 1 dog (9.1%) with focal signs affecting the left trigeminal and left facial nerves (Table 1).

159

160 *Diagnostic findings*

161 One dog had a moderate neutrophilia 24.5 x 10^9/l (reference interval: 3.0 x 10^9/l - 11.5 x 10^9/l) suspected to
162 be a result of chronic steroid treatment for presumed otitis interna/media. White blood cell counts of the remaining
163 10 dogs were within normal limits (reference interval: 6-17.1 x 10^9/l). Nine dogs (81.8%) had a peripheral
164 eosinophilia with a median cell number of 1.60 x 10^9/l (Ranging from: 0.2 x 10^9/l – 3.81 x 10^9/l, reference
165 interval 0.0 x 10^9/l – 1.3 x 10^9/l), with all dogs having an increased proportion of eosinophils in the white blood
166 cell count (Table 1). Cisternal CSF analysis demonstrated an eosinophilic pleocytosis in all dogs with a median
167 TNCC of 671 WBC/mm^3 (Ranging from: 6–5400 WBC/mm^3, reference interval <5 WBC/mm^3). The median
168 differential cell count included 85% eosinophils (Ranging from: 12% - 95%, reference interval <1%). CSF median
169 TP concentration was 0.61 mg/dl (Ranging from: 0.23-1.06 mg/dl, reference interval <25mg/dl). There was a
170 significant positive correlation between eosinophil percentage in the CBC and eosinophil percentage in the CSF

171 (r = 0.683, p = 0.01). There was also a strong positive correlation between eosinophil numbers on CBC and the
172 TNCC (r = 0.618, p = 0.032).
173
174 Serology and/or PCR analysis of CSF for *Toxoplasma gondii*, *Neospora caninum* were available and negative in
175 11 dogs (100%). PCR of CSF for canine distemper virus was available and negative in three dogs (27.3%). Five
176 dogs (45.5%) tested negative for *Angiostrongylus vasorum*, *Ehrlichia canis, Borrelia burgdorferi, Anaplasma*
177 *phagocytophilum* and *Anaplasma platys* with SNAP 4Dx ELISA™ (Idexx), serology for *Cryptococcus spp.* was
178 negative in 3 dogs (27.3%). A further 8 dogs (72.7%) were negative for *Angiostrongylus vasorum* by Angio
179 Detect™ (Idexx) and faecal analysis was normal for 3 dogs (27.3%). All 11 dogs tested negative for
180 *Angiostongylus vasorum* by one or more tests.
181
182 *MRI findings*
183 MR imaging was performed between 3 and 40 hours after admission. In 10 dogs (90.9%) MR images
184 demonstrated a T1W iso/hypointense to normal grey matter, T2W and FLAIR hyperintense signal affecting the
185 cortical grey matter (Fig 1 a,b,c). Cerebral sulci appeared grossly enlarged with a reduction in the size of the
186 cortical gyri (Fig 1a,c). In all 10 dogs the lesions were bilateral and symmetrical and confined to the cerebral
187 cortex on MR images. T1W images retrieved after gadolinium contrast administration revealed diffuse contrast
188 uptake affecting both the pachymeninges and leptomeninges in 10 dogs (Fig 1d, Table 1). Abnormalities detected
189 in the MRI studies were felt to be most consistent with a diffuse meningitis and atrophy or necrosis of the cortical
190 grey matter. In the remaining dog there was marked masticatory muscle atrophy with regions of T2W and FLAIR

hyperintensity within the masseter and temporalis muscles which also showed moderate contrast uptake (Fig 2). The left trigeminal nerve was T2W and FLAIR hyperintense compared to the right side and showed mild homogenous contrast enhancement (Fig 2). There was also a left mandibular lymphadenopathy. Significant MRI abnormalities could not be detected in the left facial nerve.

Treatment

All dogs survived initial general anaesthesia for MR imaging, whereafter 10 dogs (90.9%) received a single IV dose of dexamethasone (0.3–0.5 mg/kg) within hours of diagnosis. In 8 dogs (72.7%) initial dexamethasone treatment was followed by high dose oral prednisolone therapy (4 mg/kg/day) that was reduced after 48 hours (2 mg/kg/day). In 2 dogs the initial starting dose of oral prednisolone was 2mg/kg/day and was reduced to 1mg/kg/day after 48 hours. In 1 dog the initial oral prednisolone dose was 1mg/kg/day until results for *Cryptococcus* were returned negative, at which time the oral prednisolone dose was increased to 2mg/kg/day for 6 weeks before dose reduction to 1mg/kg/day. The final dog did not receive steroid therapy and died the day after diagnosis (Case 11, Table 1). Dogs typically remained on the same dose of oral prednisolone for 3 weeks after which the dose was reduced by 50% depending on clinical progression. At the time of diagnosis 7 dogs (63.6%) received additional treatment with cytosine arabinoside, given as subcutaneous injections (50 mg/m^2 SC every 12 hours for 2 consecutive days). Dogs that started cytosine arabinoside treatment received a second course 3 weeks after initial treatment and treatment intervals were then typically extended by a week at each presentation depending on clinical progression. In 8 dogs a 14-day course of clindamycin was also provided (11-14mg/kg BID) until results for protozoal infectious disease testing returned negative.

211

212 *Outcomes*

213 Ten dogs (90.9%) survived to discharge and one dog died of respiratory arrest the day after presentation (Table
214 1). One dog was euthanised as a result of disease progression at 31 days post-diagnosis (Table 1). Two dogs were
215 lost to follow-up at 60 days and 732 days, both were alive and considered to be neurologically improved at that
216 time. One of the dogs lost to follow-up was receiving oral prednisolone only (1.5mg/kg/day) whilst the other dog
217 was not receiving any immunosuppressive treatment (Table 1). Median survival time was 762 days (Ranging
218 from: 31 to 3631 days). At the time of data capture 7 dogs were alive and had full follow-up information. Three
219 dogs (42.8%) were considered to be neurologically normal and 3 dogs (42.8%) were considered improved but
220 with mild deficits in menace response. The dog with left sided facial and trigeminal nerve deficits initially
221 responded well to immunosuppressive treatment, but relapsed after the first prednisolone dose reduction at three
222 weeks requiring a return to 2mg/kg/day oral prednisolone after which time it continued to improve (Table 1). Of
223 the 7 dogs for which follow-up information was available 2 dogs were not receiving any immunosuppressive
224 therapy, 2 dogs were receiving only cytarabine arabinoside (50 mg/m^2 SC every 12 hours for 2 consecutive days)
225 at eight weekly intervals, 2 dogs were receiving a combination of oral prednisolone (1-2mg/kg/day) and cytarabine
226 arabinoside, 1 dog was receiving oral prednisolone only (0.5mg/kg, EOD) (Table 1). Case 5 had repeat MRI and
227 cisternal CSF sampling performed 1173 days after diagnosis at the owner's request. Neurological examination
228 was within normal limits although the dog was felt by the owner to be slower to respond to commands and often
229 seemed confused during normal daily life. CSF reported a TNCC of zero and a protein level of 0.13mg/dl
230 (reference interval <25mg/dl). MRI findings included worsening vetriculomegaly, further widening of the

subarachnoid space, a reduction in size of the interthalamic adhesion and increased T2W hyperintense signal surrounding the cortical grey matter (Fig 3). These findings were felt to be consistent with a worsening diffuse meningitis and atrophy or necrosis of the cortical grey matter.

Discussion

Eosinophilic MUO has been sporadically reported in dogs and to date no clear consensus has been reached on the clinical presentation and MRI findings, making effective diagnosis and treatment challenging (Bennett *et al.* 1997; Salvadori *et al.* 2007; Smith-Maxie *et al.* 2008; Williams *et al.* 2008; Henke *et al.* 2009; Windsor *et al.* 2009; Granger *et al.* 2010; Olivier *et al.* 2010). The aim of this study was to better describe the clinical presentation, diagnostic test results, MRI findings and treatment outcomes of dogs with eosinophilic MUO without evidence of infectious disease.

Eosinophils are secretory cells that provide a host defense against parasites but also have the potential to modulate inflammatory responses by producing cytokines and can contribute to chronic inflammation in a wide range of tissues (Lilliehook *et al.* 2000). Eosinophils differentiate from myeloid precursor cells and enter the circulation after maturation where they last for only minutes to hours and are rapidly recruited in response to chemokines into target tissues or inflammatory sites where they can survive for up to two weeks (Young *et al.* 2006). Eosinophils release neurotoxic proteins, such as eosinophilic cationic protein, major basic protein, and eosinophil-derived neurotoxin and also produce reactive oxygen and nitrogen metabolites causing severe tissue damage (Oliveira & Lukacs 2003; Temkin *et al.* 2004). In the brain, neurons and myelinated axons are highly susceptible to eosinophilic-induced neurotoxicity (Williams *et al.* 2008).

Consistent with previous reports the most common neurological examination findings included one or more of the following: mentation changes, seizures, proprioceptive deficits, visual deficits and reduced or absent menace

255 response consistent with a diffuse forebrain neuroanatomic localisation (Bennett *et al.* 1997; Salvadori *et al.* 2007;
256 Smith-Maxie *et al.* 2008). Central vestibular system lesions have only previously been reported in one case of
257 eosinophilic MUO, however in the current study four dogs showed neurological signs consistent with a central
258 vestibular system localisation, including head tilt, nystagmus, mentation changes and vestibular ataxia (Henke *et*
259 *al.* 2009). Although no lesions in the central vestibular system were detected on MR imaging in the current study,
260 previously reported histopathological findings demonstrated an eosinophilic infiltrate throughout the brainstem
261 and cerebellum which could account for the central vestibular system signs observed (Olivier *et al.* 2010). Nine
262 dogs (81.8%) had a peripheral eosinophilia and all dogs had an increased proportion of eosinophils in the CBC
263 (Table 1). This compares with previous reports where 51% of dogs had a peripheral eosinophilia. CSF analysis in
264 all dogs showed an eosinophilic pleocytosis and increased TP concentration consistent with previous reports. Case
265 2 is notable in that although all values were within the inclusion criteria the TNCC and differential cell count were
266 lower than other cases (Table 1). This dog is unique in that it had received a 90 day tapering dose of corticosteroids
267 prior to diagnosis and it is believed that the low TNCC, in comparison to the other cases, is reflective of a response
268 to immunosuppressive corticosteroid treatment.
269
270 MRI findings have previously been reported in 16 dogs with presumed eosinophilic meningoencephalitis of
271 unknown origin and were considered normal in 6 dogs (37.5%). Two dogs (12.5%) had bilateral, symmetrical
272 cortical atrophy and diffuse meningeal contrast uptake (Salvadori *et al.* 2007; Henke *et al.* 2009; Windsor *et al.*
273 2009). In the current study 10 dogs (90.9%) demonstrated a T2W and FLAIR hyperintense signal affecting the
274 cortical grey matter, T1W iso/hypointense to normal grey matter, with enlarged cerebral sulci, a reduction in

cortical gyri and diffuse meningeal contrast uptake (Fig 1). These MRI findings are identical to previous case reports where the accompanying histopathology was consistent with a severe eosinophilic and macrophagic inflammation resulting in a diffuse meningitis and atrophy or necrosis of the cortical grey matter (Salvadori *et al.* 2007; Henke *et al.* 2009; Windsor *et al.* 2009). Based on the histopathological findings reported by other authors and MRI findings in the current study it is suggested that the majority of dogs with eosinophilic MUO suffer from a diffuse cerebrocortical meningitis and cortical (polio)encephalitis which can be clearly identified on MRI (Smith-Maxie *et al.* 2008; Williams *et al.* 2008; Henke *et al.* 2009; Olivier *et al.* 2010). Although no histopathology was available for dogs in the current study it is hypothesized that MRI findings are due to migration of eosinophils via the meningeal vasculature into the meninges and cerebral cortex leading to an inflammatory response and neuronal necrosis in the cerebral cortex (Bennett *et al.* 1997; Smith-Maxie *et al.* 2008;). In other forms of non-infectious meningoencephalitides (granulomatous meningoencephalitis (GME), necrotising meningoencephalitis (NME), necrotising leukoencephalitis (NLE)) lesions reflect the neuropathologies associated with each disorder often with significant overlap between conditions (Talarico 2010). Consequently MR images are rarely pathognomonic with variables such as the presence or absence of necrosis, topographical distribution, mass effect and the level of meningeal contrast uptake allowing some differentiation between diseases (Coates 2014, Talarico 2010). GME, NLE and NME frequently produce multifocal, asymmetrical, intraaxial lesions that variably affect grey and white matter. This is in contrast to MR images from the current study in which lesions are most often bilateral, symmetrical, limited to the cerebral cortex and associated with diffuse meningeal contrast uptake (Coates 2014, Talarico 2010). Other potential differential diagnoses for the bilateral cortical changes noted on MRI could include storage diseases such as neuronal ceroid lipofuscinosis (Nakamoto *et al.* 2011), canine

295 cognitive dysfunction (Hasegawa *et al.* 2005) or cerebrocortical necrosis secondary to metabolic or nutritional
296 abnormalities (Singh *et al.* 2005, Dewey & da Costa 2016). However, the signalment of dogs in the current study,
297 normal biochemical test results, CSF analysis demonstrating an eosinophilic pleocytosis and the specific nature
298 of the MRI findings were not felt to be consistent with alternate differential diagnoses.
299
300 The final dog in the current study had marked masticatory muscle atrophy, left facial nerve and trigeminal nerve
301 dysfunction and an enlarged and contrast enhancing left trigeminal nerve on MRI (Table 1, Fig 3). There were
302 also T2W and FLAIR hyperintense lesions within the muscle and mild patchy contrast uptake post-gadolinium
303 administration on T1W images. Focal involvement of cranial nerves in dogs with eosinophilic MUO has
304 previously been reported in a 4-year-old Golden Retriever and a 13-year-old German Shepherd Dog. As in the
305 current study both dogs reported in the literature were the oldest in their respective studies (Smith-Maxie *et al.*
306 2008; Windsor *et al.* 2009). Atrophy and MRI changes of masticatory muscles have been reported with conditions
307 causing a focal myositis or neuritis affecting the trigeminal nerve including immune-mediated masticatory
308 myositis, myositis or neuritis as a result of *Neospora caninum*, trauma, or neoplasms. Serum biochemistry and
309 tests for infectious agents were negative making an infectious aetiology less likely. Further diagnostic tests that
310 could have been performed to further exclude neoplastic or inflammatory/infectious differentials include muscle
311 biopsy and histopathology, 2M antibody testing and fine needle aspirates of muscle or nerve (Cauduro *et al.* 2013;
312 Dewey & da Costa 2016).
313
314 Due to the small sample size and lack of histopathology it is challenging to draw robust conclusions on outcomes

315 for dogs diagnosed with eosinophilic MUO. However in the current study the outcome is overall good, with over
316 90% of dogs surviving to discharge and 75% of dogs, for which long-term follow-up was available, considered
317 neurologically normal or markedly improved. It should be noted that for two dogs included in the survival analysis
318 the follow-up time from diagnosis was 60 days or less (Case 3, Case 8) which may potentially bias the analysis
319 of median survival time and highlights the need for a larger sample size in studies of this type. One dog died
320 because of disease progression and a further two were euthanised as a result of persistent clinical signs which is
321 similar to previous reports where 25% of dogs with eosinophilic MUO died or were euthanized because of the
322 condition (Windsor *et al.* 2009). Only two of the 7 dogs for which follow-up was available were not receiving any
323 treatment whilst the remaining 5 dogs received long-term low doses of prednisolone, cytarabine arabinoside or a
324 combination of the two. While it is challenging to make direct comparisons with treatment protocols used in other
325 forms of MUO the outcome data in this study compare favorably to other non-infectious meningoencephalitides
326 where mortality rates are as high as 26% in the first week following diagnosis (Cornelis, Volk & De Decker 2016;
327 Cornelis *et al.* 2016). The good response to long-term immunosuppressive drugs observed in many dogs with
328 eosinophilic MUO, and no evidence of infectious disease, further supports an underlying immune-mediated
329 condition that may be affecting cortical neurons (Windsor *et al.* 2009; Talarico & Schatzberg 2010). It is of note
330 that in the single dog that had follow up MRI 1173 days after diagnosis the MRI findings had shown a significant
331 progression, including worsening cortical atrophy and ventriculomegaly, despite a normal CSF sample (Fig 4).
332 This suggests that the cortical damage caused by eosinophilic MUO is permanent and potentially progressive
333 despite effective treatment of the disease as determined by CSF sampling.
334

This study is limited by its retrospective nature and the small number of included cases preventing robust conclusions being drawn on outcomes and prognostic indicators for dogs with eosinophilic MUO. To date no dogs have been available for post-mortem examination due to the small number of cases and the relatively favourable outcomes following immunosuppressive treatment. A key area of future work is to obtain histopathology to corroborate MRI findings and gain a better understanding of the underlying pathology of eosinophilic MUO.

To the best of the authors' knowledge, this is the first study describing clinical, imaging and outcome data for a series of dogs with eosinophilic MUO. The majority (83%) of eosinophilic MUO cases present as a severe diffuse cerebrocortical meningitis and cortical encephalitis which can be clearly identified on MRI. Seventy five percent of dogs in this study have a favorable response to immunosuppressive therapy in the medium to long term. These distinct MRI findings may serve as an aid in the diagnosis of eosinophilic MUO in dogs.

Figure Legends:

Fig 1. Dog 7: Transverse magnetic resonance images at the level of the interthalamic adhesion (a,b,c,d) showing a T2W hyperintense (a) to grey matter, FLAIR hyperintense (b) and T1W iso/hypointense (c) signal bilaterally affecting the sub-meningeal cortical grey matter (white asterisks). Cerebral sulci appeared grossly enlarged with a reduction in the size of the cortical gyri (a,c). Post-gadolinium contrast administration there was moderate diffuse contrast uptake affecting both the pachymeninges and leptomeninges (c,d) (white arrows). Mild ventricular enlargement is also evident (black asterisk).

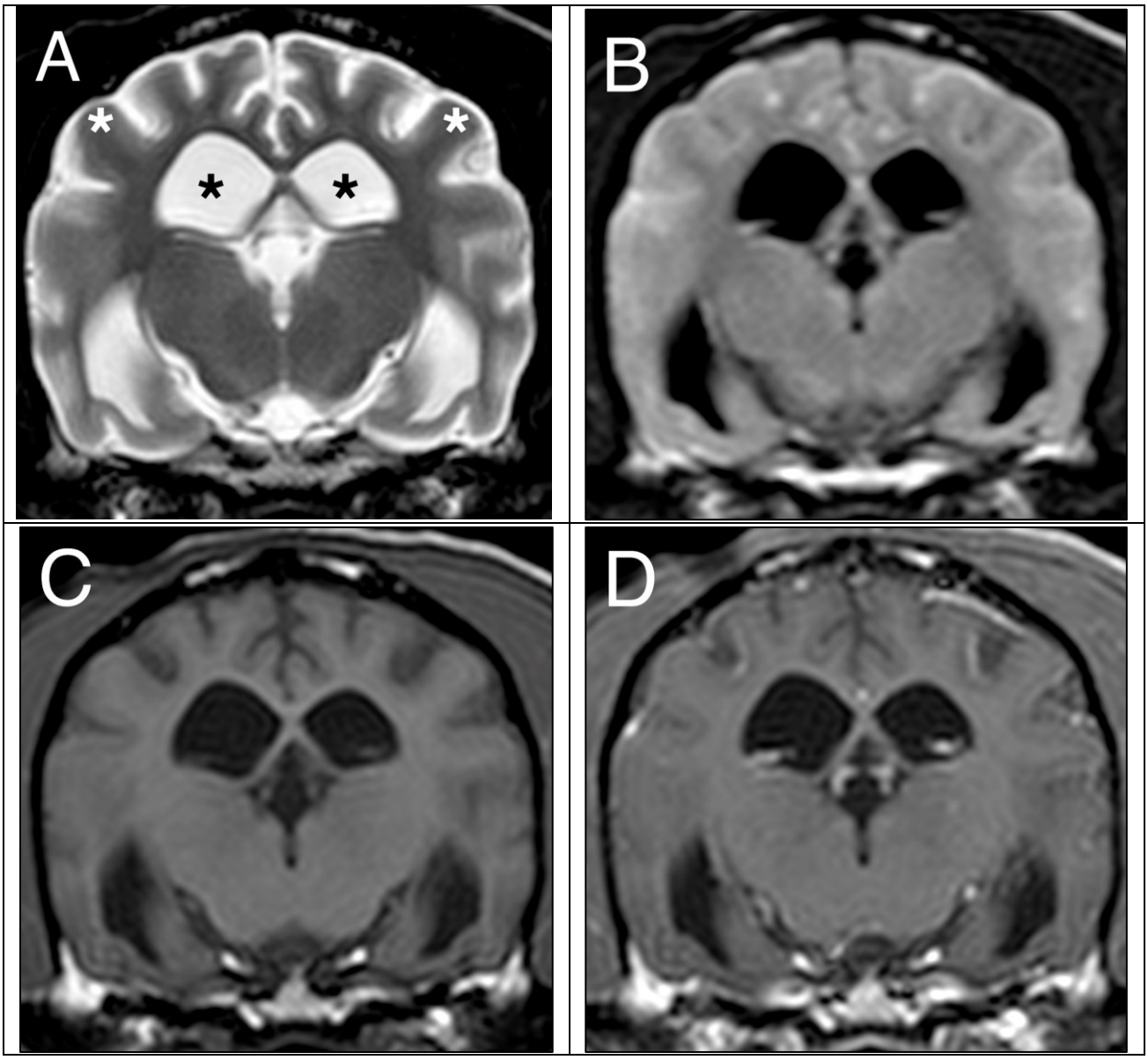
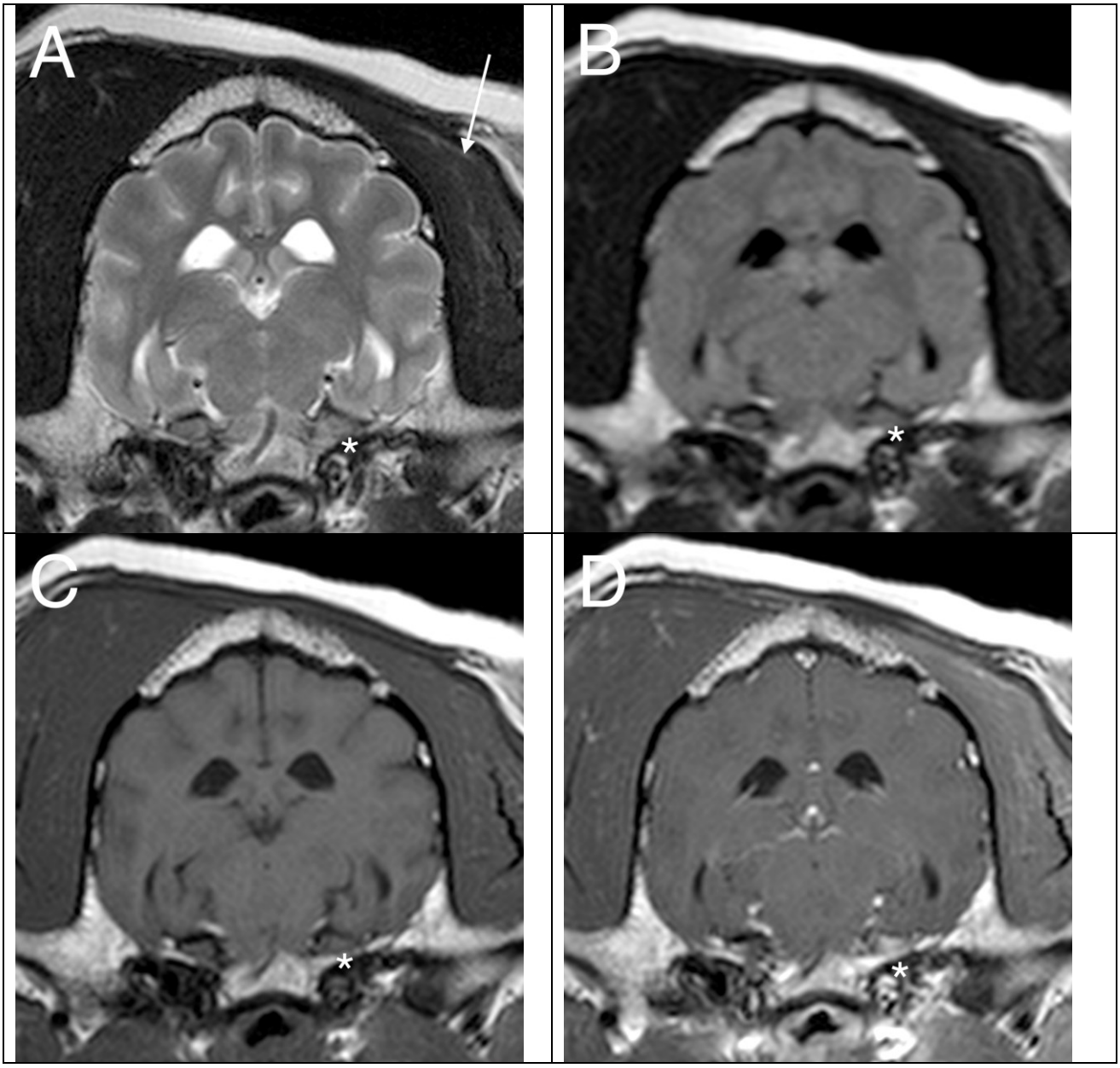


Fig 2. Dog 9: Transverse magnetic resonance images at the level of the thalamus (a,b,c,d) showing masticatory muscle atrophy (white arrow) with regions of T2W and FLAIR hyperintensity within the masseter and temporalis muscles which showed moderate contrast uptake. The left trigeminal nerve was T2W and FLAIR hyperintense compared to the right side, subjectively enlarged and showed mild homogenous contrast enhancement (white asterisk).



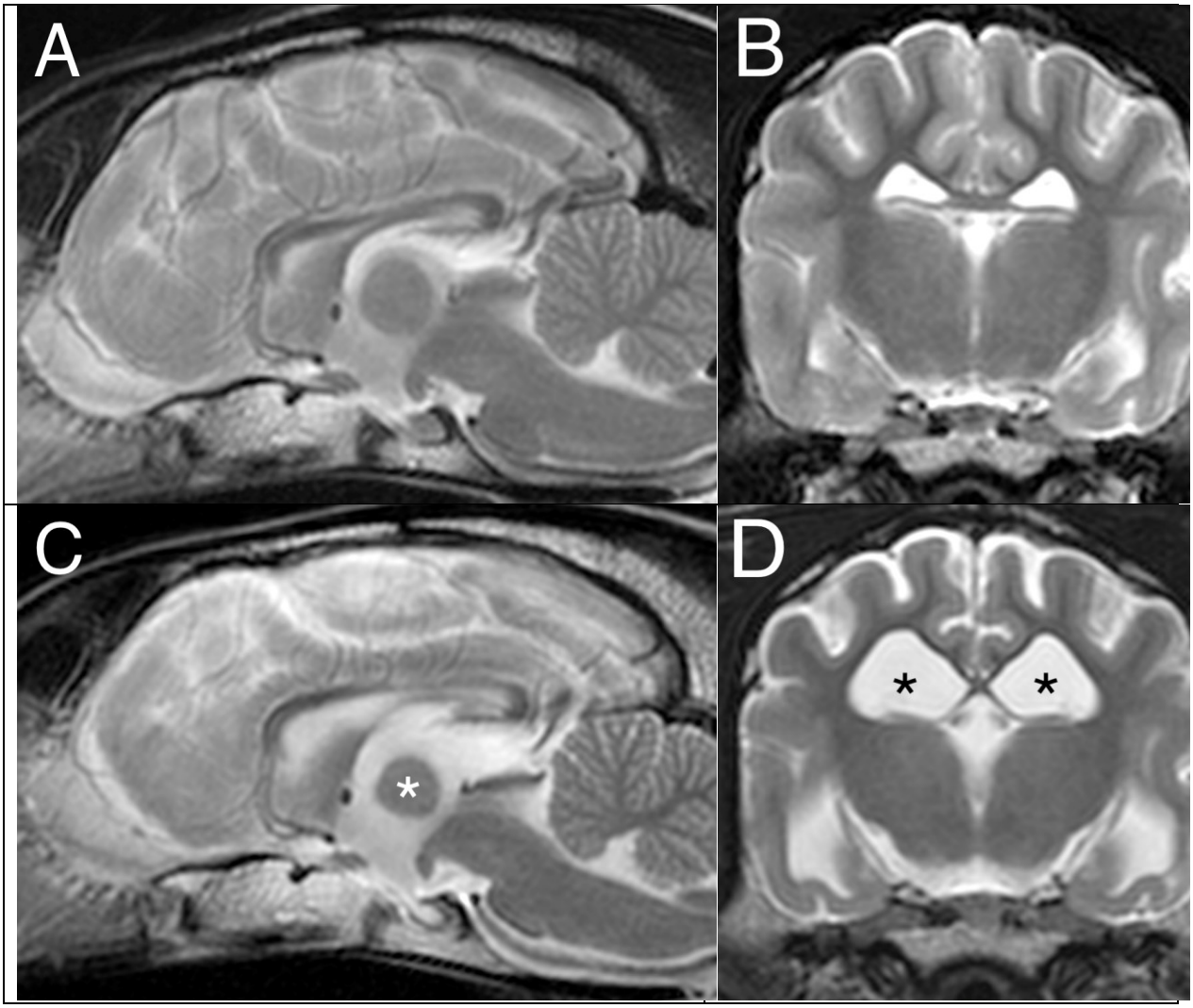
362 Fig 3. Dog 6: Sagittal midline (a, c) and transverse magnetic resonance images at the level of the thalamus (b, d)

363 demonstrating increased T2W hyperintense to normal grey matter signal bilaterally affecting the sub-meningeal

364 cortical grey matter, increased ventriculomegaly (black asterisk) and a reduction in size of the interthalamic

365 adhesion (white asterisk) when images at 1173 days after diagnosis (c, d) are compared to those taken at the time

366 of diagnosis (a, b).



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Table 1: Summary of clinical presentation, MRI findings and treatment outcomes of dogs diagnosed with suspected eosinophilic meningoencephalitis of unknown origin

No.	Breed	Weight (kg)	Age (mths)	Sex / Neuter	TTP (days)	Previous treatment	Neurological examination (abnormal findings stated)	Neuro-anatomical localisation	WBC (x10^9/l)	Blood Eos (x10^9/l) (%)	CSF TNCC (mm3) (%)	CSF Protein (mg/dl)	MRI findings	Treatment (at time of data capture)[b]	Outcome (survival since diagnosis)
1	Flat-Coated Retriever	31.0	34	M	24	None	Obtunded, reduced paw positioning pelvic limbs, reduced menace response (OU), blind.	Diffuse forebrain	10.90	2.07 (19%)	222 (78%)	0.33	T2W and FLAIR hyperintense signal affecting cortical grey matter Cerebral sulci enlarged Meningeal contrast uptake	Prednisolone 2mg/kg/day	Euthanised (remained blind) (31 days)
2	Jack Russell Terrier	8.9	25	M	78	Prednisolone (Tapering to 0.35mg/kg PO SID, 90 days)	Obtunded, reduced paw positioning all limbs, reduced menace response (OU), reduced PLR (OU), generalised ataxia, blind	Multifocal brain	27.10	0.20 (2%)	6 (12%)	0.21	T2W and FLAIR hyperintense signal affecting cortical grey matter Cerebral sulci enlarged Meningeal contrast uptake	None	Alive (3631 days)
3	Rottweiler	36.0	12	M	14	None	Obtunded, reduced menace response (OU), reduced PLR (OU), generalised ataxia, cervical hyperaesthesia, blind	Multifocal brain	9.00	2.07 (23%)	1,005 (90%)	0.73	T2W and FLAIR hyperintense signal affecting cortical grey matter Cerebral sulci enlarged Meningeal contrast uptake	Prednisolone 1.5mg/kg/day	Lost to follow up at 60 days
4	Bichon Frise	9.1	23	MN	8	None	Obtunded, vestibular ataxia (falling to left), positional vertical nystagmus	Central vestibular system	15.20	3.81 (25%)	1,839 (93%)	0.97	T2W and FLAIR hyperintense signal affecting cortical grey matter Cerebral sulci enlarged Meningeal contrast uptake	None	Alive (1642 days)
5	Welsh Terrier	7.7	11	F	32	None	Seizures, obtunded, absent paw positioning pelvic limbs, absent menace response (OU), vestibular ataxia (falling to right)	Central vestibular system	11.09	0.91 (12%)	542 (76%)	0.52	T2W and FLAIR hyperintense signal affecting cortical grey matter Cerebral sulci enlarged Meningeal contrast uptake	Cytarabine arabinoside: every 8 weeks	Alive (1173 days)
6	Welsh Terrier	10.3	17	M	8	None	Obtunded, reduced paw positioning all limbs, reduced menace response (OU), generalised ataxia, cervical hyperaesthesia	Multifocal brain	12.81	1.55 (10%)	837 (85%)	0.38	T2W and FLAIR hyperintense signal affecting cortical grey matter Cerebral sulci enlarged Meningeal contrast uptake	Cytarabine arabinoside: every 8 weeks	Alive (820 days)
7	Cross breed	4.9	18	FS	36	None	Obtunded, absent paw positioning all limbs, absent menace response (OU), vestibular ataxia, positional vertical nystagmus, cervical hyperaesthesia, blind	Central vestibular system	7.36	1.41 (15%)	16 (80%)	0.15	T2W and FLAIR hyperintense signal affecting cortical grey matter Cerebral sulci enlarged Meningeal contrast uptake	Prednisolone 0.5mg/kg every other day.	Alive (214 days)
8	Kerry Blue Terrier	22.0	99	MN	44	Prednisolone (0.5mg/kg/day, 34 days. Stopped 8 days prior to presentation)	Absent corneal and facial sensation (left), absent palpebral reflex (left), left masticatory muscle atrophy	Left trigeminal and facial nerves (or their nuclei)	11.41	2.17 (19%)	5,400 (91%)	1.06	Left masticatory muscle atrophy with moderate contrast uptake Enlarged left trigeminal nerve with contrast uptake	Prednisolone 2mg/kg/day Cytarabine arabinoside: every 3 weeks	Alive (57 days)
9	Boxer	26.0	26	F	62	None	Obtunded, reduced menace response (OU), generalised ataxia, cervical hyperaesthesia	Multifocal brain	7.33	1.39 (19%)	4,960 (86%)	0.93	T2W and FLAIR hyperintense signal affecting cortical grey matter Cerebral sulci enlarged Meningeal contrast uptake	Prednisolone 1mg/kg/day Cytarabine arabinoside: every 3 weeks	Alive (120 days)

10[a]	Flat-Coated Retriever	32.4	22	F	23	None	Seizures, obtunded, reduced paw positioning pelvic limbs, absent menace response (OU), blind	Diffuse forebrain	12.09	1.62 (14%)	451 (95%)	0.7	T2W and FLAIR hyperintense signal affecting cortical grey matter Cerebral sulci enlarged Meningeal contrast uptake	None	Lost to follow-up 732 days
11[a]	Belgian Shepherd Dog (Tervueren)	18.0	7	F	2	Prednisolone (0.9mg/kg/day, 1 day)	Obtunded, absent paw positioning all limbs, absent menace response (OU), ataxia, positional vertical nystagmus, blind.	Central vestibular system	13.60	1.73 (13%)	671 (85%)	0.76	T2W and FLAIR hyperintense signal affecting cortical grey matter Cerebral sulci enlarged Meningeal contrast uptake	N/A	Died (1 day)

[a] Ghent University

[b] all Cytarabine arabinoside doses: 50 mg/m^2 SC every 12 h for 2 consecutive days

BID: Twice daily, Eos: Eosinophils, F: Female entire, FS: Female spayed, IHC: Immunohistochemistry, M: male entire, MN: Male neutered, N/A: Not available, OU: Oculus uterque, PLR: pupillary light reflex, TID: every 8 hours, TNCC: Total nucleated cell count, TTP: time to presentation (time from clinical signs first being noticed by owner to diagnosis), WNL: Within normal limits.

368 **References**

369 Bennett, P.F. *et al.*, 1997. Idiopathic eosinophilic meningoencephalitis in rottweiler dogs: three cases (1992-
370 1997). *Australian Veterinary Journal*, 75(11), pp.786–789.

371 Bosch, I. & Oehmichen, M., 1978. Eosinophilic granulocytes in cerebrospinal fluid: Analysis of 94
372 cerebrospinal fluid specimens and review of the literature. *Journal of Neurology*, 219(2), pp.93–105.

373 Cardy, T.J.A. *et al.*, 2015. Clinical reasoning in canine spinal disease: what combination of clinical information
374 is useful? *Veterinary Record*, 177(7), pp.171–171.

375 Cauduro, A. *et al.*, 2013. Use of MRI for the early diagnosis of masticatory muscle myositis. *Journal of the*
376 *American Animal Hospital Association*, 49(5), pp.347–352.

377 Coates JR, Jeffery ND., 2014 Perspectives on meningoencephalomyelitis of unknown origin. *Vet Clin North Am*
378 *Small Anim Pract*, 44. pp.1157–1185.

379 Cornelis, I., Volk, H.A. & De Decker, S., 2016. Clinical presentation, diagnostic findings and long-term
380 survival in large breed dogs with meningoencephalitis of unknown aetiology. *Veterinary Record*, 179(6),
381 pp.147–147.

382 Cornelis, I., Volk, H.A., Van Ham, L., *et al.*, 2016. Prognostic factors for 1-week survival in dogs diagnosed
383 with meningoencephalitis of unknown aetiology. *The Veterinary Journal,* 214, pp.91–95.

384 Dewey, C. W., & da Costa, R. C. (2016) Practical Guide to Canine and Feline Neurology. 3rd edn. Eds C. W.
385 Dewey and R. C. da Costa. Wiley Blackwell, West Sussex, UK. pp 64-65

386 Di Terlizzi, R. & Platt, S.R., 2009. The function, composition and analysis of cerebrospinal fluid in companion
387 animals: part II - analysis. *Veterinary journal,* 180(1), pp.15–32.

388 Dorta-Contreras, A.J. & Reiber, H., 1998. Intrathecal synthesis of immunoglobulins in eosinophilic
389 meningoencephalitis due to Angiostrongylus cantonensis. *Clinical and Diagnostic Laboratory Immunology*,
390 5(4), pp.452–455.

391 Graeff-Teixeira, C., da Silva, A.C.A. & Yoshimura, K., 2009. Update on eosinophilic meningoencephalitis and
392 its clinical relevance. *Clinical microbiology reviews*, 22(2), pp.322–48.

393 Granger, N., Smith, P.M. & Jeffery, N.D., 2010. Clinical findings and treatment of non-infectious
394 meningoencephalomyelitis in dogs: A systematic review of 457 published cases from 1962 to 2008. *The*
395 *Veterinary Journal*, 184(3), pp.290–297.

396 Hasegawa, D. *et al.*, 2005. Measurement of interthalamic adhesion thickness as a criteria for brain atrophy in
397 dogs with and without cognitive dysfunction (dementia). *Veterinary Radiology & Ultrasound*, 46(6), pp.452–
398 457.

399 Henke, D. et al., 2009. Eosinophilic granulomatous meningoencephalitis in 2 young Belgian Tervueren
400 Shepherd dogs. *Journal of Veterinary Internal Medicine*, 23(1), pp.206–210.

401 Kuberski T. 1981. Eosinophils in cerebrospinal fluid: criteria for eosinophilic meningitis. *Hawaii Med J,* 40,
402 *pp.*97-98

403 Lilliehook, I. *et al.*, 2000. Diseases associated with pronounced eosinophilia: a study of 105 dogs in Sweden.
404 *The Journal of small animal practice*, 41(6), pp.248–253.

405 Lowrie, M., Smith, P.M. & Garosi, L., 2013. Meningoencephalitis of unknown origin: investigation of
406 prognostic factors and outcome using a standard treatment protocol. *Veterinary Record*, 172(20), pp.527–527.

407 Nakamoto, Y. *et al.*, 2011. Neuronal ceroid-lipofuscinosis in longhaired Chihuahuas: clinical, pathologic, and
408 MRI findings. *Journal of the American Animal Hospital Association*, 47(4), pp.e64–70.

409 Oliveira, S.H.P. & Lukacs, N.W., 2003. The role of chemokines and chemokine receptors in eosinophil
410 activation during inflammatory allergic reactions. *Brazilian Journal of Medical and Biological Research*,
411 36(11), pp.1455–1463.

412 Olivier, A.K. *et al.*, 2010. Idiopathic eosinophilic meningoencephalomyelitis in a Rottweiler dog. *Journal of*
413 *Veterinary Diagnostic Investigation*, 22(4), pp.646–648.

414 Salvadori, C. et al., 2007. Magnetic resonance imaging and pathological findings in a case of canine idiopathic
415 eosinophilic meningoencephalitis. *Journal of Small Animal Practice*, 48(8), pp.466–469.

416 Singh, M. *et al.*, 2005. Thiamine deficiency in dogs due to the feeding of sulphite preserved meat. *Australian*
417 *Veterinary Journal*, 83(7), pp.412–417.

418 Smith-Maxie, L.L. *et al.*, 2008. Cerebrospinal fluid analysis and clinical outcome of eight dogs with
419 eosinophilic meningoencephalomyelitis. *Journal of Veterinary Internal Medicine*, 3(3), pp.167–174.

420 Talarico, L.R. & Schatzberg, S.J., 2010. Idiopathic granulomatous and necrotising inflammatory disorders of the
421 canine central nervous system: a review and future perspectives. *Journal of Small Animal Practice*, 51(3),
422 pp.138–149.

423 Temkin, V. *et al.*, 2004. Eosinophil major basic protein: first identified natural heparanase-inhibiting protein.
424 J*ournal of Allergy and Clinical Immunology*, 113, 703-709

425 Williams, J.H. *et al.*, 2008. Review of idiopathic eosinophilic meningitis in dogs and cats, with a detailed
426 description of two recent cases in dogs. *Journal of the South African Veterinary Association*, 79(4), pp.194–204.

427 Windsor, R.C. *et al.*, 2009. Cerebrospinal Fluid Eosinophilia in Dogs. *Journal of Veterinary Internal Medicine*,
428 23(2), pp.275–281.

429 Young, B., ODowd, G. & Woodford, P., 2006. Blood. In *Wheater's Functional Histology*. Elsevier Health
430 Sciences, pp. 46–64.

431