MANUSCRIPT

Structured summary

Objectives - Although difficult to clinically diagnose, presumptive meningomyelitis of unknown origin (MMUO) is an important differential diagnosis for dogs presenting with signs of spinal cord dysfunction. The aim of this study was to evaluate clinical presentation, diagnostic findings and long-term outcome for dogs clinically diagnosed with MMUO.

Methods - Medical records were reviewed for dogs diagnosed with presumptive MMUO between 2006 and 2015.

Results - Twenty-one dogs met the inclusion criteria. The majority of dogs presented with an acute (43%) or chronic (52%) onset of neurological signs. Ambulatory paresis was the most common neurological presentation (67%). Neurological examination most commonly revealed a T3-L3 myelopathy, and spinal hyperaesthesia was a common finding (71%). A spinal cord lesion was visible in 90% of cases on MR imaging. Lesions were typically extensive, ill-defined, hyperintense on T2-weighted and isointense on T1-weighted images. Eighteen lesions (86%) showed parenchymal contrast enhancement and 17 lesions (81%) showed contrast enhancement of overlying meninges. All dogs were treated with immunosuppressive doses of glucocorticosteroids, sometimes combined with cytosine arabinoside. At time of data capture, 10/21 dogs (48%) had died or been euthanized because of MMUO. Overall median survival time was 669 days.

Impact - MMUO should be considered in the differential diagnosis of dogs presenting with an acute or chronic, progressive, and potentially painful myelopathy.

MRI features can possible help to distinguish presumptive MMUO from other more common spinal diseases. Overall, long-term survival is guarded, approximately 50% of dogs will die or be euthanized because of MMUO regardless of immunosuppressive treatment.

Keywords

MRI, inflammatory CNS disease, GME, cytosine arabinoside, glucocorticosteroids

Introduction

Pure myelitis (inflammation of spinal cord parenchyma) or meningomyelitis (inflammation of spinal cord parenchyma and surrounding meninges) are rare diseases in small animals and occur commonly in combination with inflammatory brain disease (Tipold and Stein 2010). Viruses (canine distemper virus, feline coronavirus), bacteria (*Staphylococcus* spp., *Streptococcus* spp., *Pasteurella,* coliforms, *Actinomyces, Nocardia* spp.), fungi (*Cryptococcus, Coccidioides* spp., *Blastomyces, Histoplasma*), rickettsiae (*Ehrlichia, Rickettsia,* Rocky Mountain spotted fever), protozoa (*Toxoplasma gondii*, *Neospora caninum*), parasites (*Dirofilaria immitis, Cuterebra*, *Angiostrongylus vasorum*) and algae (*Prototheca wickerhamii, Prototheca zopfii*) are known causes for meningomyelitis in dogs and cats, with or without concurrent intracranial signs (Dewey 2016; Csebi *et al.* 2010; Parry *et al.* 2009; Griffin *et al.* 2008). Apart from infectious causes, non-infectious meningomyelitis including granulomatous meningoencephalomyelitis, pyogranulomatous meningoencephalomyelitis and steroid-responsive meningitis-arteritis (SRMA) are described (Dewey 2016; Parry *et al*. 2009; Griffin *et al.* 2008; Meric 1988). In agreement with the terminology for meningoencephalitis of unknown origin (MUO), dogs clinically diagnosed with non-infectious inflammatory myelitis that did not have positive infectious disease testing, that were not classified as SRMA or eosinophilic meningomyelitis, and that were not histopathologically confirmed, were named meningomyelitis of unknown origin (MMUO). A clinical diagnosis of MMUO is typically made by a combination of clinical presentation, imaging of the vertebral column, and results of cerebrospinal fluid (CSF) analysis (Griffin *et al.* 2008).

Currently, only one previous study has focused specifically on the clinical presentation, diagnostic findings, and outcome in dogs with meningomyelitis caused by a variety of underlying aetiologies (Griffin *et al.* 2008). Twenty-eight cases were included, of which 15 dogs were diagnosed with MMUO. Clinical signs were reflected by the affected spinal cord segments, and younger dogs, toy breeds, and hound breeds were suggested to be predisposed for meningomyelitis. Although results of myelography, computed tomography (CT), and CT-myelography have been reported, little is known about magnetic resonance imaging (MRI) findings in dogs with MMUO. The aims of this study were therefore to describe the signalment, clinical presentation, diagnostic findings, including results of MRI, and long-term survival in dogs diagnosed with presumptive MMUO without concurrent clinical signs of intracranial (Deleted: It was hypothesized that dogs diagnosed with MMUO could be of any breed, gender or age, that they would present generally with severe neurological dysfunction but without spinal hyperaesthesia and that their long-term prognosis is guarded to good).

Materials and methods

Case selection

The electronic medical database was searched between March 2006 and February 2015 for dogs diagnosed with “MUA”, “MUO”, “GME”, “myelitis”, “inflammatory spinal cord disease”. Dogs were included based on the criteria used by Granger et al. (2010), if they had (1) complete medical records available, (2) a complete neurological examination performed leading to a spinal cord localisation, (3)

inflammatory CSF analysis, (4) MRI of the spinal cord, and if (5) long-term follow-up information was available through revision of medical records or through contacting the referring veterinarian by telephone. Dogs were excluded if (1) the clinical records or imaging studies were incomplete or not available for review, (2) dogs showed clinical or neurological signs of intracranial involvement at time of presentation, (3) they had a peracute onset of clinical signs that were not progressive after 12-24 hours, (4) they had signs of extradural or extradural/intramedullary spinal cord compression on MRI and if (5) they had positive infectious disease titres or if clinical presentation, CSF analysis or necropsy findings were suggestive of SRMA or eosinophilic meningoencephalomyelitis (>10% eosinophils in CSF) (Dewey 2016). Typical clinical presentation for SRMA was considered to be a dog less than 2 years of age of a typical dog breed (Boxer, Beagle, Bernese Mountain dog, Nova Scotia Duck Tolling Retriever, Golden Retriever, German Shorthaired Pointed) presenting with pyrexia and cervical hyperesthesia. CSF analysis in SRMA is typically revealing a predominantly neutrophilic pleocytosis (Dewey 2016). Dogs with histopathological confirmation of the disease (granulomatous meningo(encephalo)myelitis (GMEM) or necrotising meningo(encephalo)myelitis (NMEM)) only needed to fulfil inclusion criteria (1) and (5). Information retrieved from the medical records included breed, gender, age at diagnosis, body weight, results of neurological examination including neuroanatomical localisation, duration of clinical signs prior to diagnosis, results of complete blood count (CBC) and biochemistry profile, results of CSF analysis including total nucleated cell count (TNCC), white blood cell differentiation and total protein (TP) concentration, treatment received, and outcome. (Deleted: Based on body weight, dogs were divided in small/medium (<15kg) and large breed dogs (>15kg). For dog breeds in which the body weight varied around 15kg, mean body weight for

male and female dogs as reported on the Kennel Club website (http://www.thekennelclub.org.uk/services/public/breed/standard-find.aspx) were used to consider them large or medium/small breed dogs). Duration of clinical signs prior to diagnosis (days) was classified as peracute (<2 days), acute (2–7 days) or chronic (>7 days). For dogs that had CSF analysis performed, site of collection (cisternal or lumbar), TNCC, TP and cytological differentiation were recorded. Total nucleated cell count was considered normal if < 5 cells/mm^3. Protein concentration was considered normal for a cisternal collection if < 0,25 g/l and for a lumbar collection if < 0,4 g/l.

Neurological assessment

The neurological status was classified from 0 to 5 according to the clinical examination (adapted from Scott *et al.* 1997): grade 0 = neurologically normal; grade 1 = spinal hyperesthesia without neurological deficits; grade 2 = ataxia, ambulatory para- or tetraparesis; grade 3 = non- ambulatory para- or tetraparesis; grade 4 = para- or tetraplegia with or with- out bladder control, and intact deep pain sensation; grade 5 = para- or tetraplegia, urine retention or overflow, and deep pain sensation loss. Possible neuroanatomical localizations included C1-C5, C6-T2, T3-L3 or L4-S3 spinal cord segments. Dogs were diagnosed with a focal lesion if only one spinal cord segment was affected, and with a multifocal lesion if more then one spinal cord segment appeared to be affected on the neurological examination.

Magnetic resonance imaging

Magnetic resonance imaging was performed under general anaesthesia with a permanent 1.5T magnet (Intera, Philips Medical Systems, Eindhoven, the

Netherlands) and all images were reviewed by the corresponding author using Osirix Dicom viewer (Osirix Foundation, V.5.5.2 Geneva, Switzerland). Sequences could vary, but studies included a minimum of T2-weighted (T2W) (repetition time (ms) (TR)/echo time (ms) (TE), 3000/120) and T1-weighted (T1W) (TR/TE, 400/8) images of the affected spinal cord region in a sagittal and transverse plane. The T1W images were acquired before and after IV administration of paramagnetic contrast medium (0,1 mg/kg, gadoterate meglumine, Dotarem, Guerbet). If MR images of the brain were present, they were reviewed concurrently. Variables recorded were lesion intensity on T2W and T1W images, lesion localization and distribution, lesion length and presence of parenchymal and/or meningeal contrast enhancement. Lesion length was measured using Osirix Dicom viewer, and performed on sagittal T2W images for dogs that had focal lesions. Lesion length was measured twice, and the mean value between both was used. To compensate for differences in body size, values were corrected towards length of vertebral body of C6 (for cervical lesions) or L2 (for thoracolumbar lesions). Vertebral body length was measured on T1W sagittal images.

Treatment and follow-up

For all dogs, the specific treatment protocol was recorded. During hospitalisation, all dogs underwent daily at least one general physical and complete neurological examination by a board-certified neurologist or neurology resident. The results of the neurological examination as well as response to treatment (improvement, deterioration or static status) were systematically recorded on the kennel sheets. Follow-up information during hospitalisation was collected from the medical records, and afterwards through medical records of re-examination visits or telephone contact with the referring veterinarian. A successful outcome was defined as the dog being

ambulatory, fecal and urinary continent and, according to the owners, without signs of overt spinal hyperaesthesia. An unsuccessful outcome was defined as (1) deterioration in neurological status by one or more grades after diagnosis and treatment, or (2) if the dog was not independently ambulatory, possibly with previously non-existing or worsening fecal and/or urinary incontinence, or was experiencing spinal hyperaesthesia as defined by the owner.

Statistical analysis

Data analysis was performed with the aid of a standard statistical software package (Prism, Graphpad Software Inc). (Deleted: A Mann-Whitney *U* test was used to compare age, duration of clinical signs prior to diagnosis, and TNCC in CSF between small/medium and large breed dogs. A fisher's exact test was used to compare differences in sex and neuroanatomical localization between small/medium and large breed dogs.) Regarding outcome, a Mann-Whitney *U* test was used to evaluate effect of relative lesion length on long-term outcome. A fisher's exact test was used to evaluate the effect of pain, presence of lymphopenia and additional administration of cytosine arabinoside on outcome. Numeric variables were expressed as median and interquartile ranges (IQR). Values of $P<0.05$ were considered significant. Survival analysis was performed using both a Log-rank (Mantel-Cox) and Gehan-Breslow-Wilcoxin test, resulting in median survival time (MST) calculation and a Kaplan-Meier survival curve. (Deleted: comparing survival percentage in small/medium and large breed dogs, and presenting overall survival

One-way NOVA was used to evaluate significant differences between affected regions on neurological examination.) Survival was defined as time from diagnosis to death or euthanasia, including whether this happened because of disease progression

or due to unrelated causes, or time from diagnosis to data collection for dogs that were alive at time of data capture. Dogs that died because of unrelated causes and dogs that were still alive at time of data capture were censored for survival analysis.

Results

Signalment

Twenty-one dogs were included in the study. (Deleted: Thirteen dogs (62%) were large breeds and 8 dogs (38%) were considered small/medium breed dogs) Represented breeds included French Bulldog (n=2), Jack Russell Terrier (n=2), Lhasa Apso (n=2) and one each of Akita, Bearded Collie, Boxer, Bull Mastiff, Chihuahua, cross breed, English Springer Spaniel, Giant Schnauzer, Labrador Retriever, Maltese Terrier, Rhodesian Ridgeback, Rottweiler, Shih Tzu, West Highland White Terrier and Yorkshire Terrier. Overall, median age at presentation was 56 months (10 – 128 months). (Deleted: There was no significant difference in age at presentation between small/medium and large breed dogs (P=0.358).)Thirteen dogs (62%) were male and 8 dogs (38%) were female. Compared to the general hospital population between March 2006 and February 2015, there was no significant difference in sex distribution in the group of dogs with MMUO (P=0.075). Median duration of clinical signs prior to diagnosis was 8 days (ranging from 1-90 days). One dog (5%) presented with peracute, 9 dogs (43%) with acute and 11 dogs (52%) with a chronic onset of neurological signs.

Neurological examination

Thirteen (62%) and 8 (38%) dogs were diagnosed with a focal and multifocal spinal lesion on neurological examination, respectively. (Deleted: Small/medium breed dogs

presented significantly more with a focal spinal cord lesion on the neuroanatomical localisation compared to large breed dogs (P=0.049).) Regarding dogs with focal spinal lesions (n=13), 3 dogs were diagnosed with a lesion affecting the C1-C5 spinal cord segments, 2 dogs with a lesion affecting the C6-T2 spinal cord segments, 6 dogs with a lesion affecting the T3-L3 spinal cord segments and 2 dogs with a lesion affecting the L4-S3 spinal cord segments. At time of diagnosis, no dogs presented as grade 0; 2 dogs (10%) were grade 1; 14 dogs (67%) grade 2; and 5 dogs (24%) grade 3. No dogs were found to have paraplegia or tetraplegia at time of presentation. Pain on direct spinal palpation was present in 15 (71%) dogs. Urinary retention was seen in 2 dogs (10%), and a combination of urinary and faecal incontinence was noticed in 2 dogs (10%). One dog (5%) developed seizures 669 days after diagnosis of MMUO. An overview of the clinical findings of the 21 included dogs, can be consulted in table 1.

Diagnostic findings

As required by the inclusion criteria, CSF collection revealed a pleocytosis in all cases. Overall, median TNCC was 209 cells/mm^3 (ranging from 6 – 6000). Total protein measurement was performed in all but 3 CSF samples, and was above reference values in 17/18 dogs (94%). The median TP concentration was 1.67 g/l (ranging from 0.21-16.3 g/l). Complete blood count and serum biochemistry results were available in 16 dogs (76%). Leucocytosis was only present in 2 dogs (10%) and lymphopenia was present in 6 dogs (29%). Infectious disease testing based on serology and/or polymerase chain reaction (PCR) on CSF for Canine Distemper Virus (CDV), *Toxoplasma gondii*, and *Neospora caninum* was not performed in 2 (10%)

dogs and was negative in the remaining 19 (90%) dogs. In the 2 dogs with lacking infectious disease testing, full necropsy was performed, revealing GMEM.

Magnetic resonance imaging

Magnetic resonance imaging of the spinal cord was available in all cases, revealing a focal lesion in 15 dogs (71%), a multifocal lesion in 4 dogs (19%) and no lesion was visible on sagittal T2W or T1W images in 2 dogs (10%). Lesion length was measured in the focal cases only. Median lesion/vertebral body ratio was 4.8 (ranging from 0.6 – 10.9). All visible lesions were ill-defined, intramedullary, hyperintense on T2W images and isointense on T1W images (Figure 1 and 2). Lesions showed parenchymal contrast enhancement in 18 dogs (86%), and contrast enhancement of overlying meninges in 17 dogs (81%). In dogs presenting with spinal hyperaesthesia (n= 15), there was no significant association with the presence of meningeal contrast enhancement on MRI (P=0.24). In the 2 cases where no lesion was visible on sagittal T2W and T1W images, no parenchymal contrast enhancement was seen, but 1 dog only showed meningeal contrast enhancement. In 2 dogs (10%) intracranial images were present within the field of view of the cervical spinal cord images (T2W transverse and sagittal images), revealing multiple T2W hyperintensities in the forebrain and/or brainstem. Neither of those dogs had clinical or neurological signs of intracranial involvement at time of diagnosis. The first dog, a 56-month-old Jack Russell Terrier, never recovered from general anaesthesia after diagnostic procedures, and full necropsy revealed GMEM. The second dog, a 123-month-old Rhodesian Ridgeback, developed seizures 669 days after diagnosis and was euthanized without further investigations.

Treatment and outcome

As required by the inclusion criteria, outcome was available in all dogs. As described above, one dog never recovered from general anaesthesia for MRI of the spinal cord, and this dog was censored for survival analysis. Mean duration of hospitalisation was 5 days (ranging from 1 – 19 days), with 17 dogs (81%) showing improvement in neurological status within those days. One dog (5%) remained neurologically stable (no improvement nor deterioration), and 3 dogs (14%) showed deterioration of their neurological status. All dogs, including the dog that never recovered from anaesthesia, were treated with immunosuppressive doses of glucocorticosteroids immediately after diagnosis. This consisted of IV dexamethasone (dose ranging from 0.3 – 0.5 mg/kg/day) in 9 dogs (43%), and oral prednisolone (dose ranging from 2 – 4 mg/kg/day) in 12 dogs (57%). Fourteen dogs (67%) received additional treatment with cytosine arabinoside as a constant rate infusion (CRI) of 200mg/m^2 over 8 hours in 1 dog (7%) and as 4 subcutaneous (SC) injections of 50mg/m^2 every 12 hours for 2 consecutive days in 13 dogs (93%).

Twenty dogs (95%) survived to discharge. Of these dogs, 9 dogs (45%) were still alive at time of data capture. Of these 9 dogs, 8 dogs were neurologically normal according to follow-up information, and 1 dog was still showing ataxia and ambulatory paraparesis. Of the 8 normal dogs, 2 dogs were still receiving cyclosporine 5mg/kg every 24 hours, 1 dog was receiving cytosine arabinoside 50mg/ m^2 every 12 hours for 2 consecutive days every 9 weeks, 1 dog was receiving prednisolone 0.2mg/kg every 24 hours, 1 dog was receiving prednisolone 1mg/kg every 24 hours and cytosine arabinoside 50mg/ m^2 every 12 hours for 2 consecutive days every 4 weeks, and 3 dogs were not receiving any treatment at time of data capture. The dog that was still showing neurological abnormalities was receiving

0.5mg/kg prednisolone every other day and cytosine arabinoside 50mg/ m^2 every 12 hours for 2 consecutive days every 5 weeks. Regarding the 11/20 dogs (55%) that had deceased at time of data capture, 3 dogs died or were euthanized because of disease progression, 6 dogs were euthanized because of acute neurological deterioration after initial neurological improvement, and 2 dogs were euthanized because of unrelated causes (complications after stifle surgery and development of aggression). Dogs that showed acute neurological deterioration after initial improvement did so within a median of 171 days after diagnosis (ranging from 30 – 669 days). Of those 6 dogs, 1 dog showed acute deterioration after discontinuation of prednisolone treatment, and 5 dogs were still receiving treatment consisting of prednisolone 1mg/kg every 24 hours, prednisolone 0.5mg/kg every 24 hours, prednisolone 2mg/kg every 24 hours and azathioprine 2mg/kg every 24 hours, or cytosine arabinoside 50mg/ m^2 every 12 hours for 2 consecutive days every 7 weeks. Overall, we can conclude that 10/21 dogs (48%) died or were euthanized because of MMUO.

No difference was seen in long-term survival between dogs receiving sole prednisolone therapy or combination therapy with cytosine arabinoside (P=0.31). Overall, the MST was 669 days (ranging from 1 – 2250 days) (Figure 3). (Deleted: There was no difference in survival time (ST) between small/medium and large breed dogs with MMUO (P=0.47) (Figure 2).) No significant difference was seen in relative lesion length on MR imaging between dogs that are alive and dogs that died or were euthanized because of MMUO (P=0.91). Post mortem confirmation was available in 3 dogs, revealing GMEM in 2 dogs and necrotising meningomyelitis in 1 dog. All clinical data are summarized in tables 1 and 2.

Discussion

This study evaluated the clinical presentation, diagnostic findings and long-term survival in 21 dogs diagnosed with presumptive MMUO. Dogs had a median age of 5 years at time of diagnosis. A lesion affecting the T3-L3 spinal cord segments resulting in ambulatory paraparesis was considered the most common clinical presentation. However the overall MST was 669 days, 48% of dogs diagnosed with MMUO died or were euthanized because of MMUO, indicating a guarded long-term prognosis.

To be included in the study, dogs were not allowed to have clinical signs or neurological examination abnormalities suggestive of intracranial involvement. Interestingly, additional MR images of the brain were included in the field of view of the cervical MRI in 2 dogs, showing additional lesions in both cases. One of those dogs, a 123-month-old Rhodesian Ridgeback, developed seizures 669 days after diagnosis despite on-going cytosine arabinoside treatment, and was therefore euthanized. No necropsy was performed, but because intracranial lesions were already present at time of diagnosis, development of MUO was assumed. The other dog, a 56-month-old Jack Russell Terrier, never recovered from general anaesthesia for MR imaging. Necropsy was performed, revealing the presence of GMEM. Because intracranial MR images were only available in 2 dogs, it is currently unclear (1) if these brain abnormalities represent a multifocal nature of the disease or cranial extension of the cervical inflammatory lesions, and (2) if inflammatory brain lesions are currently underdiagnosed in dogs with MMUO and if MMUO could therefore be considered a more generalised inflammatory disease process, a meningoencephalomyelitis.

Pain on direct spinal palpation was present in 71% of dogs. Spinal pain reflects the involvement of the meninges, and/or vertebrae (vertebral periosteum), and/or nerve roots or spinal nerves (Da Costa 2012). In the present study, the lesions showed meningeal contrast enhancement in 18/21 dogs, but there was no significant association between spinal hyperaesthesia and the presence of meningeal enhancement on MR imaging.

MRI of the spinal cord revealed no lesion on sagittal T2W and T1W images in 10% of dogs (n=2), which appears similar to the 7% described for the brain in dogs with MUO (Granger *et al.* 2010). In the retrospective study of Griffin *et al*. (2008), only 1 dog with meningomyelitis had MRI performed, revealing no abnormalities. Based on these findings, presence of MMUO cannot be ruled out based on unremarkable MRI findings. The first dog was a 42-month-old Bull Mastiff with a one-month history of slowly progressive T3-L3 spinal cord lesion. After diagnostic procedures, the dog was treated with oral prednisolone but continued to deteriorate and was euthanized after 6 days. No necropsy was performed. The second dog was a 136-month-old Bearded Collie with a one-week history of a progressive multifocal spinal cord neuroanatomical localisation (T3-S3 spinal cord lesion). The dog showed improvement on treatment with prednisolone and cytosine arabinoside (see table 1) after diagnostic investigations, and was still alive without current treatment 1100 days after diagnosis. Both dogs had inflammatory CSF analysis (increased TNCC and TP concentration). For both dogs, the presence of vascular, degenerative and neoplastic spinal cord lesions can’t be excluded. As both dogs had a progressive disease course, a vascular (ischaemic) lesion seemed less likely. A neoplastic lesion cannot be excluded, although this seems rather unlikely in the Bull Mastiff considering his very

young age. The second dog had a lymphocytic pleocytosis on CFS analysis, but no signs of lymphoma were seen on microscopical examination, however no specific test to look for clonality was performed.

If a lesion was visible on MRI, all lesions were extensive, ill-defined, intramedullary, hyperintense on T2W images and isointense on T1W images. Other spinal conditions, including acute non-compressive nucleus pulposus extrusions (ANNPE) and ischaemic myelopathy (IM), are also associated with intraparenchymal hyperintensities on MRI. These conditions are however associated with other clinical and MRI characteristics, which could potentially aid in differentiating between these conditions (Cardy *et al.* 2015; Fenn *et al.* 2016). Looking into a recent study (Cardy *et al*. 2015), the clinical presentation of dogs with spinal cord dysfunction, IM (most commonly fibrocartilagenous embolic myelopathy (FCEM)) and ANNPE are typically characterised by a peracute onset of non-progressive clinical signs and affected dogs do not commonly demonstrate overt spinal hyperaesthesia at time of admission. This is in contrast with the clinical presentation of dogs with MMUO, which was characterised by an acute onset of progressive and mainly symmetrical neurological deficits, with pain on spinal palpation or manipulation in 86% of dogs (Cardy *et al.* 2015), which is comparable with the 71% of dogs presenting with spinal hyperaesthesia in the presented study. (Deleted: Typical MRI characteristics of dogs with ANNPE include a focal area of intramedullary spinal cord hyperintensity on T2W images overlying an intervertebral disc space, a reduction in volume of the T2W hyperintense nucleus pulposus signal, mild narrowing of the associated disk space, presence of extradural material or signal intensity change with minimal or no spinal cord compression at this level, and are more likely to be lateralised (De Risio *et al.* 2015; Fenn *et al.* 2016). Diagnosis of IM (or presumed FCEM) is based on the

presence of a focal, relatively well-demarcated intramedullary T2W hyperintense lesion, mainly affecting grey matter, with an absence of the above criteria used to diagnose ANNPE and mainly showing no lateralization (De Risio *et al*. 2015; Fenn *et al.* 2016). In the presented study, dogs diagnosed with MMUO all showed presence of an extensive, ill-defined, intramedullary hyperintensity over multiple vertebral bodies, without concurrent presence of disk space narrowing or reduction in the nucleus pulposus signal. In a study looking at MRI findings in dogs with suspected ischemic myelopathy, contrast enhancement was seen in a small proportion of affected dogs (De Risio *et al.* 2007). Additionally, lesions of dogs with IM have been reported to have a median lesion to vertebral body ration of 1.6 and 2.2 for lesions in the cervical (compared to C6) and thoracolumbar region (compared to L2), respectively (De Risio *et al.* 2007). Compared to the present study, revealing an overall lesion/vertebral body ratio of 4.8, the lesions in dogs with MMUO seem to be remarkably longer than the intraparenchymal hyperintense lesions seen in dogs with IM or ANNPE.) Although CSF analysis in dogs with IM is most often within normal limits, affected dogs can demonstrate an increased TP concentration and mild neutrophilic or mixed cell pleocytosis with a median TNCC of 12 WBC/microL (De Risio *et al.* 2007). A marked pleocytosis with a median TNCC of 209 WBC/mm^3 was seen in the presented study, although results should be interpreted with caution as presence of a CSF pleocytosis was considered one of the inclusion criteria. To conclude, the presentation of a dog with an acute or chronic onset of a progressive and painful T3-L3 myelopathy in combination with an extensive, ill-defined, intramedullary lesion with presence of parenchymal and/or meningeal contrast enhancement on MRI, and presence of a marked pleocytosis on CSF analysis, can be presumptively diagnosed with MMUO. The importance of differentiating between these conditions is

highlighted by the differences in treatment and prognosis between dogs with presumptive MMUO and dogs with ANNPE or IM.

A previous study demonstrated that short tau inversion recovery (STIR) hyperintensities in the cervical epaxial musculature of dogs with meningoencephalomyelitis had a sensitivity of 78% and a specificity of 92% in predicting inflammatory CSF results (Eminaga *et al.* 2013). In the presented study, STIR images were unfortunately only available in 3/21 cases. Adding this sequence to the protocol in dogs with presence of a focal or multifocal, ill-defined T2W intramedullary hyperintensity might be considered in the future.

Several studies have evaluated survival times of dogs diagnosed with MUO (Granger *et al.* 2010, Coates and Jeffery 2014). Overall, dogs with MUO appear to have a guarded prognosis. A large meta-analysis of dogs with MUO revealed an overall reported MST of 240-590 days in 96 dogs treated with corticosteroids plus any other immunosuppressive protocol, compared to a MST of 28-357 days for 43 dogs receiving corticosteroids alone (Granger *et al.* 2010). In the presented study, dogs with presumptive MMUO had a MST of 669 days (2 years), but ultimately, 48% of dogs died or were euthanized because of MMUO, indicating a more guarded long-term prognosis.

Limitations of this study are the relative small sample size and retrospective character, which limited standardisation of patient assessment and treatment. Although dogs were all treated with glucocorticosteroids, it cannot be excluded that specific differences in treatment have influenced our results. Despite including cases over a

relative large period and from a busy referral hospital, only 21 dogs could be included. This could indicate that MMUO should be considered a rare disorder and this is in agreement with previously reported findings (Cardy *et al.* 2015), which indicated that MMUO represents approximately 6% of all spinal disorders in dogs.

Conclusion

Presumptive MMUO can be diagnosed in every dog breed of every age that is presented with signs of a mainly acute or chronic, possibly painful, myelopathy. Although clinical signs can vary, affected animals most typically present with ambulatory paraparesis and ataxia, localizing to T3-L3 spinal cord segments. MRI typically reveals an extensive, ill-defined and intramedullary lesion that appears hyperintense on T2W images and isointense on T1W images. Most lesions showed parenchymal contrast enhancement and/or enhancement of the overlying meninges on post-contrast T1W images which can possibly differentiate dogs with MMUO from other more common spinal diseases. In 10% of cases, no lesion was visible on sagittal T2W and T1W images. Almost 50% of dogs died or were euthanized because of MMUO, with a MST of 669 days for all dogs. Future studies should be performed looking into intracranial imaging in dogs diagnosed with presumptive MMUO and its prognostic value, extensive infectious disease testing in all cases and outcome using a standard treatment protocol to give more information about this condition.

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FIGURE LEGENDS

Figure 1

Figure 1: T2W transverse (left image) MR image of the vertebral column and spinal cord at the level of C3, and mid sagittal (right image) MR image of the cervical and cranial thoracic vertebral column and spinal cord of a 56-month-old Jack Russell Terrier. There is presence of a large, ill-defined, intramedullary hyperintensity extending from cranial C2 until cranial C6.

Figure 2

Figure 2: T2W sagittal (top image) and transverse (bottom left image), and T1W transverse (bottom right image) of the vertebral column and associated spinal cord of a 13-month-old French Bulldog. There is presence of a large, ill-defined, intramedullary lesion that is hyperintense on T2W images and isointense on T1W images. The lesion is extending from mid T10 until caudal L1.

Figure 3

Figure 3: Kaplan-Meier survival curve for overall survival in dogs diagnosed with MMUO. Results were censored for dogs that were still alive at time of data capture and dogs that died because of unrelated causes (single little blocks).

TABLES

Table 1

Table 1: Clinical details of the 21 dogs diagnosed with MMUO. FE = female entire, FN = female neutered, ME = male entire, MN = male neutered, CSF = cerebrospinal fluid, TNCC = total nucleated cell count, SC = subcutaneous, CRI = constant rate infusion, NA = not applicable, NP = not performed, GMEM = granulomatous meningoencephalomyelitis, NMEM = necrotising meningoencephalomyelitis.

Case	Breed	Gender	Age (months) at presentation	Clinical presentation	Neuroanatomical localisation	Spinal hyperesthesia	CSF TNCC (cells/µl)	MRI lesion	Initial treatment	Cytosine arabinoside dose (mg/m2), SC or CRI	Initial response to treatment	Long-term follow-up and treatment	Death or euthanasia because of MMUO	Overall ST (days)	Post mortem findings
1	Akita	FE	36	Non ambul paraparesis	Multifocal	Yes	1740	Focal	Prednisolone 2mg/kg /day	50 mg/m2 SC	Improvement	Euthasia because of acute deterioration after discontinuation of prednisolone treatment	Yes	380	NP
2	Rottweiler	ME	123	Ataxia	T3-L3	No	209	Focal	Prednisolone 2mg/kg /day	50 mg/m2 SC	Deterioration	Euthanasia because of disease progression	Yes	20	NP
3	Bull Mastiff	ME	42	Ambulatory paraparesis	T3-L3	Yes	6	No lesion visible	Prednisolone 2mg/kg /day	No cytosine arabinoside	Deterioration	Euthanasia because of disease progression	Yes	6	NP
4	Labrador	MN	105	Ambulatory paraparesis	L4-S3	Yes	123	Focal	Dexamethasone 0,3mg/kg/day	50 mg/m2 SC	Improvement	Euthanasia because of acute deterioration, was still receiving 1mg/kg of prednisolone every day	Yes	30	NP
5	JRT	MN	89	Ambulatory paraparesis	T3-L3	No	200	Focal	Prednisolone 2mg/kg /day	No cytosine arabinoside	Improvement	Normal dog, still receiving 0,2 mg/kg/day prednisolone	No	237	NA
6	Lhasa Apso	FE	48	Ambulatory tetraparesis	C1-C5	Yes	900	Focal	Prednisolone 4mg/kg /day	50 mg/m2 SC	Improvement	Euthasia because of acute deterioration, was still receiving 0,5 mg/kg prednisolone per day	Yes	171	GMEM
7	Shih Tzu	MN	50	Ambulatory tetraparesis	C6-T2	Yes	5	Focal	Prednisolone 2mg/kg /day	50 mg/m2 SC	Improvement	Normal dog, receiving cyclosporine 5mg/kg/day	No	2250	NA
8	Giant Schnauzer	ME	32	Non ambul paraparesis	Multifocal	No	1345	Focal	Prednisolone 2mg/kg /day	50 mg/m2 SC	Improvement	Euthanasia because of agression, was only receiving cytosine arabinoside every 5 weeks	No	752	NP
9	Yorkshire Terrier	FN	36	Ambulatory tetraparesis	C1-C5	Yes	7	Focal	Prednisolone 2mg/kg /day	No cytosine arabinoside	Improvement	Euthanasia because of acute deterioration, was still receiving 1mg/kg of prednisolone per day	Yes	202	NMEM
10	English Springer Spaniel	ME	85	Ataxia	Multifocal	No	455	Focal	Prednisolone 2mg/kg /day	No cytarabine	Improvement	Euthanasie because of post-operative infection after stifle surgery, dog normal and on no medication	No	304	NP
11	Rhodesian Ridgeback	FE	123	Normal gait	C1-C5	Yes	89	Focal*	Dexamethasone 0,3mg/kg/day	50 mg/m2 SC	Improvement	Euthanasie because development of seizures, was still receiving cytarabine 50mg/m2 SC every 7 weeks	Yes	669	NP
12	Bearded Collie	MN	136	Ambulatory paraparesis	Multifocal	Yes	162	No lesion visible	Prednisolone 2mg/kg /day	50 mg/m2 SC	Improvement	Normal dog, receiving no current treatment	No	1100	NA
13	Boxer	ME	26	Normal gait	Multifocal	Yes	6000	Focal	Prednisolone 2mg/kg /day	50 mg/m2 SC	Improvement	Normal dog, receiving cytarabine 50mg/m2 SC every 9 weeks	No	1460	NA
14	Lhasa Apso	MN	128	Ambulatory paraparesis	L4-S3	Yes	1540	Multifocal	Prednisolone 2mg/kg /day	50 mg/m2 SC	Stable	Euthanasia because of disease progression	Yes	33	NP
15	Chihuahua	ME	19	Ataxia	T3-L3	Yes	9	Multifocal	Dexamethasone 0,3mg/kg/day	No cytosine arabinoside	Improvement	Normal dog, receiving no current treatment	No	635	NA
16	Cross Breed	FN	83	Ambulatory paraparesis	Multifocal	No	1230	Multifocal	Dexamethasone 0,3mg/kg/day	200 mg/m2 CRI	Improvement	Euthanasia because of acute deterioration, was still receiving 2mg/kg of prednisolone every day, combined with 2mg/kg azathioprine	Yes	93	NP
17	French Bulldog	ME	13	Ambulatory paraparesis	T3-L3	No	250	Multifocal	Prednisolone 2mg/kg /day	No cytosine arabinoside	Improvement	Normal dog, receiving no current treatment	No	791	NA
18	Maltese Terrier	FN	104	Ataxia	Multifocal	Yes	95	Focal	Dexamethasone 0,3mg/kg/day	50 mg/m2 SC	Improvement	Normal dog, still receiving 1mg/kg of prednisolone per day, and cytarabine 50mg/m2 SC every 4 weeks	No	577	NA
19	Jack Russell Terrier	FN	56	Non ambulatory tetraparesis	C6-T2	Yes	2690	Focal*	Dexamethasone 0,5mg/kg/day	No cytosine arabinoside	Dog never recovered from general anaesthesia for MRI	Dog never recovered from GA	Yes	0	GMEM
20	French Bulldog	ME	10	Non ambulatory paraparesis	T3-L3	Yes	43	Focal	Dexamethasone 0,3mg/kg/day	50 mg/m2 SC	Improvement	Ataxia and ambulatory paraparesis, still receiving 0,5mg/kg of prednisolone every other day and cytarabine 50mg/m2 every 5 weeks	No	90	NP
21	West Highland White T.	FE	103	Non ambulatory tetraparesis	Multifocal	Yes	1980	Multifocal	Dexamethasone 0,3mg/kg/day	50 mg/m2 SC	Improvement	Normal dog, receiving cyclosporine 5mg/kg/day	No	210	NA

Table 2

Table 2: summary of the most important demographic, treatment and outcome data in dogs diagnosed with MMUO. IQR = interquartile range, CSF = cerebrospinal fluid, TNCC = total nucleated cell count, TP = total protein, IV = intravenous, CRI = constant range infusion, SC = subcutaneous.

Variable	Number (%) or median (IQR)
Signalment	
Age (months)	56 (10 – 128)
Male / female	13 (62%) / 8 (38%)
Duration of clinical signs prior to diagnosis (days)	8 (1 – 90)
Onset of neurological signs	
Peracute	1 (5%)
Acute	9 (43%)
Chronic	11 (52%)
Neurological examination	
Focal / multifocal lesion	13 (62%) / 8 (38%)
Focal lesion localisation	
C1-C5	3 (23%)
C6-T2	2 (15%)
T3-L3	6 (47%)
L4-S3	2 (15%)
Neurological grade	
Grade 0	0
Grade 1	2 (10%)
Grade 2	14 (67%)
Grade 3	5 (24%)
Grade 4	0
Grade 5	0
Pain on spinal palpation	15 (71%)
Urinary retention	2 (10%)
Urinary and faecal incontinence	2 (10%)
CSF examination	
TNCC (cells/mm^3)	209 (6 – 6000)
TP concentration (g/l)	1.67 (0.21 – 16.3)
Treatment	
Glucocorticosteroids	21 (100%)
IV dexamethasone	9 (43%)
Oral prednisolone	12 (57%)
Cytosine arabinoside	14 (67%)
CRI	1 (7%)
SC injections	13 (93%)
Outcome	
Survival to discharge	20 (95%)
Alive at time of data capture	9 (45%)