UNIVERSIDADE DE LISBOA

Faculdade de Medicina Veterinária

CANINE INTRACRANIAL PACHYMENINGEAL ENHANCEMENT: A STUDY OF 2 CLINICAL CASES

INÊS DE AVELAR TEIXEIRA CALIFÓRNIA QUINTAS

CONSTITUIÇÃO DO JÚRI

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DISSERTAÇÃO DE MESTRADO INTEGRADO EM MEDICINA VETERINÁRIA

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LISBOA

“Quando perdemos a capacidade de nos indignarmos com as atrocidades praticadas contra outros, perdemos também o direito de nos considerarmos seres humanos civilizados”

(Vladimir Herzog)

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RESUMO

INTENSIFICAÇÃO DO SINAL PAQUIMENÍNGICO CRANIANO CANINO: ESTUDO RETROSPECTIVO DE 2 CASOS CLÍNICOS

A intensificação do sinal paquimeníngico pós-contraste, sinónimo de intensificação do sinal dural ou dural-aracnóide pós-contraste, é um sinal imagiológico melhor apreciado na ressonância magnética (RM) com contraste. No Homem, pode surgir associado a vários processos clínicos benignos ou malignos, como é o caso de alterações pós-cirúrgicas transitórias, hipotensão intracraniana ou neoplasias primárias como meningioma e linfoma do sistema nervoso central e ainda de doença metastática.

O presente estudo descreve dois casos clínicos de cães com diagnóstico diferencial primário de meningite por toxoplasma e meningite eosinofílica idiopática, que apresentaram intensificação do sinal paquimeníngico difuso na RM pós-contraste.

Ambos os canídeos apresentaram sinais clínicos compatíveis com doença intracraniana e da medula espinhal e uma análise do líquido cefalorraquidiano compatível com doença inflamatória do sistema nervoso central.

A instituição de corticoterapia permitiu em ambos os casos uma melhoria clínica, a qual foi agravando à medida que se diminuía a dose do medicamento a que o doente era sujeito.

Apesar da terapia instituída, ambos os doentes apresentaram uma evolução negativa do seu quadro neurológico, tendo os cuidadores optado pela realização do acto de eutanásia ao final de um período de 2 e 7 meses após o diagnóstico clínico ter sido estabelecido.

Palavras-chave:

Cão; Neurologia; Encéfalo; Realce Paquimeníngico; Ressonância Magnética; Meningite.

ABSTRACT

CANINE INTRACRANIAL PACHYMENINGEAL ENHANCEMENT: RETROSPECTIVE STUDY OF 2 CLINICAL CASES

Post-contrast pachymeningeal enhancement, synonymous of post-contrast dural or dura-arachnoid enhancement, is an imaging feature best appreciated on a contrast-enhanced magnetic resonance imaging (MRI). In humans, it may arise from various benign or malignant clinical processes, such as transient postoperative changes, intracranial hypotension or primary neoplasms, including meningiomas and secondary central nervous system lymphoma, and metastatic disease.

The present study describes two clinical cases of canine diffuse intracranial pachymeningeal enhancement as the only intracranial imaging abnormality in a dog with toxoplasma suspected meningitis and in a dog with presumed idiopathic eosinophilic meningitis.

Both dogs presented clinical signs compatible with intracranial and spinal disease. Both cases had cerebrospinal fluid analysis results compatible with central nervous system inflammatory disease. Clinical improvements were observed with corticosteroid therapy and worsening of the clinical signs appeared to be associated with corticosteroid tapering in both dogs.

Despite the therapy instituted, both patients presented a negative evolution of their neurological condition, and the tutors opted for the euthanasia act at the end of a period of 2 and 7 months after the clinical diagnosis was established.

Keywords: Dog; Neurology; Brain; Magnetic Resonance; Pachymeningeal Enhancement; Meningitis.

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LIST OF ABBREVIATIONS

CNS – Central nervous system

CSF – Cerebrospinal fluid

CT – Computed tomography

EME – Eosinophylic meningoencephalitis

FLAIR – Fluid attenuation inversion recovery

GME – Granulomatous meningoencephalitis

Ig G – Immunoglobulin G

Ig M – Immunoglobulin M

IHP – Idiopathic hypertrophic pachymeningitis

LMN – Lower motor neuron

MR – Magnetic resonance

MRI – Magnetic resonance imaging

MUE – Meningoencephalomyelitis of unknown etiology

NIME – Non-infectious meningoencephalitis

NLE – Necrotizing leukoencephalitis

NME – Necrotizing meningoencephalitis

PCR – Polymerase chain reaction

PmE – Pachymeningeal enhancement

PL – Pelvic limbs

PLR – Pupillary light reflex

PNS – Peripheral nervous system

RF – Radiofrequency

RT-PCR – Reverse transcription polymerase chain reaction

SE – Spin-echo

SRMA – Steroid-responsive meningitis-arteritis

TE – Echo Time

Ti – Inversion time

TL – Thoracic limbs

TR – Repetition time

T1-W – T1-weighted

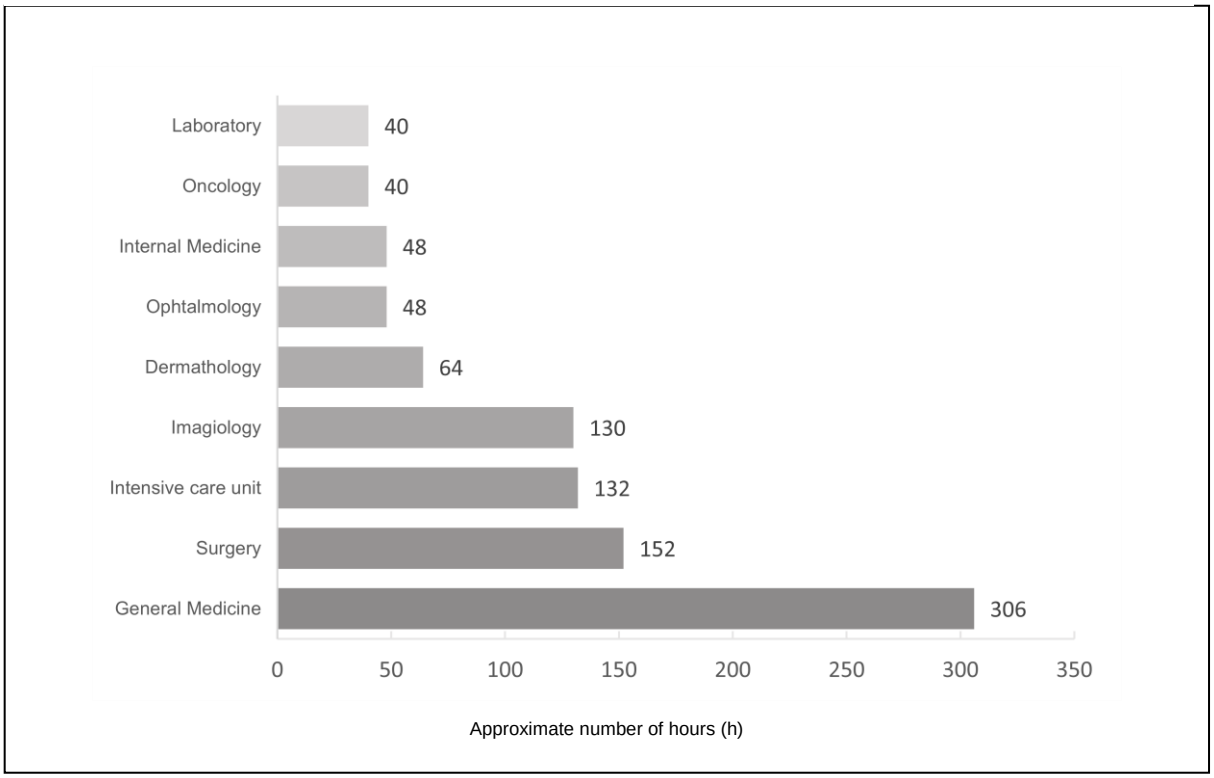
T2-W – T2-weighted

UMN – Upper motor neuron

PART I: CURRICULAR INTERNSHIP REPORT

The curricular internship curricular training for the Integrated Masters in Veterinary Medicine degree was held at the Veterinary Teaching Hospital of Faculty of Veterinary Medicine - University of Lisbon (*Hospital Escolar Veterinário* – HEV), from March 2018 to August 2018 summing up to a total of approximately 960 hours, distributed in rotational shifts by different departments as shown in Chart.

Chart 1. Approximate number of hours spent in each department during the curricular internship.



During the general medicine context, it was possible to attend a reasonable number of consultations to practice the gathering of information such as patient main complaint, the anamnesis, as well as, to perform the general physical examination of the patient and some specific procedures such as biologic sample collection. Differential diagnostic list, complementary exams and treatment plans, were always discussed with the attending veterinary surgeon. Also, I had the possibility to implement prophylactic plans, such as, vaccinations and internal and external deworming.

In the surgery rotation, I was able to admit the patients for surgery, help to preparing them for surgery, administer the therapeutics, assisting a different types of surgeries and monitoring the patient during and after the surgery, which enabled the improvement of concepts of surgical asepsis, surgical simple techniques, anaesthesia and analgesia.

On the intensive care unit, it was possible to participate in the care of pets with life-threatening disease conditions or with special needs (such as recovery from major surgery, advanced pain management, complex drug therapy, etc). The tasks in this service included the frequent patient monitorization through general and specific examinations and performing procedures such as catheterization, administration of fluid therapy and specific drugs, nutritional support, wound care, urinary output monitoring and discussing the home care with the patient's tutor's regarding medical discharge of the patient.

In the imagiology rotations it was possible to improve the radiographic patient positioning, ultrasound technique and, especially, radiographic and ultrasound image interpretation skills.

Regarding dermatology, ophthalmology and oncology rotations, the training of specific examinations and specialized procedures, together with discussion of the cases with the referral veterinary surgeon, contributed to a deeper understanding on these subjects.

In order to better understand the processing and analysis of biological samples, one of the rotations was spent in *Braço Forte* Laboratory, which is an in-house laboratory where the majority of the blood and urine samples of HEV are analysed. This week allowed to process several samples of blood and urine and to improve the microscopic evaluation of these biological samples.

Besides canine and feline patients, this internship also allowed to be in contact with some other species of small mammals, birds and reptiles – the exotic consulting.

The continuous monitoring and supervision by the hospital's clinical staff, namely physicians, nurses and auxiliaries, contributed to the execution of clinical procedures in a pedagogical and safe context. The development of soft skills during this internship is also a noteworthy learning point provided by this multidisciplinary, pedagogical and safe environment.

PART II: INTRODUCTION

1 Nervous system general considerations

The nervous system informs an animal about its internal and external environment, setting responses that fits all the situations. Other body systems are specialized to perform various life-sustaining functions, such as locomotion, digestion, respiration, and circulation, and it is essential that these functions be regulated and coordinated by the nervous system (Evans, DeLahunta, & Miller, 2013).

Nervous tissue parenchyma consists of neurons and supportive cells called neuroglia. Neurons are the structural and functional units of the nervous system (Eurell, Frappier, & Dellmann, 2006) being unique electrically excitable cells responsible for transmitting signals throughout the body. Their membrane is capable of generating electrical impulse (action potentials), which travel from one neuron to the next via specialized contact areas known as synapses and to effector cells or organs at neuroeffector junctions (Uemura, 2015). A typical neuron has a single axon, and multiple dendrites originating as processes from its cell body. The axon, also known as nerve fiber, is responsible for transmitting signals away from the neuron body, while the dendrites allow the neuron to receive signals from their surroundings (Eurell et al., 2006).

The nervous system may be divided into central nervous system (CNS) and peripheral nervous system (PNS). The CNS consists of the brain and spinal cord and the PNS is composed of cranial, spinal and named nerves, including associated nerve roots and ganglia. The PNS conveys sensory signals about the external and internal environment to the CNS, and motor signals from the CNS to the peripheral effectors (de Lahunta, Glass, & Kent, 2015; Eurell et al., 2006).

Four general groups of neurons are recognized: primary afferent neurons, interneurons, projection neurons, and final efferent neurons. A primary afferent neuron conducts impulses into the CNS. Projection pathways conduct this afferent (sensory) information cranially to higher centres in the prosencephalon of the brain. When a motor response results, projection pathways referred to as upper motor neurons (UMNs) course caudally from these higher centres in the prosencephalon and brainstem to activate the final efferent neurons referred to as the lower motor neurons (LMNs) located in the brainstem and spinal cord. Then, these LMNs are distributed in the PNS to the effector organ (striated, smooth or cardiac muscle or gland) where a response occurs. Interneurons are activated at various levels of this pathway (Evans et al., 2013).

Certain neural components of the CNS and PNS regulate the visceral organs, smooth muscles and glands. These neural components are collectively referred to as the autonomic nervous system (ANS) (Uemura, 2015).

1.1 Regional neuroanatomy and neurofunction

In veterinary medicine the primary aims of the neurological examination are to establish whether a neurological disease exists and, if it does, to localise the lesion. Localising the lesion is done by assessing the results of the neurological examination. Lesion localisation is essential as diseases are often region-specific and determining which region(s) is involved allows the clinician to establish a list of possible causes and then pursue appropriate diagnostic tests (Thomson, Hahn, & Johnson, 2012).

1.2 Central Nervous System

The CNS comprises the brain and the spinal cord which are organized through a series of developmental events. Starting as a thickened neural plate then transforming into a simple tubular structure, the neural tube, where the cranial end enlarges to become the brain, whereas the remaining develops into the spinal cord. The CNS is therefore a tubular structure and its cavity, filled with cerebrospinal fluid (CSF), comprises brain chambers and canals (referred to as ventricular system) and a spinal canal (Uemura, 2015).

The CNS is protected from the external environment by bone (skull, vertebrae), meninges, and CSF. The bony skull and vertebrae function as major barriers against physical trauma to the CNS. The meninges lie between the bone and the CNS and support blood vessels entering the nervous tissue. The CSF provides a cushioning effect that helps protecting the CNS from trauma. It also helps maintaining the optimal chemical environment of the brain and removes waste products from the brain (Uemura, 2015).

CNS tissue is divided into white and gray matter, based on the gross appearance of freshly sectioned CNS parts. The gray matter consists primarily of cell bodies of neuronal cells, neuroglia, intertwined dendrites and both myelinated and non-myelinated axons. The white matter consists of myelinated axons and the neuroglia associated with the white matter (Evans et al., 2013).

1.3 Meninges

The brain, the spinal cord and the roots of peripheral nerves are enveloped by three membranes of connective tissue termed meninges. Meninges also surround the entire optic nerve (Eurell et al., 2006). Proceeding from the outer surface inward, the meninges are organized into three layers: the dura mater, arachnoid, and pia mater (de Lahunta et al., 2015; Evans & DeLahunta, 2010; Uemura, 2015). The meningeal layers can also be classified based on embryological origin into the pachymeninx, i.e. the dura mater derived from the ectomeninx (mesodermal derivative) and the leptomeninx, i.e. the pia and arachnoid mater derived from the endomeninx (mesodermal and ectodermal derivative) (Patel & Kirmi, 2009).

As cranial and spinal nerves leave the osseous foramina, there is a transition from meninges surrounding the nerve root (meningeal sheaths) to neural connective tissue around the nerve.

Dura mater is continued by fibrous perineurium and epineurium and pia mater is continued by endoneurium (Eurell et al., 2006).

1.3.1.1 Pachymeninx

The most superficial of the meningeal layers is the dura mater (pachymeninx), which is a relatively thick, tough structure composed of dense connective tissue consisting mainly of fibroblasts, collagen bundles arranged in variably oriented planes, some elastic fibers, occasional blood vessels are also present, as well as lymphatic supply and a parasympathetic and sympathetic innervation system (Evans et al., 2013; Tipold, 2003).

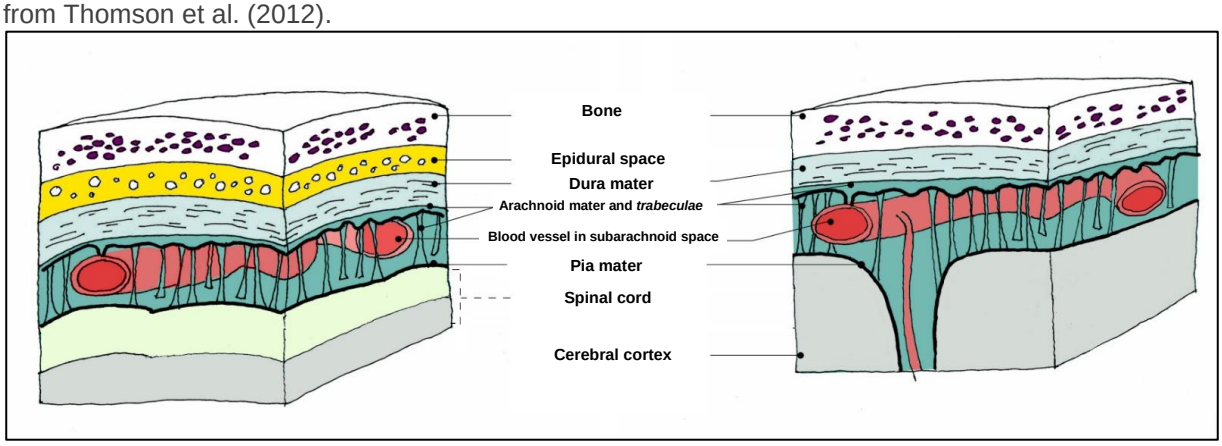
The intracranial dura mater lines the cranial cavity and is composed of two layers (figure 1).

The outer layer is not of meningeal origin and serves as the true periosteum of the inner table of the calvaria. It contains meningeal arteries and veins as well as nerves. It is adherent to the inner surface of the skull, with especially tight attachments to the sutures and skull base. The inner and deeper layer is known as the meningeal layer. This layer is derived from the meninx and is responsible for forming reflections that divide the brain into compartments (Patel & Kirmi, 2009). Three major partitions of these reflections of the dura can be identified in dogs. The *falx cerebri* is the largest one and is a sickle-shaped dural reflection that originates from the dorsal median plane of the calvaria and that extends ventrally into the longitudinal fissure which separates right and left cerebral hemispheres (figure 2). The dorsal sagittal venous sinus is present along its dorsal margin. Caudally, the *falx cerebri* meets the surface of the *tentorium cerebelli membranaceum*, a dural partition inserted into the transverse fissure that forms a tent-like roof over the caudal cranial fossa covering the rostral cerebellum and separating it from the cerebral hemispheres (Evans et al., 2013; Uemura, 2015). This transverse reflection of dura mater encloses a core osseous *tentorium* (a process of parietal and occipital bones) and extends beyond it as *tentorium cerebelli membranaceum*. The *diaphragma sellae*, also formed by reduplication of the inner meningeal layer of the dura (Tipold, 2003), is the dural partition that separates brain from the hypophysis and the cavernous venous sinuses. It is penetrated by the infundibulum of the hypophysis and by internal carotid arteries (Evans et al., 2013). Apart from when they separate to form endothelium-lined spaces called dural venous sinuses (de Lahunta et al., 2015; Evans et al., 2013), there is no distinct macroscopic border between the meningeal and periosteal dura (Patel & Kirmi, 2009).

The meningeal layer of the dura mater can be microscopically differentiated from the periosteal layer by the lower fibroblasts and collagen content in the meningeal layer. Deep to the meningeal layer is a unique layer of fibroblasts referred to as the dural border cell layer, subdural mesothelium, neurothelium, subdural cells, inner dural cell layer or the intermediate cellular layer in the human medicine literature (Patel & Kirmi, 2009). This layer of flattened fibroblasts is continuous externally with the meningeal dura and internally with the arachnoid fibroblasts to which it adheres by desmosomes. Therefore, in the cranial cavity, subdural space

is normally non-existent but a potential space instead that may develop under certain pathologic circumstances once the dura–arachnoid desmosomal attachments may be easily disrupted (e.g. subdural hematoma after injury or inadverted injection of contrast agents into the dura–arachnoid) (de Lahunta et al., 2015).

Figure 1. Sections through meninges surrounding the spinal cord (left) and brain (right) (modified from Thomson et al. (2012).



The outer layer of the intracranial dura mater terminates at the foramen magnum, where it fuses with the periosteum lining the foramen and with the periosteum along the floor of the vertebral canal within the atlas and axis (Evans et al., 2013). The inner layer continues into the spinal canal to form the only layer of the spinal dura mater (Sze, 1993) which extends laterally into the intervertebral foramina to form the dural root sleeves that cover the spinal nerve roots and spinal ganglia (Dietemann et al., 2005). Within most of the vertebral canal, the dura mater forms a long sleeve free of any other attachments to the vertebrae, separated from the periosteum of the bony canal by the loose connective tissue of the epidural space (de Lahunta et al., 2015). Caudally, the spinal cord dura mater tapers to a slender filament, the *filum durae matris spinalis*, which envelops the *filum terminale* and can extend until the middle of the tail. Collagen and elastic fibers in the spinal dura mater are oriented parallel to the long axis of the spine, providing the dura with longitudinal tensile strength, stiffness, and the property of relaxation (Eurell et al., 2006).

1.3.1.2 Leptomeninges

The arachnoid membrane and pia mater are collectively designated leptomeninges because they are delicate relative to the dura mater and connected both physically and embryologically (Eurell et al., 2006).

The arachnoid membrane consists of outer layers of flattened fibrocytes and inner, loosely arranged, flattened fibrocytes associated with small bundles of collagen fibers. Arachnoid *trabeculae* are thin strands of inner arachnoid composed of collagen fibers coated by flattened fibroblasts that cross the subarachnoid space and establish continuity with pia mater.

Arachnoid *villi* are microscopic projections of arachnoid that penetrate walls of dural venous sinuses and act as one-way valves for drainage of CSF (Eurell et al., 2006).

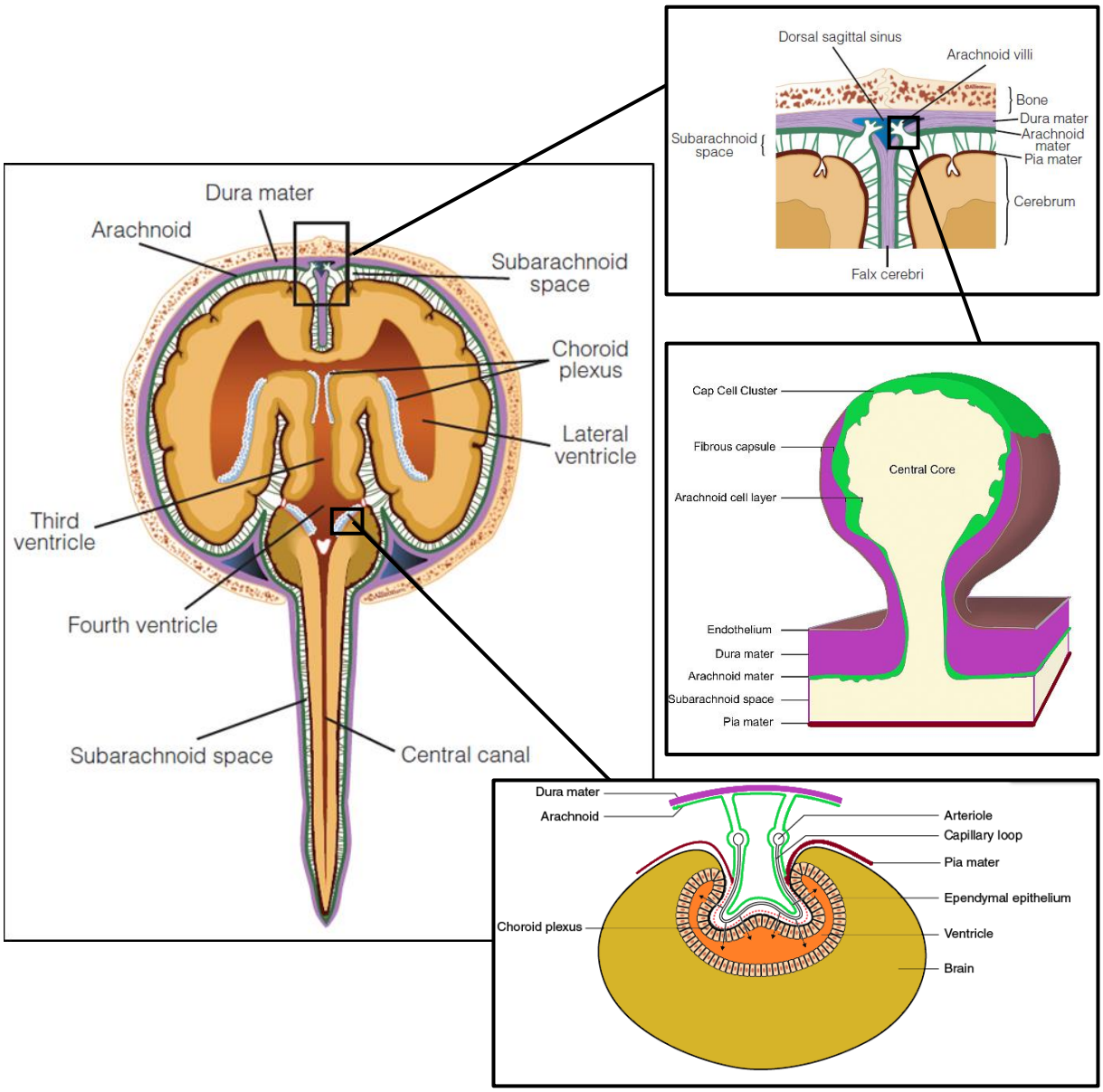
The pia mater consists of a thin layer of connective tissue attached to the surface of the brain, the spinal cord, nerve roots, and the optic nerve. It is characterized by wide intercellular spaces containing variable amounts of interlacing collagen fibers and fine elastic networks with a few fibrocytes, lymphocytes, and mast cells. The collagen fibers of pia mater make contact with a basal lamina which separates the pia mater collagen from the underlying glial limiting membrane (astrocyte processes) (Eurell et al., 2006). Because it is intimately attached to the surface of the CNS, the pia mater extends into the depths of various *sulci*, fissures, and crevices of the CNS (Evans et al., 2013). The pia mater is relatively vascular, because all vessels entering and leaving the CNS must travel in pia mater (Evans et al., 2013).

Along each midlateral surface of the spinal cord, an increase in the amount of pia mater collagen creates a ligament called the denticulate ligament (Eurell et al., 2006). Each denticulate ligament is an elongated longitudinal cord of connective tissue with periodic series of projections extending laterally to attach to spinal dura mater, midway between the roots of adjacent spinal cord segments. These bilateral denticulate ligaments act to suspend the spinal cord within the dura mater so the spinal cord is completely surrounded by the CSF within the subarachnoid space (Eurell et al., 2006). Caudally, each denticulate ligament terminates in a process that connects to the dura mater between the entrances of the L5 and L6 or between the L6 and L7 spinal roots into dural sheaths system (Evans et al., 2013).

Besides covering the CNS surface, the leptomeninges may also be found more centrally around or within the ventricular system where they participate on the secretion of CSF via choroid *plexus* (figure 2). A choroid *plexus* develops where neural tube neuroepithelium did not proliferate to form parenchyma but remained as a single layer of ependymal neuroepithelial cells, referred to as a roof plate. The layer of ependyma and the adjacent pia are referred to as *tela choroidea*. At these sites, the vessels in the pia covering the single layer of ependymal cells proliferate to form a dense *plexus* of capillaries intimately related to these neuroepithelial cells. This proliferation of capillaries is called choroid *plexus*. However, in common usage, the term choroid *plexus*, usually comprises all three components (pia mater, choroid *plexus* and ventricular ependyma). Choroid *plexi* are found in the ventricular system within the medulla *oblongata* (roof plate of the fourth ventricle), the diencephalon (roof plate of the third ventricle), and the telencephalon (roof plate of the lateral ventricle) (de Lahunta et al., 2015). Small amounts of CSF from the brain parenchyma enter the ventricles through their ependymal lining of the ventricular system and enter the subarachnoid space through the pial-glial membrane on the external surface of the parenchyma. Leptomeningeal capillaries in the subarachnoid space are an additional small source of CSF (de Lahunta et al., 2015; Tipold, 2003).

In normal animals, CSF pressure is regulated primarily by its absorption through the arachnoid *villi* which are projections of arachnoid membrane into dural venous sinuses (figure 2) (Tipold, 2003).

Figure 2. The ventricular system depicting the neuroanatomical origins of CSF production and sites of absorption (modified from Patel and Kirmi (2009), Platt and Olby (2012) and Skerritt and King (2018))



1.3.1.3 Extra-axial compartments of the central nervous system

The meninges define the extra-axial (i.e., outside neural axis) compartments of the CNS. The epidural space exists in the spinal canal and contains epidural fat and, particularly along the floor of the canal, blood vessels (figure 1). Inside the cranial cavity, it represents only a potential space because the dura mater serves as the intracranial periosteum, being fused to the adjacent skull bone, as previously mentioned (Evans et al., 2013).

The subarachnoid space lies between the pia and the arachnoid maters and it is filled with CSF that surrounds the entire surface of the brain and spinal cord. Besides CSF, the subarachnoid space also contains blood vessels, spinal nerve roots, and arachnoid trabeculations (de Lahunta et al., 2015; Evans & DeLahunta, 2010).

The depth of the subarachnoid space is variable, because arachnoid membrane contacts dura mater and the pia mater follows every irregularity of the brain surface (Evans et al., 2013). Subarachnoid cisterns occur in areas where the arachnoid and pia are more widely separated (Evans & DeLahunta, 2010). The largest and most important is the cerebellomedullary cistern, formerly *cisterna magna*, located in the angle where the caudal surface of the cerebellum meets the dorsal surface of the *medulla oblongata* (Evans et al., 2013). CSF is most commonly collected in the veterinary practice from this cistern by means of a needle puncture through the atlanto-occipital membrane (Evans & DeLahunta, 2010).

Normally, CSF is a clear, slightly alkaline colourless ultrafiltrate of plasma with low protein content and few cells (Di Terlizzi & Platt, 2006; Evans et al., 2013). It is mostly located in the ventricular system, central canal of the spinal cord and the subarachnoid space (Tipold, 2003; Uemura, 2015). CSF protects the CNS through its physical support, with the parenchyma suspended in this fluid medium present within ventricles and the subarachnoid space (de Lahunta et al., 2015) and provides a cushioning effect that helps protect the CNS from trauma (Uemura, 2015). It also protects the CNS through its role in modulating pressure changes that occur, especially in the closed cranial cavity (de Lahunta et al., 2015). The skull is a rigid structure with a fixed volume containing three components: brain tissue, intracranial vascular volume and intracranial CSF. A volume increment in any of these intradural components will be opposed and must be balanced by reciprocal volume changes in one or both of the other intradural components. Only the CSF and blood can be displaced to maintain constant intracranial volume and pressure without causing damage to the brain tissue (Di Terlizzi & Platt, 2006). In conjunction with cerebral blood flow, CSF helps regulating the normal and abnormal variations in intracranial pressure (de Lahunta et al., 2015; Di Terlizzi & Platt, 2006). CSF is separated from extracellular fluid of nervous tissue by glial limiting membrane and either pia mater or ependyma. Solutes can be exchanged between the two fluid compartments and CSF is normally augmented by flow of extracellular fluid from nervous tissue (Evans et al., 2013). This way, solutes entering the brain through the blood–brain barrier, as well as those synthesised by the brain, diffuse freely from the CNS brain extracellular fluid into the CSF (Di Terlizzi & Platt, 2006). CSF acts as a source of nourishment by being a medium for the transport of metabolites and nutrients between blood and the CNS parenchyma. CSF also transports extraneous particles, such as cells and bacteria, from the brain into the subarachnoid space and has a filtration function allowing movement of water-soluble substances, especially large molecules, from the CNS parenchyma into the CSF, which thus functions like an alternative lymphatic drainage system for the CNS (Di Terlizzi & Platt, 2006).

It plays a role in maintaining the consistent ionic composition necessary for neuronal function by acting as a chemical buffer for the CNS parenchyma (de Lahunta et al., 2015). It is also a vehicle for the intracerebral transport of biologically active substances, like hormone-releasing factors formed in the hypothalamus and discharged into the CSF of the third ventricle, (Di Terlizzi & Platt, 2006), neuroendocrines and neurotransmitters within the parenchyma (de Lahunta et al., 2015).

Rosenberg (1990), accordingly to de Lahunta et al. (2015) and Di Terlizzi and Platt (2006), refers that 35% of the CSF production is derived from the third and lateral ventricles, 23% from the fourth ventricle, and 42% from the subarachnoid space. The CSF flows from the lateral ventricles, through the interventricular foramens into the third ventricle, then it flows caudally through the mesencephalic aqueduct to the fourth ventricle (Thomson et al., 2012). From the fourth ventricle, a fraction of CSF leaves the ventricular system via small openings, the lateral apertures, to enter the subarachnoid space. The remaining part of the CSF in the fourth ventricle flows into the central canal of the caudal *medulla oblongata* and spinal cord (Uemura, 2015) and, ultimately, the terminal ventricle of the spinal cord (Evans et al., 2013). The CSF in the subarachnoid space circulates both cranially and caudally in order to surround the surface of the CNS in its entire length, however its flow in this space is variable.

The two major drainage routes for return of CSF to blood classically described are the arachnoid *villi* at the venous sinus system and the lymphatic system along cranial and spinal nerve (Evans et al., 2013; Tipold, 2003). The mechanism for the bulk flow reabsorption into the venous system depends upon the hydrostatic pressure of CSF within the subarachnoid space (Tipold, 2003). In normal animals, CSF pressure is regulated primarily by its absorption through the arachnoid *villi*. At a *villus*, CSF is separated from blood by flattened fibroblasts and endothelial cells. Each *villus* functions as a one-way valve regulating flow of CSF into the venous sinus. When CSF pressure exceeds venous sinus pressure, *villi* expand and spaces between cell processes increase, allowing more fluid to flow from the subarachnoid space to the bloodstream. When pressure in the venous sinus exceeds CSF pressure, the *villi* collapse, effectively blocking blood reflux. Arachnoid *villi* have also been reported in association with veins located at intervertebral foramina. CSF can also drain into lymphatics of peripheral nerves. At the distal recesses of meningeal sheaths surrounding nerve roots, where roots continue as spinal and cranial nerves, CSF escapes across the arachnoid membrane into nerve lymphatics. There is also considerable CSF drainage associated with the optic and olfactory nerves (Evans et al., 2013). Although a few isolated lymphatic vessels were reported around the cranial nerves and dural blood vessels, the CNS and its meningeal linings were long believed to be devoid of lymphatic vasculature system. Contrary to this belief, Aspelund et al. (2015) observed that the mechanism of CSF flow into the deep cervical lymph nodes in mice is made directly via an adjacent dural lymphatic network, which may be important for the clearance of macromolecules and immune cells from the brain. The existence of an extensive

meningeal lymphatic vessel network that serves macromolecular clearance and immune cell trafficking functions in the brain (Aspelund et al., 2015; Louveau et al., 2015) leads us to conclude that malfunction of the meningeal lymphatic vessels could be a root cause of a variety of neurological disorders in which altered immunity is a fundamental player (Louveau et al., 2015).

CSF analysis may be useful as an important component of the diagnostic evaluation of patients with central and peripheral neurological disease. Inflammatory, degenerative, metabolic, traumatic, and neoplastic brain lesions may alter the normal CSF. Focal, multifocal, and diffuse spinal cord lesions, as well as any disease, especially one that is inflammatory in nature, that affects the spinal nerve roots may also lead to changes in the CSF. For that reason, CSF analysis should be considered whenever an inflammatory, infectious, traumatic, neoplastic or degenerative disorder of the brain and the spinal cord is suspected (Di Terlizzi & Platt, 2009). Abnormalities in the colour, cellularity, and protein level obtained by a routine CSF analysis may contribute to a diagnosis, but rarely by themselves provide a specific diagnosis. It is rare for tumour cells or organisms to be visualized in CSF samples, but when this does occur, a definitive diagnosis can be made. CSF analysis is very sensitive, in that it is often abnormal in patients with neurologic disease being very inespecific, i.e., the results do not point to just one specific disease and can be explained by a group of diferent neurological diseases, in most cases (Dewey & Da Costa, 2016).

Blood vessels passing through the subarachnoid space are covered by leptomeningeal tissue derived from arachnoid *trabeculae* or pia mater (Eurell et al., 2006). When a vessel penetrates the CNS, it is surrounded by a perivascular space (i.e., a space situated between the vessel wall and the glial limiting membrane). Leptomeninges, particularly pia mater collagen fibers, fill the perivascular space so that a space is not obvious, except when it fills with inflammatory cells under pathologic conditions. Communication between perivascular spaces and the subarachnoid space is blocked by a continuous barrier of leptomeningeal cells, formed by cells on surfaces of vessels uniting with those on the pia mater surface. As a vessel proceeds and divides into smaller branches within the CNS, the size of its perivascular space is progressively reduced and the basal lamina associated with the glial limiting membrane merges with vascular basal lamina. Ultimately, capillaries are surrounded only by basal laminae, the outer surface of which is contacted by astrocyte end feet. In contrast to most endothelial cells in the body, endothelial cells of CNS capillaries are generally non-fenestrated and joined by tight junctions. These endothelial features are responsible for the blood-brain barrier, which impedes diffusion of hydrophilic molecules from the bloodstream to the CNS (polar molecules must be specifically transported into the CNS). A blood-brain barrier is not present either neonatally or at the few sites in the adult brain where modified ependymal cells are found (choroid *plexi* and circumventricular organs). A blood-brain-barrier is also present in peripheral nerves (but not ganglia), where capillaries within the endoneurium exhibit tight junctions. Perineural epithelioid

cells surrounding nerve fascicles also have tight junctions establishing a blood-nerve barrier for hydrophilic molecules (Eurell et al., 2006).

2 Canine intracranial pachymeningeal enhancement

Post-contrast pachymeningeal enhancement (PmE), synonymous of post-contrast dural or dura-arachnoid enhancement is an imaging feature best appreciated on a contrast-enhanced magnetic resonance imaging (MRI). Intracranial PmE can be either a normal finding or indicative of pathology (Antony, Hacking, & Jeffree, 2015).

2.1 Introduction to magnetic resonance imaging of the meninges

Plain skull radiographs play a virtually non-existent role in the diagnosis of extra-axial disease. Computed tomography (CT) is the initial modality in the setting of trauma and is most sensitive for identifying acute hemorrhage and calcification (Kirmi, Sheerin, & Patel, 2009). In the evaluation of meningeal processes in humans, contrast-enhanced MR imaging appears to be far more sensitive than contrast-enhanced CT for meningeal enhancement, especially when most of the enhancement lies against the skull vault. Small lesions at the inner table of the skull and subtle meningeal enhancement can be obscured in CT due to beam hardening artifact. With MRI, even small lesions are easily detected, making MR the modality of choice for imaging of meningeal disease (Sze, Soletsky, Bronen, & Krol, 1989).

Hydrogen is the optimal element for MRI as it is the most common element in the body being present in all tissues and fluids. Its nucleus consists of a single proton and has the strongest magnetic dipole moment of any element suitable for MRI. Most pathologic processes affecting the CNS result in alteration of content, distribution, and ambient environment of hydrogen protons facilitating differentiation of diseased from normal tissue (Dewey & Da Costa, 2016). Hydrogen protons in biological tissues are not static but spin around their axis, generating their own micromagnetic environments. In the absence of an external magnetic field the magnetic moments of the spinning protons are randomly oriented. However, when brought into a strong external magnetic field (i.e. an MR scanner), they rearrange under its influence (Dewey & Da Costa, 2016).

The alignment of individual protons may be parallel or antiparallel with the external magnetic field. A slight majority of protons will align with the magnetic field, generating a magnetic vector ("net magnetization vector") which is utilized during MRI. The main magnetic field is denoted by B_0, the tissue magnetization vector by M_0. As long as M_0 is parallel with the much stronger B_0 it cannot be easily separated out and cannot be used for imaging. MRI manipulates tissue magnetization in a way that it can be distinguished from the external magnetic environment (Dewey & Da Costa, 2016).

In addition to a spinning motion around their individual axes, hydrogen protons wobble like a spinning top - a phenomenon called precession - under the influence of B_0. The precession

frequency of the spinning protons is dependent on the strength of the external magnetic field. The magnetic field strength is denoted by the unit “tesla” (T). The strength of clinically used MR scanners ranges from approximately 0.18T (low-field) to 3T (high-field) (Dewey & Da Costa, 2016).

In order to manipulate tissue magnetization so it can be separated from the main magnetic field and create an image, radiofrequency (RF) pulses are applied. Once the nuclei are exposed to an RF pulse exactly matching their precessional frequency they can absorb energy and start precessing in synchrony, also known as *in phase*. As a result of the energy gain, some protons change their alignment with the magnetic field, causing the magnetic net vector to move away from B_0, or “flip.” The most common flip angle of the tissue magnetization vector is 90-degree used in spin-echo sequences. Hydrogen protons flipped into this “transverse plane” (x-y plane), which is in perpendicular orientation to the main magnetic field (z axis or the “longitudinal plane”), continue to precess *in phase*.

Any change in the magnetic environment of a coil of wire will cause an electric signal. Strategic placement of receiver coils in the MR unit allows detection and measurement of magnetization in the transverse plane, which is the basis of image formation in MRI. After the RF pulse is switched off, the signal induced in the receiver drops off rapidly due to two concurrent processes: 1) T1-relaxation (spin-lattice relaxation) where excited hydrogen protons return to lower energy states, and realign their electromagnetic fields in relation to the magnetic field and 2) T2-relaxation (spin-spin relaxation), where hydrogen protons quickly precess out of synchrony so that they become *out of phase*, resulting in a signal drop-off (Dewey & Da Costa, 2016; Pooya, Séguin, Tucker, Gavin, & Tobias, 2004). Three important tissue parameters for MRI include T1- and T2-relaxation times and proton density, i.e. the actual amount of hydrogen protons in a certain tissue volume (Dewey & Da Costa, 2016). Different tissues emit signals of different intensity according to their relaxation properties, and the computer assigns a shade of gray to each voxel (unit of volume) according to the intensity of the collected signals, translating these inherent tissue differences into image contrast, making it possible to display different tissues on a given image.

Several different MRI protocols (pulse sequences) that emphasize T1 and/or T2 relaxation effects have been developed in order to optimize anatomic and physiologic information (Pooya et al., 2004). Additionally, a contrast agent, most often gadolinium, can be used to modify the relaxation properties of tissues and make two tissues that would otherwise have similar relaxation characteristics appear more distinct (Pooya et al., 2004).

The most common pulse sequence currently used is the spin-echo (SE) pulse sequence. With conventional SE pulse sequences, an image can be T1, T2, or proton-density weighted if most of the contrast between tissues is due to a difference in T1 relaxation, T2 relaxation, or proton concentration, respectively (Pooya et al., 2004). This consists of a 90-degree RF pulse that flips the longitudinal magnetization from the z-axis to the x-y axis. This is followed by a 180-

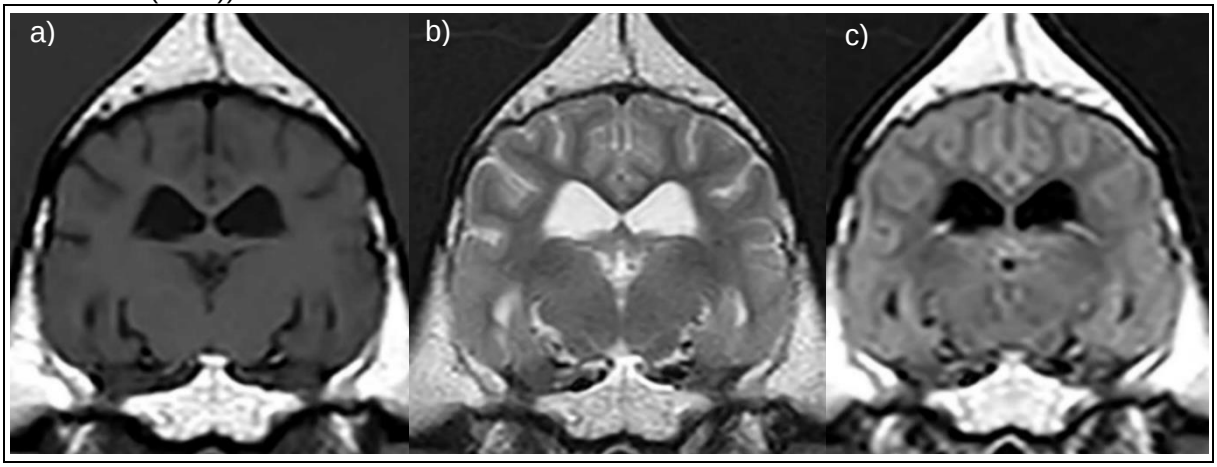
degree pulse applied exactly halfway between the initial 90-degree pulse and the generation of the signal (echo), which rephases the protons that are dephased because of external magnetic field inhomogeneities, essentially reversing any effects an external disturbance might have on proton alignment and resultant tissue signal, resulting therefore in a true T2 relaxation contributing to image contrast (Dewey & Da Costa, 2016; Grossman & Yousem, 2003). As the MR signal generated during a single episode of proton excitation is too small to create an image, the process is repeated many times until enough data have been collected (Dewey & Da Costa, 2016).

Variations in the length of repetition time (TR), which is the time between 90-degree pulses, and the echo-time (TE), which is the time between the 90-degree pulse and the echo, determines the weighting of an SE sequence. In T1-weighted (T1-W) sequence, a short TR (<1000 milliseconds) is chosen to maximize the differences in T1 relaxation between tissues. This is combined with a short TE (<45 milliseconds) to minimize T2 effects (Dewey & Da Costa, 2016; Grossman & Yousem, 2003). Fat has a short T1 relaxation time and appears hyperintense, while fluid has a long T1 relaxation time and appears hypointense in T1-W images (Dewey & Da Costa, 2016) (figure 3). Soft tissues have somewhat variable, intermediate T1 relaxation times and are medium in intensity in T1-W images. T1-W images are used for tissue discrimination and in conjunction with paramagnetic contrast agents because physiologically contrast-enhancing tissues (e.g. pituitary gland) and contrast-enhancing pathologic lesions (e.g.certain brain tumours) become hyperintense (Dewey & Da Costa, 2016; Grossman & Yousem, 2003).

In T2-weighted (T2-W) sequence, a long TE (>60 milliseconds) is chosen to maximize differences in T2 relaxation between tissues, combined with a long TR (>2000 milliseconds) to minimize T1 relaxation effects (Dewey & Da Costa, 2016; Grossman & Yousem, 2003). A long TE ensures that tissues with a short T2 relaxation time will have completely lost their transverse magnetization and have a low signal at the time of image acquisition, while tissues with longer T2 relaxation times still maintain their transverse magnetization and appear hiperintense. Fluid has a long T2 relaxation time and therefore is hyperintense on T2-W images. Soft tissues have intermediate T2 relaxation times. Fat has a short T2 relaxation time and appears hypointense on conventional T2-W images (figure 3). A T2-W sequence can be considered a “pathology” scan, because abnormal fluid collections and tissues with abnormal increased fluid content (e.g. edema, inflammation, neoplasia) will appear hyperintense compared to normal tissues. Proton density weighting is achieved by choosing a long TR in combination with a short TE to minimize T1 and T2 effects on image contrast. Proton density weighting images provide a good contrast between gray and white matter, and although their value in the neuroimaging of small animals is limited, anecdotal evidence suggests they may be helpful in the evaluation of patients with degenerative brain disease (Dewey & Da Costa, 2016).

Another factor in scanning is the inversion time (TI), which is the length of time before the 90-degree pulse that a 180-degree inversion pulse is placed. This parameter can be set to various values to generate contrast and/or to null the signal of a specific tissue in the brain, spine, or head and neck. The most frequent uses in neuroradiology are in supressing fat in the orbits, neck or bone marrow (short tau inversion recovery), or in supressing CSF signal in the brain (fluid attenuation inversion recovery [FLAIR] – figure 3) (Grossman & Yousem, 2003).

Figure 3. T1-W (a), T2-W (b) and FLAIR (c) transverse images of a normal canine brain (modified from Imaios (2019))



High-field MRI not only provides increased spatial resolution, but also offers greater potential to enhance contrast resolution through sequence protocols that include chemical fat suppression not available at lower field strength (D'Anjou, Carmel, Blond, Beauchamp, & Parent, 2012).

After a bolus injection of contrast material into a large peripheral vein, the blood level of the agent rises rapidly, creating a gradient across the capillary endothelial membrane, since the extravascular interstitial fluid does not have the compound. In regions with relatively free capillary permeability, the contrast agent will leak across the vessel wall and begin to accumulate in the perivascular interstitial fluid. In the brain, spinal cord, and proximal cranial and spinal nerves, the intact blood-brain barrier will prevent leakage of contrast material. Interstitial enhancement is related to alterations in the permeability of the blood-brain-barrier, whereas intravascular enhancement is proportional to increases in blood flow or blood volume (Smirniotopoulos, Murphy, Rushing, Rees, & Schroeder, 2007).

Post-contrast T1-W images have been emphasized as very important both in humans and in dogs to examine the meninges (D'Anjou, Carmel, Blond, Beauchamp, & Parent, 2012; Keenihan, Summers, David, & Lamb, 2013; Mellema, Samii, Vernau, & LeCouteur, 2002; Penning, Suckling, Evans, Chandler, & Cappello, 2008; Smirniotopoulos, Murphy, Rushing, Rees, & Schroeder, 2007; Sze, 1993) but enhancement of the meninges varies depending on the field strength, sequence parameters, and the individual scanner. FLAIR may be also useful in the settings of conspicuous subarachnoid pathology by eliminating flow artefacts and differentiating CSF from blood or other pathologic processes (Kirmi, Sheerin, & Patel, 2009).

In human medicine, methods to increase the conspicuity of meningeal enhancement include suppression of the signal from fat in bone marrow, making subtraction images by postprocessing the T1-W pre- and post-contrast images, and contrast-enhanced T1-W FLAIR which, although less commonly used, also further increases the sensitivity of subtle leptomeningeal pathology in humans (Kirmi et al., 2009). Delayed image acquisition following contrast medium injection (Kremer et al., 2006), using a high-dose of gadolinium (Kallmes, Gray, & Glass, 1998) as well as increasing the volume of injected material, reducing the slice thickness, selecting an optimal plane of section (Meltzer, Fukui, Kanal, & Smirniotopoulos, 1996; Quint, Eldevik, & Cohen, 1996) have also been associated with increased detection of meningeal pathology in humans.

In dogs, studies in MRI techniques focusing on intracranial meninges are very limited. According to the study of Keenihan et al. (2013), post-contrast T1-W images, subtraction images, and T2-W images have comparable accuracy for diagnosis of meningeal lesions, and moderate agreement with results of histopathology and subtraction images did not achieve significantly improved accuracy of detecting meningeal lesions compared to the post-contrast T1-W images on which they were based. Post-contrast T1-W images were significantly more accurate than T2-W FLAIR images, which appear to have limited diagnostic utility for meningeal lesions, according to the same authors. The authors conclude that only post-gadolinium images and subtraction images allow the normal meninges to be distinguished from the adjacent brain and that, although T2-W images and FLAIR images are sensitive to lesions causing cytotoxic or vasogenic edema of neural tissues, they do not clearly depict the meninges.

In the study of Lora-Michiels et al. (2008) with a 1.5T MR, contrast enhanced T1-W FLAIR proved to be superior to conventional T1-W in dogs with brain lesions that included meningeal diseases, by increasing the contrast between contrast-enhancing lesions and both normal white matter and CSF. It also allowed greater differentiation between gray and white matters.

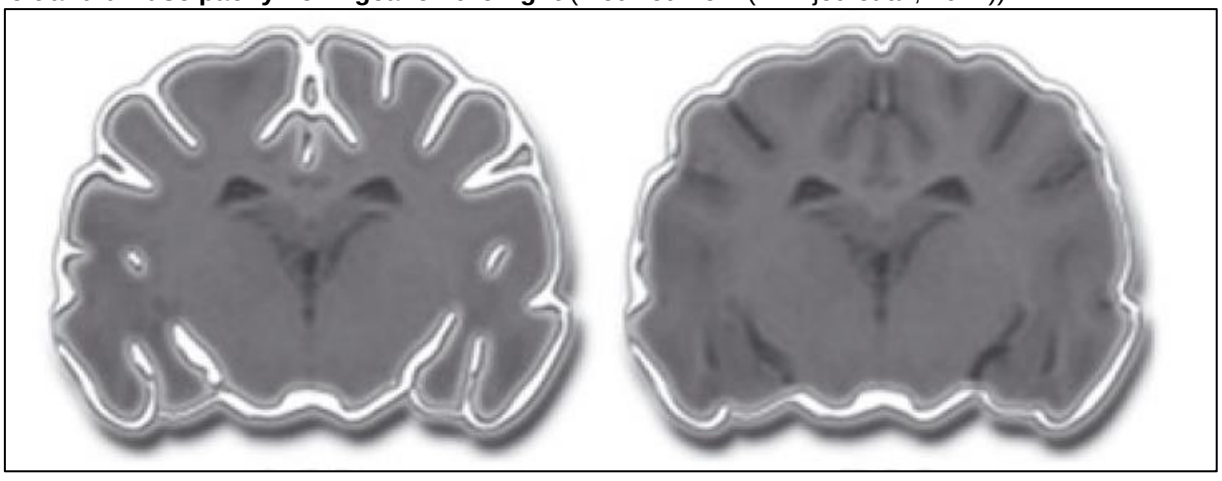
Gadolinium-enhanced T1-W FLAIR with fat supression was the most useful pulse sequence 50% of the time for definite diagnoses and/or characterization of meningeal enhancement in the study of D'Anjou et al. (2012). This sequence was associated with the highest inter-observer repeatability, and the greatest chances to obtain a definite diagnosis of meningeal enhancement. The authors refer this sequence allowed more confident detection and characterization of meningeal enhancement, particularly PmE or in areas with more abundant adipose tissue in the adjacent bone marrow. In the same study, delayed sequences were acquired 5 min and 15-20 minutes post contrast injection and did not bring additional information in most dogs for the detection and characterisation of meningeal enhancement. In the study of Joslyn et al. (2011) a post-contrast T1-W sequence obtained 60-90 seconds immediately after administration was shown to be preferable to a 10-minute delayed post-contrast T1-W sequence to study meningeal enhancement in normal dogs (higher contrast-to-

noise ratio supported by substantial inter- and intra-observer agreement in favour of the initial postcontrast series). In a study with dogs with experimentally induced bacterial meningitis in an early stage, the authors found that acquisition of images immediately following contrast medium injection as well as a high-contrast dose (0.3 mmol/kg) both improved the detection of meningeal enhancement significantly (Runge et al., 1995).

In dogs, meningeal contrast enhancement is recognized with acceptable repeatability. Recognition of contrast enhancement patterns and other MRI characteristics may enhance the role of MRI in the detection, diagnosis, and follow up of neoplastic and non-neoplastic disorders affecting the meninges (D'Anjou et al., 2012).

Based on the anatomical classification of the meninges, extra-axial enhancement in the CNS can be described as pachymeningeal or leptomeningeal (figure 4) (Smirniotopoulos et al., 2007). Because the normal, thin arachnoid membrane is attached to the inner surface of the dura mater, the pachymeningeal pattern of enhancement is also described as dura-arachnoid enhancement and leptomeningeal enhancement is also described as pial or pia-arachnoid enhancement. Pachymeningeal, dural or dura-arachnoid enhancement pattern consists of curvilinear enhancement overlying the brain, manifesting up against the bone, immediately deep to the inner table of the calvaria, or it may involve the dural reflections of the *falx cerebri*, *tentorium cerebelli membranaceum*, and cavernous sinus. Leptomeningeal, pial or pia-arachnoid enhancement pattern consists of a curvilinear enhancement that closely follows along the pial surface of the brain, filling the subarachnoid spaces of the *sulci* and cisterns. This pattern is usually described as having a “gyriform” or “serpentine” appearance (D'Anjou et al., 2012; Keenihan, Summers, David, & Lamb, 2013; Smirniotopoulos et al., 2007). Each type of enhancement can be further classified according to the enhancement distribution in focal, regional, or diffuse (D'Anjou et al., 2012).

Figure 4. Schematization of type of meningeal enhancement with diffuse leptomeningeal on the left and diffuse pachymeningeal on the right (modified from (D'Anjou et al., 2012)).



However, leptomeningeal pathology does not always follow sulcal indentations, hence it may resemble primarily dural lesions on MR leading to misclassification (Sze, 1993). It is worth

mentioning that, as PmE is usually considered when meningeal enhancement does not extend into the *sulci* of the cerebrum or cerebellum and leptomeningeal enhancement is considered when regions of contrast enhancement extend into these *sulci*, it is possible that concurrent PmE may coexist in the latter classification and some cases classified has having leptomeningeal enhancement may represent enhancement of all three layers of the meninges (Kioumehr et al., 1995; Mellema, Samii, Vernau, & LeCouteur, 2002).

In the retrospective study of Keenihan et al. (2013) with 60 dogs with MRI of the brain and histopathologic examination including the meninges, correct anatomic classification of lesions occurred in 14/16 (88%) dogs with pachymeningeal lesions and 6/35 (17%) dogs with leptomeningeal lesions on basis of T1-W post-contrast images, and in 12/15 (80%) dogs with pachymeningeal lesions and 3/32 (9%) dogs with leptomeningeal lesions on basis of subtraction images. According to this study, a correct anatomic classification of lesions seems to occur more often for pachymeningeal than leptomeningeal lesions. The authors also observed six instances of dogs with leptomeningeal and brain lesions that were incorrectly classified as having pachymeningeal lesions, and examples of meninges correctly classified as unaffected (e.g. malignant oligodendroma) and leptomeninges incorrectly classified as unaffected (e.g., high-grade glioma).

The almost uniform character of leptomeningeal or PmE in the presence of infective, inflammatory, neoplastic, vascular or reactive changes is the reason for the restricted specificity of MRI, so that with the imaging process alone, only very limited information about the etiology is available. Clinical data, laboratory findings, examination of the CSF and histopathological examination are therefore essential for interpreting the meningeal changes and making a differential diagnosis (Schumacher & Orszagh, 1998).

2.2 Normal pachymeningeal enhancement

The *falx cerebri* and *tentorium cerebelli membranaceum* enhance in approximately 50% of normal human patients receiving gadolinium (Patel & Kirmi, 2009). On post-contrast T1-W images, thin and frequently discontinuous (i.e., not visualized on multiple contiguous sections), enhancement along the inner table, *falx cerebri* and *tentorium cerebelli membranaceum*, can be observed (Dietemann et al., 2005; Sze et al., 1989). Since the normal dural reflections near the superior sagittal sinus usually show considerable enhancement, it is recommended to avoid examination of midline sagittal sections only since this enhancement may be mistaken for abnormal meningeal enhancement (Sze et al., 1989).

A slight degree of enhancement of both the pachymeninges and the leptomeninges is normal in dogs. In the study of Joslyn et al. (2011), a degree of meningeal enhancement was a consistent finding in normal dogs. In this study, the authors found a small amount of enhancement, appearing as short thin meningeal segments, in all the 22 normal dogs studied.

In the study of Penning, Suckling, Evans, Chandler, and Cappello (2008) with 200 dogs, PmE occurred over the cerebral convexities and *falx cerebri* in over half the dogs with normal brains. Contrast material enhancement is a combination of two primary processes: intravascular enhancement and interstitial (extravascular) enhancement. If intact, the blood-brain barrier will prevent leakage of contrast material to the perivascular interstitial fluid. In regions with free capillary permeability, the contrast agent will leak across the vessel wall and begin to accumulate in the perivascular interstitial fluid. The vessels of the dura mater have a discontinuous endothelial cell layer, making them permeable (Kioumehr et al., 1995) but the irregularly arranged, tightly packed, collagenous fibers of the dura mater predominate over other intracellular and extracellular structures. Therefore, even though blood vessels of the dura facilitate exchange of compounds into and out of the dura mater, because the extracellular compartment of dural tissues is limited, relatively little contrast agent can accumulate extravascularly in this tissue (Quint et al., 1996). Even if a small amount of gadolinium chelate does manage to accumulate in the extravascular space of the dura mater, there is little imageable free water in the dense collagenous fibres to undergo the T1 shortening required for enhancement on MR images (Quint et al., 1996; Smirniotopoulos et al., 2007). It appears that the short segment convexity meningeal enhancement observed on contrast enhanced MR images do not represent intrinsic (extravascular) but extrinsic (intravascular) enhancement of meninges and should, therefore, be considered a normal finding (Quint et al., 1996). Intravascular pachymenigeal enhancement may be especially evident in case of oblique sectioning of the dural vessels (Penning et al., 2008).

2.3 Abnormal pachymeningeal enhancement

Intravascular enhancement is proportional to increases in blood flow or blood volume, and pathological situations may reflect neovascularity, vasodilatation or hyperemia, shortened transit time or shunting (Smirniotopoulos et al., 2007). It is hypothetised that PmE may be due to increased vascularity or angiogenesis secondary to malignant infiltration and inflammatory processes or due to dural hypertrophy secondary to meningeal irritation (Antony et al., 2015). Physical or chemical irritants may result in meningeal vascular dilatation and/or proliferation that contrast-enhances (Schumacher & Orszagh, 1998). In humans, the decrease in CSF pressure causing secondary fluid shift resulting in vasocongestion and interstitial edema of the dura mater is also hypothesized (Antony et al., 2015).

In human patients, PmE is considered pathological if present in multiple contiguous slices, if its signal intensity or thickness (>3 cm) is increased or if a nodular pattern is present (Kirmi et al., 2009; Patel & Kirmi, 2009; Quint et al., 1996). This pattern of enhancement is mainly detected at the intracranial level (whereas leptomeningeal enhancement is seen with similar frequency at the intracranial and intraspinal levels) and appears as an area of linear

enhancement along the inner table of the cranial convexity with involvement of the *falx* and *tentorium* (Dietemann et al., 2005).

In the study of D'Anjou et al. (2012) with 155 dogs consecutive dogs imaged for suspected brain disease with 1.5 tesla MRI equipment, the maximal thickness of contrast-enhancing meninges, excluding *sulci*, in dogs with neoplasia was significantly greater than in dogs with inflammation. In the same study, the mean ratio of meningeal signal intensity/normal thalamus signal intensity was greater in dogs with neoplasia. Kuchiwaki, Inao, Ishii, Ogura, and Sakuma (1995), measured the dural thickness of 12 normal dogs under atmospheric pressure and observed a mean value of 0.235 ± 0.023 mm, however, to our knowledge, there are no other studies reporting values of dural thickness in normal dogs based on direct measurement of the dura mater or on indirect measurement via MRI, therefore the cut-value for considering the dura mater as thickened does not exist. Qualitative, subjective criteria are used to detect contrast-enhancing dural thickening on MR images.

Several physiologic and pathological processes can lead to post-contrast pachymeningeal enhancement (Smirniotopoulos et al., 2007). In human medicine PmE and thickening can be “secondary,” when identifiable causes coexist, although their definite relationship may be debatable, including infectious agents, inflammatory or auto-immune processes, neoplastic diseases and several other possible causes, or it can be “idiopathic” when no possible cause is identified (table 1).

In humans, post-contrast leptomeningeal enhancement is generally associated with inflammatory or infectious meningitis, especially if diffuse, whereas PmE is more often indicative of meningeal neoplastic infiltration (Smirniotopoulos et al., 2007). In the study of Kioumehr et al. (1995) with 83 human subjects, 83% (25/30) of the cases with meningeal carcinomatosis, 100% (14/14) of the reactive (due to surgery, shunt or trauma), 100% (3/3) of the inflammatory and 13% (1/8) of the chemical (cysticercosis or dermoid cyst rupture, post intra-thecal chemotherapy) meningitis subgroups demonstrated PmE, while 100% (28/28) of the infectious meningitis and 87% (7/8) of the chemical meningitis subgroups had leptomeningeal enhancement. The diffuse or focal nature of the PmE and its location are also useful for differential diagnosis in humans. Regular and diffuse enhancement is suggestive of inflammation or intracranial hypotension either spontaneous or following spinal tap, whereas irregular or focal enhancement is suggestive of tumour (Dietemann et al., 2005). A trend for type of meningeal enhancement was not recognized in the study of Mellema et al. (2002) with 15 dogs and 3 cats with evidence of meningeal enhancement on MR images. In the study of D'Anjou et al. (2012), meningeal enhancement in dogs was more commonly leptomeningeal and diffuse with inflammatory disease while this enhancement was more variable in type and distribution in the neoplastic group. Although meningeal enhancement varied in type and distribution between inflammatory and neoplastic diseases in this study, there was no significant difference noted.

Table 1. Etiology of pachymeningeal enhancement and thickening in humans (adapted from Antony et al. (2015) Fain and Mekinian (2017), Kupersmith, Martin, Heller, Shah, and Mitnick (2004), Sylaja, Cherian, Das, Radhakrishnan, and Radhakrishnan (2002))

Infectious
Bacterial
Neurosyphilis
Tuberculous meningitis
Lyme disease
Meningococcus
Propionibacterium acnes
Complication of otitis or sinusitis
Fungal
Candida tropicalis
Cryptococcus neoformans
Pettriellidium boydii
Aspergillus flavus
Histoplasma
Coccidioides
Viral
Human T-cell lymphocytic virus I (HTLV-1)
Parasitic
Cysticercosis
Non-infectious
Inflammatory or autoimmune/vasculitic
Neurosarcoidosis
Granulomatosis with polyangeiitis
Erdhein-Chester disease
Ig4-related meningeal disease
Rheumatoid arthritis
Sharp syndrome
Behçet disease
Antiphospholipid syndrome
Giant cell arteritis
Sjögren syndrome
Temporal Arteritis
Neoplasic
Meningioma
Dural carcinomatosis
Lymphoma
Intracranial plasmocytome
POEM syndrome
Amylose-AL
Myelofibrose
Metastatic
Miscellaneous
Intracranial hypotension
Spontaneous
Post spinal fluid drainage
Neuro-surgical intervention
Peritoneo-ventricular derivation
Chronic intratecal drug administration
Brain radiotherapy
Chronic hemodyalisis
Mucopolysaccharidoses
Idiopathic cranial or spinal pachymeningitis

PmE was observed in association with otitis interna with extension to the brainstem (2 dogs and 1 cat), cryptococcal meningitis (2 dogs) bacterial meningitis (1 dog), and feline infectious peritonitis (1 cat) in the study of Mellema et al. (2002), however a trend for type of meningeal enhancement was not recognized in this study with 15 dogs and 3 cats.

Sampling of the CNS at necropsy may vary from case to case depending on the suspected disease process and priorities of the pathologist. At necropsy, the dura mater is retained routinely with spinal cord tissue samples, but only leptomeninges may be retained with brain slices unless it is macroscopically evident that the lesion also involves the pachymeninges. This practice allows the possibility that histologic changes affecting the pachymeninges might be missed in some cases (Keenihan et al., 2013). This fact makes it difficult to understand the histopathological results of the scientific articles, since the absence of results related to the dura mater can be due to the absence of pathological alterations or to the absence of the histopathological evaluation of this meninx. Furthermore, the classification of meningeal contrast pattern in MRI is not nearly common in the veterinary medicine scientific literature. Since the study of Mellema et al. (2002) was published, few authors have tried to incorporate it in their research, making articles that mention the observation of leptomeningeal or pachymeningeal enhancement very scarce.

2.3.1 Inflammatory pachymeningitis

Meningitis refers to inflammation of the meninges. In common usage, the term meningitis generally refers to inflammation of the leptomeninges (the pia mater, subarachnoid space, and adjacent arachnoid mater) in contrast to inflammation of the dura mater, which is referred to as pachymeningitis. Inflammation of specific parts of the dura mater of the cranial cavity can occur in the external periosteal dura after osteomyelitis, formation of extradural abscesses and pituitary abscesses, skull fracture and involve the inner dura in association with leptomeningitis (Zachary & McGavin, 2011).

The term inflammation can be used in a broad sense covering a wide range of reactive processes resulting from tissue injury, or in the traditional sense of the word comprising lesions characterized by strong participation of the immune system. In the CNS, morphological evidence of such an immune reaction consists of accumulation of inflammatory cells in the meninges, around the blood vessels and in the parenchyma (Marc Vandevelde, Higgins, & Oevermann, 2012). In chronic leptomeningeal inflammatory disease, the fibroblastic proliferation and fibrous collagenous thickening may become very extensive and potentially occlusive. Fibroblastic proliferation of the inner surface of the dura can occur in response to chronic dural irritation (tumours, vertebral subluxation, meningitis, etc.) (Marc Vandevelde et al., 2012).

Encephalitis refers to inflammation involving the brain parenchyma itself (Muñana, 1996). Because of the close anatomical association between the brain parenchyma and meninges,

an inflammatory process often involves both structures, causing a meningoencephalitis (Dewey & Da Costa, 2016; Muñana, 1996). Myelitis refers to an inflammatory process involving only the spinal cord parenchyma but not the meninges, whereas meningomyelitis is an inflammation of both the spinal cord and its surrounding meninges. Clinically, the distinction between the two diseases is based on the presence or absence of spinal pain. Dogs with pure myelitis have no spinal pain as the spinal cord (and brain) has no nociceptors (i.e. pain receptors) (Dewey & Da Costa, 2016). Pure myelitis and meningomyelitis are rare in small animals and mostly occur in a combination with inflammatory brain disease (Tipold, 1995; Tipold & Stein, 2010). Many inflammatory CNS diseases involve the brain, spinal cord and associated meninges, resulting in meningoencephalomyelitis (Sorjonen, 1992).

Some of these lesions are associated with infectious diseases but other result from derangement of the immune system (Marc Vandevelde et al., 2012), therefore inflammatory conditions affecting the meninges can be included in two broad categories: infectious inflammatory diseases of the CNS and non-infectious inflammatory diseases of the CNS.

Infectious inflammatory diseases of the CNS include viral, protozoal, rickettsial, bacterial, fungal, parasitic and algal agents. Leptomeninges and the pachymeninges can be involved mainly as meningitis in infections of the CNS associated with infection of the subarachnoid space (Marc Vandevelde et al., 2012). Some viruses such as canine distemper virus and rabies virus and some protozoans such as systemic *Toxoplasma gondii* and *Neospora caninum* are neurotropic, resulting primarily in diseases characterized by parenchymal inflammation. Other agents that are non-neurotropic become microembolized in the meningeal vasculature as a result of their systemic spread to many tissues. They cause disease as a consequence of their induction of vasculitis and meningitis with secondary parenchymal injury (Greene, 2012). The predominant clinical signs in viral, protozoal, and parasitic diseases relate to parenchymal involvement of the brain and/or spinal cord with associated meningeal reaction. Clinical signs in these conditions are more typical of encephalitis and/or myelitis than meningitis. Rickettsial and fungal diseases may display parenchymal as well as meningeal signs. Bacterial diseases most commonly present with meningeal signs, which may spread to display parenchymal signs (Luttgen, 1988).

The most frequently encountered viral infection in the dog brain is canine distemper. Rabies, canine herpesvirus, pseudorabies and West Nile virus are other less-common causes of meningoencephalitides in dogs. There are also reports of CNS involvement associated with canine adenovirus, canine parainfluenza virus, and canine parvovirus (Dewey & Da Costa, 2016). Encephalomyelitis has been reported in dogs after canine distemper and rabies vaccination (Greene, 2012).

Protozoal meningoencephalitis is most commonly caused by *Toxoplasma gondii* and *N. caninum* which occur worldwide. These organisms can be disseminated and cause

encephalomyelitis, destructive myositis, dermatitis or multifocal dissemination (Dewey & Da Costa, 2016; Muñana, 1996).

Canine meningoencephalitis due to a Sarcocystis-like organism (Dubey & Slife, 1990), to *Acanthamoeba castelloni* (Pearce, Powell, Chandler, & Visvesvara, 1985) and *Balamuthia mandrillaris* (Foreman, Sykes, Ball, Yang, & De Cock, 2004) have also been reported. Leishmaniosis has been found to cause granulomatous meningitis. Babesia is considered to be potentially associated with CNS inflammation but the neuropathology of such infection has not been well defined (Muñana, 1996; Marc Vandevelde et al., 2012).

Rickettsial infections infrequently involve the nervous system but may cause signs suggestive of meningitis (pyrexia, hyperesthesia) along with signs of parenchymal involvement (e.g., depression, paresis, seizures). However, these diseases are more commonly associated with extra-neural clinical signs (e.g., pyrexia, petechiation, anemia, lymphadenopathy, splenomegaly) (Luttgen, 1988). Erlichiosis, caused by *Ehrlichia canis* in many countries with warm climate around the world and Rocky Mountain Spotted Fever, caused by *Rickettsia rickettsii* in North America, are tick-borne diseases that can cause meningoencephalitis in dogs (Dewey & Da Costa, 2016; Meric, 1988; Muñana, 1996). A cerebellar meningitis caused by *Neorickettsia helminthoeca* present in salmon is also reported in dogs and foxes on the American Pacific coast (Muñana, 1996; Marc Vandevelde et al., 2012).

Bacteria by their nature are non-neurotropic, causing infection by producing meningitis or abscess formation (Greene, 2012). In contrast to humans, bacterial meningitis in veterinary patients does not seem to be caused by microorganisms with a predilection for the nervous system, therefore is relatively uncommon (Greene, 2012). In canine bacterial meningitis, organisms gain access to the subarachnoid space through hematogenous spread, contiguous infection from adjacent structures (middle/inner ear, nasal cavity, sinuses, orbit, skull, vertebrae and intervertebral disks), direct inoculation (trauma, bite wound, surgery, spinal needles), migration of foreign bodies or aberrant parasites (Dewey & Da Costa, 2016) and leukocytic trafficking (Marc Vandevelde et al., 2012). Once established, the inflammatory process usually spreads to involve parenchymal structures (meningoencephalomyelitis) (Luttgen, 1988). Additionally, CNS bacterial infection may result in focal parenchymal abscesses or empyema in the subdural or epidural space (Dewey & Da Costa, 2016). A variety of bacterial organisms have been reported in CNS of dogs, including aerobes such as *Staphylococcus* spp., *Streptococcus* spp., *Pasteurella* spp., *Actinomyces* spp., *Nocardia* spp, and anaerobes such as *Fusobacterium* spp., *Bacteroides* spp., *Peptostreptococcus* spp., and *Eubacterium* spp. (Greene, 2012; Muñana, 1996). The spirochete responsible for Lyme disease - *Borrelia burgdorferi* – as also been reported in rare cases to be involved in central neurologic abnormalities in dogs (Azuma, Kawamura, Isogai, & Isogai, 1993).

Fungal organisms usually reach the CNS via hematogenous or lymphatic routes but may also arrive via direct extension from adjacent infections, as in fungal sinusitis (Luttgen, 1988).

Although *Cryptococcus neoformans* is the most common fungal organism associated with meningoencephalitis in dogs, a wide variety of other fungal diseases may affect the CNS, including coccidioidomycosis (*Coccidioides immitis*), blastomycosis (*Blastomyces dermatitidis*), histoplasmosis (*Histoplasma capsulatum*), aspergillosis (*Aspergillus terreus*, *A.deflectus* , *A. flavipes* , and *A. fumigatus*), phaeohyphomycosis (*Cladophialophora* - formerly *Cladosporium*, *Xylohypha*, and *Exophiala*), hyalohyphomycosis (*Paecilomyces*) (Dewey & Da Costa, 2016; Greene, 2012; Meric, 1988; Muñana, 1996; W. B. Thomas, 1998).

The algae Prototheca (*Prototheca wickerhamii* and *P. zopfii*) has been reported to cause CNS disease in dogs both with and without disseminated disease (Marquez et al., 2012; Tyler, Lorenz, Blue, Munnell, & Chandler, 1980).

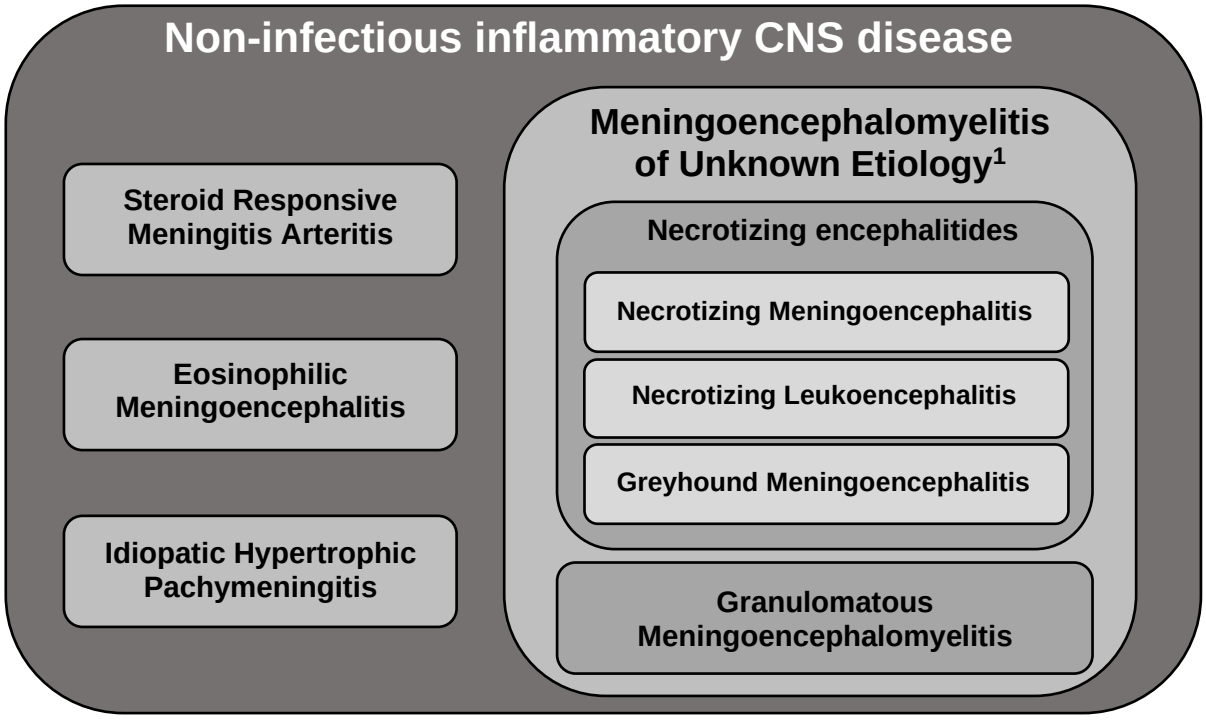
There are several reports describing aberrant parasitic migration to the brain of dogs and cats by organisms such as *Dirofilaria immitis*, *Baylisascaris procyonis*, *Angiostrongylus cantonensis*, *Cuterebra* spp*, Eucoleus (Capillaria) boehmi* (Bonawandt, Berg, Joseph, & Stefanacci, 2017; Clark et al., 2013; Dewey & Da Costa, 2016; Hamir, 1987; Lunn et al., 2012; Sartin, Hendrix, Dillehay, & Nicholls, 1986; J. S. Thomas, 1988).

Non-infectious meningoencephalomyelitis (NIME) can be divided into several subtypes, based mainly on the specific regions of the CNS that are affected and the specific histopathology (Coates & Jeffery, 2014) (figure 5). They are assumed to be immune-mediated because attempts to demonstrate an infectious ethiology have failed and also because many of these conditions are responsive to treatment with corticosteroids or other immunosuppressants (Marc Vandevelde et al., 2012). The underlying etiopathogenesis of this diseases is poorly understood (Hoon-Hanks, McGrath, Tyler, Owen, & Stenglein, 2018; Roynard, Behr, Barone, Llabres-Diaz, & Cherubini, 2012; Tipold & Schatzberg, 2010; Williams et al., 2008). Some of the most clinically relevant include granulomatous meningoencephalomyelitis (GME) (Braund, Vandevelde, Walker, & Redding, 1978), necrotizing meningoencephalitis (NME) (Cordy & Holliday, 1989; Tipold, Fatzer, Jaggy, Zurbriggen, & Vandevelde, 1993), necrotizing leukoencephalitis (NLE) (Tipold et al., 1993) and eosinophilic meningoencephalitis (EME) (Smith-Maxie, Parent, Rand, Wilcock, & Norris, 1989) and steroid-responsive meningitis-arteritis (SRMA). NME, NLE, and GME have characteristic locations of lesions on histopathologic examination and, therefore, characteristic findings from advanced imaging, that together with clinical signs and clinicopathologic data (annex 1), may aid in establishing an antemortem diagnosis. However, as these specific histologic subtypes cannot be identified on routine antemortem clinical test, in the case of presumptive clinical diagnosis without ante-mortem histopathological confirmation, the umbrella term meningoencephalomyelitis of unknown origin or meningoencephalitis of unknown etiology (MUE) should be preferred (Granger, Smith, & Jeffery, 2010; Talarico & Schatzberg, 2010).

Idiopathic eosinophilic meningoencephalitis is a rare NIME (Williams et al., 2008) but due to its fairly distinct disease signature based on clinical presentation, CSF abnormalities, and

histopathology (annex 2) it is not included by most authors in the MUE umbrella term (Coates & Jeffery, 2014).

Figure 5. Various non-infectious inflammatory central nervous system diseases (adapted from Coates and Jeffery (2014)).
1. Umbrella term used when an antemortem diagnosis of idiopathic necrotizing encephalitides or granulomatous meningoencephalomyelitis is suspected but histopathological diagnosis is not available.



Reported non-infectious inflammatory lesions affecting primarily the meninges include SRMA and the recently described idiopathic hypertrophic pachymeningitis (Roynard et al., 2012). SRMA is a common NIME also known by other names such as necrotizing vasculitis, polyarteritis, panarteritis, juvenile polyarteritis syndrome, beagle pain syndrome, corticosteroid-responsive meningitis, aseptic suppurative meningitis, steril eitrige meningitis (sterile purulent meningitis) (Tipold & Schatzberg, 2010). It is considered a systemic immune disorder that, in its acute form is characterised by an extensive suppurative leptomeningitis associated with severe arteritis and fibrinoid necrosis of the arterial wall (Annex 2). Even though massive extension into the meninges and ventricular system of the forebrain is reported, spinal cord meninges are more severely affected than the brain meninges (de Lahunta et al., 2015). Wrzosek, Konar, Vandevelde, and Oevermann (2009) reported a case of a boxer with SRMA with massive extension into the meninges and ventricular system of the forebrain showing that widespread suppurative meningo-ependymitis can be a complication of SRMA. Webb, Taylor, and Muir (2002) observed that 46% of the dogs having non-infectious, non-erosive, idiopathic immune-mediated polyarthritis and spinal pain have concurrent SRMA.

Hypertrophic pachymeningitis (HP) is a clinical disorder due to localized or diffuse thickening of the dura mater, with or without an associated inflammatory process. When the evaluation

fails to reveal a cause, idiopathic hypertrophic pachymeningitis (IHP) is considered (Kupersmith et al., 2004). Scarce studies contemplating IHP exist in veterinary medicine (Cherubini, 2007; Hess & Sellon, 1997; Luján & Foote, 2007; Roynard et al., 2012). In human medicine it is also a rare disorder, however, since its first report in the spine by Charcot and Joffroy (1869) and intracranially by Mott (1909), more than a hundred cases have been documented. Due to the lack of adequate neuroimaging techniques in the pre-MRI era, the prevalence of HP is likely to be underestimated in humans (Rossi et al., 2004) and, presumably, in dogs. HP has been increasingly recognized both in humans and in dogs thanks to the advent MRI where a thickened abnormally enhancing dura mater can be demonstrated (Friedman & Flanders, 1997; Keenihan et al., 2013; Kupersmith et al., 2004; Luján & Foote, 2007; Martin et al., 1989; Roynard et al., 2012).

In humans, HP can be classified as cranial HP, which is rarer and typically involves the posterior fossa, and spinal HP, where the thoracic and cervical regions are most commonly affected (Friedman & Flanders, 1997).

IHP is a diagnosis of exclusion. A thorough workup includes search for infectious, autoimmune and neoplastic diseases. Dural biopsy is essential to establish the diagnosis of IHP and to exclude other causes of pachymeningitis. Pathological findings of idiopathic hypertrophic cranial pachymeningitis in humans consist of thick fibrous dura often associated with chronic inflammatory cell infiltrate comprising lymphocytes and plasma cells (Friedman & Flanders, 1997).

In small animals, even though there are only a few reported cases, there are some cases of isolated idiopathic hypertrophic cranial pachymeningitis and idiopathic hypertrophic spinal pachymeningitis and cases where both are present in dogs. Roynard et al. (2012) observed that the most common intracranial site for pachymeningeal enhancement in dogs with idiopathic hypertrophic intracranial pachymeningitis was the dorsolateral part of each cerebral hemisphere, which was present in all six dogs in either the parietal or frontal cortex. However, the idiopathic nature of five the dogs studied is questionable since histopathology was not performed, therefore, exclusion of other possible causes of pachymeningitis in the other five dogs is not possible. Cherubini (2007) reported one of the cases included in the study of Roynard et al. (2012) with intracranial HP in a dog that also presented spinal hyperesthesia, however spinal MRI is not mentioned by the author. Spinal idiopathic pyogranulomatous pachymeningitis with mass effect was reported in dogs by Hess and Sellon (1997) causing right-sided spinal cord compression at C4-C5 vertebral level, and by Luján and Foote (2007) causing left-sided compression at L4 vertebral level.

Dural ossification, has been reported as a cause of thickening of the dura mater, however, even though it is also known as ossifying pachymeningitis, the latter is considered an unfortunate misnomer as there is no inflammation present in the condition (de Lahunta et al., 2015; Miller, 2013). Instead, it is characterized by the metaplastic formation of round or elliptical

bone plaques on the inner surface of the dura mater, that may coalesce until they form a solid tube within the dura, often containing hematopoietic bone marrow, and it is mostly reported in large and giant breed dogs, particularly in cervical and lumbar segments of the spinal cord (de Lahunta et al., 2015; Morgan, 1969; Marc Vandevelde et al., 2012).

2.3.1 Neoplastic pachymeningeal disease

The meninges may be a site of primary neoplasia or may be affected secondarily by local neoplastic spread or distant metastasis (Keenihan et al., 2013). Meningeal involvement or metastasis varies greatly with the location and nature of the primary growth (Schumacher & Orszagh, 1998). In humans, PmE has been observed in calvarial/vertebral lesions, extra-axial neoplasms such as meningiomas, primary and secondary brain tumours and in leptomeningeal metastases (Freilich, Krol, & DeAngelis, 1995; Paakko, Patronas, & Schellinger, 1990; Phillips, Ryals, Kambhu, & Yuh, 1990; River, Schwartz, Gomori, Soffer, & Siegal, 1996; Senegor, 1991; Smirniotopoulos et al., 2007; Sze et al., 1989; Wilms et al., 1991).

Meningioma is the most common primary meningeal neoplasm both in humans and in dogs (Smirniotopoulos et al., 2007; Sturges et al., 2008). This tumour arises from arachnoid cells and expand in the subarachnoid space on the surface of the brain. A common source is from arachnoid cap cells, which are found as a cluster of arachnoid cells on the apical surface of an arachnoid *villus* that projects into a venous sinus (de Lahunta et al., 2015). Meningiomas appear consistently as a round/ovoid or plaque-like shape extra-axial mass smoothly marginated with a broad-based contact with adjacent bone (Dewey & Da Costa, 2016). Canine menigiomas can arise anywhere along the dura within the calvarium at its floor, lateral convexities, or the *falx*, most commonly occurring in the rostral fossa. Occasionally they may arise within the ventricular system or hypophyseal fossa (Kraft & Gavin, 1999; Marc Vandevelde et al., 2012). They are presented typically as single lesions, but multiple tumours may be found on occasion. They are hypointense to isointense on T1-W images, hyperintense on T2-FLAIR images, and show strong and homogeneous to heterogeneous contrast enhancement (Dewey & Da Costa, 2016). Meningiomas can enhance after gadolinium because they lack a blood-brain barrier and have tendency to develop congestion and interstitial edema (Smirniotopoulos et al., 2007). A “dural tail sign” (thickening and enhancement of the dura adjacent to an extra-axial mass) is frequently present and is strongly suggestive of but not specific for meningioma (Dewey & Da Costa, 2016). It is thought to be caused by neoplastic infiltration within or on the surface of the meninges, loose connective tissue proliferation, or vasodilatation or hypervascularity of the dura mater (Adamo, Forrest, & Dubielzig, 2004). In humans, this finding was originally thought to represent dural infiltration by tumour, and resection of all enhancing dura mater was thought to be appropriate but later studies helped confirm that most of the linear PmE, especially when it was more than a centimeter away from the tumour bulk, was probably caused by a reactive process that

includes vasocongestion and accumulation of interstitial edema, both of which increase the thickness of the dura mater (Smirniotopoulos et al., 2007). A dural tail although highly suggestive for meningiomas is non-specific and may accompany extra-axial lesions and peripheral intra-axial lesions such as histiocytic sarcomas (Tamura, Tamura, Nakamoto, Ozawa, & Uchida, 2009), pituitary macroadenomas (Wisner, Dickinson, & Higgins, 2011), choroid plexus papilloma (Rodenas, Pumarola, Gaitero, Zamora, & Anor, 2011).

In humans, metastatic disease can demonstrate variations of pachymeningeal and leptomeningeal enhancement, particularly breast carcinoma in women and prostatic malignancy in men (Smirniotopoulos et al., 2007). Although more often pachymeningeal in type (Kioumehr et al., 1995), meningeal neoplastic infiltration can also manifest as leptomeningeal enhancement as meningeal involvement by a metastatic tumour can be initially due to hematogenous deposition in the subarachnoid space or cerebral ventricles, from which the seeding develops along the CSF routes. Later, the tumour cells can grow into dural, meningeal or ependymal structures. This is generally preceded by a hematogenous seeding in the dura, ependyma or leptomeninx (Schumacher & Orszagh, 1998).

Tumours of surrounding structures of the CNS may also spread into the CNS by extension. These include craniofacial neoplasms (e.g. nasopharyngeal carcinoma) or bony tumours of the calvaria, skull base (e.g., osteosarcoma, chondrosarcoma, and multilobular osteochondrosarcoma) in which case the foramina and fissures are frequently the route to infiltration of the nearby dura (Dewey & Da Costa, 2016; Schumacher & Orszagh, 1998). Migration along perineural or perivascular structures is less common. Direct growth forward into the meninges also occurs with primary cerebral tumours or cerebral metastases which are situated superficially in the cortex (Schumacher & Orszagh, 1998) and with pituitary, and cranial nerve tumours (Dewey & Da Costa, 2016).

Neoplastic meningitis is the term used to describe the pathological condition that results from the spread of malignant cells to the leptomeninges and subarachnoid space and dissemination of tumour cells within the CSF compartment. Neoplastic meningitis is reported in human patients with solid tumours (carcinomatous meningitis), haematological malignancies (leukaemic or lymphomatous meningitis) and primary brain tumours (Gleissner & Chamberlain, 2006).

Meningeal carcinomatosis, also known as carcinomatous meningitis, is a rare complication of extra-neural solid tumours and consists of a focal, multifocal or diffuse malignant infiltration of neoplastic cells in the leptomeninges of the brain and/or spinal cord (Grossman & Moynihan, 1991). Even though this term is usually reserved for extra-neural tumours in human literature, in veterinary medicine it is also used to describe meningeal metastases caused by choroid plexus tumours (Lipsitz, Levitski, & Chauvet, 1999; Patnaik, Erlandson, Lieberman, Fenner, & Prata, 1980).

Intracranial meningeal carcinomatosis has been reported in dogs with carcinoma of mammary (Behling-Kelly, Petersen, Muthuswamy, Webb, & Young, 2010; Mandara, Rossi, Lepri, & Angeli, 2007; Pumarola & Balasch, 1996), intestinal (Stampley, Swayne, & Prasse, 1987) and choroid plexus origins (Lipsitz et al., 1999; Patnaik et al., 1980; Westworth et al., 2008). Mateo, Lorenzo, Munoz, and Molin (2010) reported a case of intracranial meningeal carcinomatosis in a dog but it was not possible to identify the primary tumour. Spinal meningeal carcinomatosis is reported in a dog with choroid plexus carcinoma (Patnaik et al., 1980). Mandara, Pavone, Ricci, and Giovanni (2007) reported a case of a dog with a transitional carcinoma of the nasal cavity that produced a direct seeding of cancerous clusters in epidural and subarachnoid space of the brain, without neurological signs. In cats, intracranial meningeal carcinomatosis has been described in two cases with squamous cell carcinoma of the external ear (Salvadori, Cantile, & Arispici, 2004) and spinal meningeal carcinomatosis with parenchymal infiltration has been described in one case with a locally invasive pulmonary adenocarcinoma (Posporis et al., 2017).

Lymphoma may occur as a primary intracranial neoplasm (Kraft et al., 1990; Palus, Volk, Lamb, Targett, & Cherubini, 2012) or as a metastatic neoplasm, which is more likely to affect the meninges (Keenihan et al., 2013). CNS lymphoma is reported both in dogs (Snyder, Shofer, Van Winkle, & Massicotte, 2006) and cats (Troxel et al., 2004). However, to our knowledge, even though primary dural lymphoma is well-reported in humans (Iwamoto & Abrey, 2006), there are no reported cases in dogs. Conversely, neoplastic meningitis due to haematological malignancies such as lymphoma is reported to be responsible for both leptomeningeal and PmE on MRI (Mellema et al., 2002). Vernau et al. (2000) observed diffuse intracranial PmE associated with metastatic acute B-cell lymphoblastic leukaemia in a 6-year-old German Shepherd Dog. According to the authors, neoplastic cells were observed in the inner surface of the dura mater and leptomeninges over the left side and caudal half of the right side of the intracranial surface of the parietal bone and infiltrated in the dura mater of the spinal cord, and in some of the nerve roots. In the study of Morozumi et al. (1993), diffuse enhancements at the margin of cerebral cortex and *falx* cerebri was observed in 3-year-old Japanese domestic cat with lymphocytic leukaemia associated with FeLV infection and histopathological examination after necropsy confirmed leukemic cells infiltrated in the dura mater and the subarachnoid space. In this case study, extradural involvement of the cancer cells extended to the cervical spinal cord and severe myelomalacia with haemorrhage were also observed on the thoracic and lumbar spinal cord.

2.3.2 Other possible causes

Iatrogenic diffuse PmE has been reported in humans post-operatively to accompany craniotomy as a linear enhancement of the dura adjacent to the operative site and ventricular shunt placement as diffuse PmE (Antony et al., 2015). Radiotherapy and intrathecal

chemotherapy are other iatrogenic causes of PmE in humans (Kioumehr et al., 1995). Trauma, lumbar puncture and hydrocephalus are other reported causes of pachymeningeal enhancement (Kioumehr et al., 1995). Even though it is also possible to occur in dogs, further studies are necessary to confirm it.

Lazzerini et al. (2017) observed 2 dogs with ethmoidal meningoencephalocele with a thickened fibrotic dura mater. Even though the MRI findings of these 2 dogs were not specified in this study, the authors found that 82% (14/17) of the intranasal meningoencephaloceles and 50% (3/6) of the dogs with parietal or frontal meningocele presented meningeal enhancement on MRI.

Intracranial hypotension may lead to vasocongestion and interstitial edema in the dura mater and is a benign cause of localized or diffuse PmE in human patients after skull surgery or fracture or with idiopathic loss of CSF pressure (Smirniotopoulos et al., 2007). The latter is not reported in veterinary medicine.

PART III: CANINE INTRACRANIAL PACHYMENINGEAL ENHANCEMENT: A STUDY OF 2 CLINICAL CASES

1 METHODS

A retrospective evaluation of the clinical data from three dogs with PmE and thickening presented to the *Referência Veterinária* clinic (RRV) (Cascais, Portugal) between 2008 and 2018 was performed. Patients were selected if evidence of intracranial PmE and thickening was present on MR images. The following parameters were retrieved from the dog's clinical records: signalment, age, historical findings, extraneural and some neural clinical signs (e.g., seizure activity), blood and CSF analysis and imaging techniques (x-ray and MRI). Treatment and follow-up information regarding clinical outcome were reviewed as well. Ancillary laboratory procedures reviewed included, whenever available, complete blood count (CBC), blood chemical profile and cytology, protein determination, and infectious disease tests of CSF collected from the cerebellomedullary cistern with the dog in lateral recumbency under general anaesthesia after performing the MRI.

Video footages of neurological exams were reviewed to identify some of the neurological signs and localise lesions within nervous system. X-ray images were evaluated when available.

MR images of the brain of the selected patients were reviewed to determine the location and extent of PmE and thickening and to detect the existence of additional intra- or extra-axial abnormalities. PmE was considered present when regions of curvilinear enhancement overlying the brain without extending into the *sulci* manifesting up against the bone, immediately deep to the inner table of the calvaria or involving the dural reflections of the *falx cerebri*, *tentorium cerebelli membranaceum* were visualized on multiple contiguous sections on post-contrast T1-W images.

All MRI included in this study were performed at RRV with dogs under general anaesthesia in sternal recumbency in a 0.18T open permanent magnet (Vet-MR 0.2T, ESAOTE, Genova, Italy). MR images of the brain were acquired using different transmitter-receiver coils dependent on the patient's head size. Transverse T2-W images (TR = 3800 - 4370 ms, TE = 100 ms), dorsal T2-FLAIR images (TR = 5830 ms, TE = 100 ms, TI = 1400 ms) and sagittal and transverse T1-W images (TR = 400 - 1220 ms, TE = 18 - 26 ms) as well as T1-W reconstructional images in one of the cases were retrieved from the institutional image archive system for review. Sagital and transverse post-contrast T1-W images were obtained immediately after intravenous administration of a bolus of 0.1 mmol/kg gadoterate meglumine (Dotarem, Guerbet, Roissy, France).

2 CASE REPORTS

Three records of dogs with evidence of post-contrast intracranial pachymeningeal enhancement, were found. One of the records was excluded because no pre-contrast T1-W images were available and CSF was contaminated with blood.

2.1 Case 1

A 13-year-old spayed female black Labrador Retriever, weighting 39 kg, was referred to RRV with the main complaint of episodic involuntary movements of the neck and paraparesis.

Short duration episodes of involuntary movements of the neck started two days before referral, occurring multiple times a day both spontaneously and induced by visual stimuli (e.g, sudden moves). These episodes did not change since the onset.

Paraparesis started three months earlier. It worsened within the two weeks before referral and was initially treated by the referring veterinarian with firocoxib for 8 days showing no improvement. Afterwards, methylprednisolone 0.6 mg/Kg *per os* (PO) every 12 hours (q12h), a B-vitamin complex supplement and sucralfate were administered during four days and mild improvements were observed.

The tutor also reported vision deficits as sometimes she bumped into objects at home.

Previous medical history is scarce since no data was available at the first-opinion clinic. Seven years before onset of clinical signs, the patient had pneumonia. One year after that, the patient was spayed after giving birth to a litter of puppies. One year before the referral the patient suffered babesiosis. Cataracts have been present in both eyes. Sitalan and Karsivan were prescribed by the referring veterinarian for several months.

At neurologic examination the patient was bright, alert, and responsive. She was unable to transfer from sitting to standing, was able to stand and take a few steps without support, but moderate paraparesis and ataxia caused stumbling and falling.

Postural reactions (proprioceptive positioning and hopping reaction) were profoundly delayed on the PL. Hopping reaction was subtly delayed on the thoracic limbs (TL) (the latter finding was inconsistent), but proprioceptive positioning was normal in both TL. Segmental spinal reflexes were normal in the TL and normal to slightly increased in the pelvic limbs (PL), with extensor toe reflex present in both PL. Cranial nerve evaluation was normal except for the repetitive blinking at the left menace response test. Signs of pain were elicited bilaterally on palpation of the skull and when the dorsal trunk skin was pinched with a hemostat during cutaneous trunci reflex testing at its full length. Series of brief myoclonic jerk episodes lasting less than a second were seen involving the neck and face in a short head retraction movement accompanied with bilateral repetitive eye blinking. These episodes could also be elicited by visual stimuli.

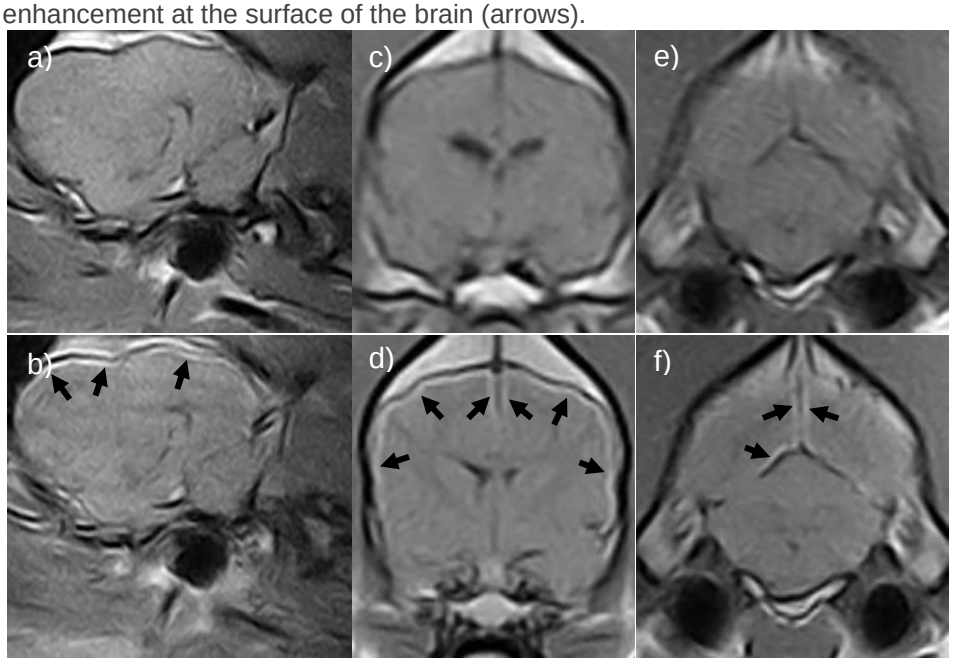
The neurologic examination revealed UMN paraparesis suggesting a lesion in spinal cord segments T3-L3. Acute, non-progressive myoclonic jerks' episodes were compatible with myoclonic seizures, therefore forebrain lesion was also considered.

The extension of the palpation areas that elicited pain, added to a multifocal or diffuse lesion, e.g., meningeal or thalamic lesion.

Based on the history, general condition and results of neurological examination, a multifocal (forebrain and T3-L3 spinal cord region) or diffuse lesion were suspected.

Thoracic, cervical and thoracolumbar spinal radiographs were unremarkable except for multiple areas of spondylosis in the thoracic and lumbar spine. Intracranial post-contrast transverse T1-W MR images revealed a symmetric, bilateral curvilinear meningeal enhancement at the frontal, parietal and temporal cortices and *falx cerebri* (figure 6). This curvilinear enhancement was also present at the ventral surface of the right occipital cortex in the *tentorium cerebelli membranaceum*. The enhanced structures did not extend into the *sulci* suggesting an altered dura mater. T2-W and FLAIR images did not show any abnormal imaging features.

Figure 6. Case 1 pre-contrast (above) and post-contrast (below) T1-W images, left parasagittal view (a,b) and transverse views at the level of the optic chiasm (c, d) and caudal fossa (e, f) at presentation. Note the curvilinear enhancement at the surface of the brain (arrows).



The studied region of the spinal cord (T3-L3) showed no significant extradural compressions that could explain clinical signs. CNS parenchyma did not show any signs of lesions in the brain and spinal cord MR images collected.

CSF collected from cerebellomedullary cistern at the end of the MRI exam showed 120 cells/µL with mixed pleocytosis with predomination of lymphoid cells.

Differential diagnoses included inflammatory diseases (including infectious meningitis and IHP) and neoplastic diseases (including lymphoma, meningioma, meningeal carcinomatosis, histiocytic sarcoma and plasma cell tumour).

A differential diagnosis of probable symptomatic epilepsy was made. The dog was started on phenobarbital 3.5 mg/kg PO q12h for a presumptive diagnosis of a focal seizure disorder of undetermined origin, methylprednisolone 2 mg/kg PO q12h as an immunosuppressive agent, as well as doxycycline 10 mg/kg PO q24h for antibiotic coverage and tramadol 3 mg/kg PO sporadically for pain management.

One and a half week after presentation, the myoclonic jerk episodes were no longer present and an initial improvement in gait was reported, however, paraparesis and pain worsened with corticosteroid dosage reduction. CSF was negative for *Mycoplasma spp*. (polymerase chain reaction (PCR)) and *N. caninum* (RT-PCR) but positive for *Toxoplasma gondii* (PCR)), therefore clindamycin 15 mg/kg PO q12h was started and prednisolone was tapered to 0.25 mg/kg PO q12h. Gabapentin 2.5 mg/kg PO every 8 hours (q8h) was added for pain management. Clinical sigs improved in the following week.

One and a half week after introducing clindamycin, prednisolone was reduced to 0.25mg PO q24h and phenobarbital was reduced to 2.5 mg/kg PO q12h. Two and a half weeks after introducing clindamycin, signs of pain were no longer observable and paraparesis improved even though it was still present. During the following two weeks, paraparesis and pain worsened and prednisolone was increased to 0.25 mg/kg PO q12h and tramadol 3mg/kg PO in the evening was added due to constant vocalizations during the night. Five weeks after introducing clindamycin, the dog presented with non-ambulatory tetraparesis and generalized hyperaesthesia. Proprioception deficits were present in both PL and TL, withdrawal reflex was reduced in all limbs however, patellar and cranial tibial reflexes were present. Cranial nerve evaluation was normal. Intracranial post-contrast MR images were similar to presentation (figure 7), additionally, a curvilinear enhancement was also present at the *tentorium cerebelli membranaceum* adjacent to the left dorsal surface of the cerebellum and a bilateral extra-axial dorso-lateral medular thickening compatible with cervical meninges was observed at the fifth cervical spinal cord segment (figure 8).

CSF collected from cerebellomedullary cistern at the end of the MRI exam showed 290 cells/µL. A mononuclear pleocytosis was observed with vacuolized mononuclear cells and reactive lymphocytes. Pandy reaction test revealed high globulin concentration (3+). *T. gondii* IgG titer and PCR were negative in CSF as well as *N. caninum* IgG and RT-PCR. Immunosuppressive dosage of prednisolone was started, however, the dog was euthanized a week later.

Figure 7. Case 1 pre-contrast (above) and post-contrast (below) T1-W images, left parasagittal view (a,b) and transverse views at the level of the optic chiasm (c, d) and caudal fossa (e, f) 6.5 weeks after presentation. Note the curvilinear enhancement at the surface of the brain (black arrows), including *falx cerebri* (arrow heads), and left *tentorium cerebelli membranaceum* (white arrow).

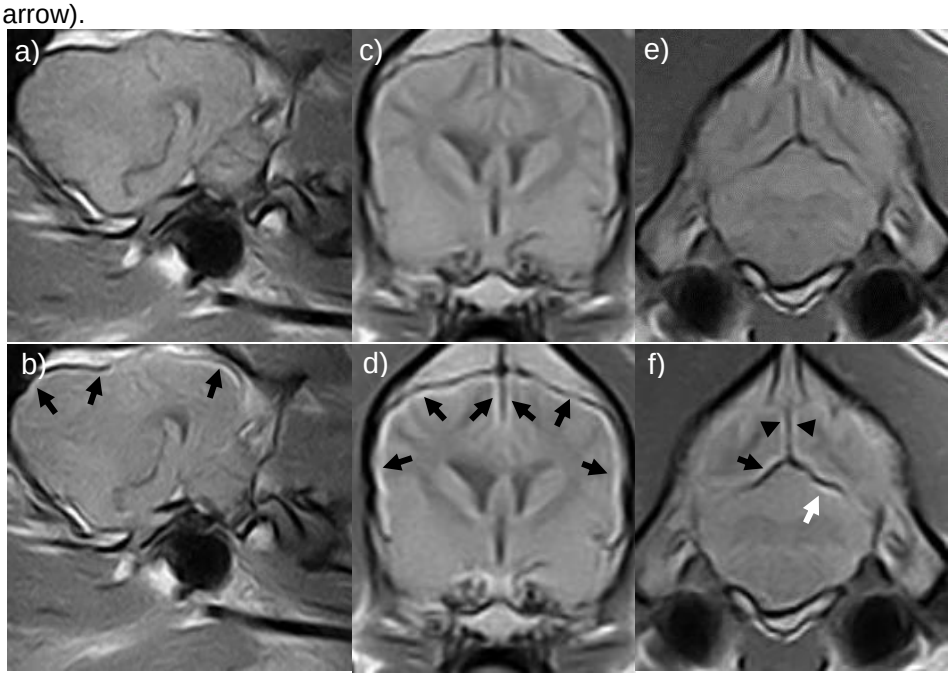
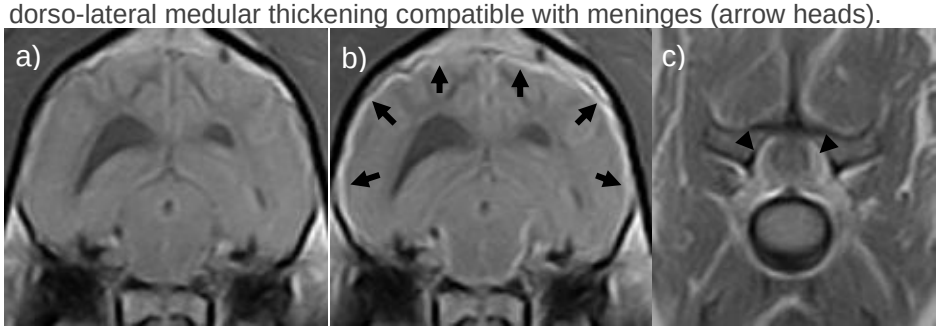


Figure 8. Case 1 pre-contrast (a) and post-contrast (b,c) T1-W images, transverse views at the level of the mesencephalic aqueduct (a,b) and fifth cervical spinal cord segment (c) 6.5 weeks after presentation. Note the curvilinear enhancement at the surface of the brain (arrows) and the extra-axial dorso-lateral medular thickening compatible with meninges (arrow heads).



2.2 Case 2

An 11-year-old spayed female brown crossbreed dog, with 16.4 kg of body mass, was referred to RRV for thoracic radiography and neurocranium MRI with history of acute unilateral blindness, progressive changes in general condition, pain and paraparesis.

Three days before presentation at RRV, the animal was observed at the referring hospital for discomfort and pain in the left side of the head. The left eye was painful at palpation, had a slight protrusion of the third eyelid and left menace response was absent. Cataracts were present in both eyes. Schirmer tear test, intraocular pressure and fundoscopy were normal for both eyes. Systolic pressure was 190 mmHg. Complete blood count (CBC) and biochemistry panel – blood urea nitrogen, creatinine, total proteins, albumin, alkaline phosphatase, alanine

aminotransferase - were normal except for glucose that was slightly raised 133 mg/dL (76-119 mg/dL). Abdominal ultrasound was also normal.

At presentation day, the dog's condition detiorated as she became lethargic, her appetite decreased and paraparesis and proprioception placing deficits on the PL were observed by the referring veterinarians. Methylprednisolone was prescribed and the dog was referred to RRV to perform head MRI.

Animal's background included up to date vaccination status for rabies virus (Nobivac Rabies®, MSD Animal Health, Ltd), canine distemper virus, canine type-2 adenovirus, canine parvovirus and canine parainfluenza virus (Nobivac DHPPi®, MSD Animal Health, Ltd) and leptospirosis (Nobivac Lepto®, MSD Animal Health, Ltd). Previous medical problems included an all-in-one surgical resolution of a right aural hematoma, tail mass and periodontal disease 7 months before and a fractured forth upper right premolar registered 2 months before presentation that was medically treated at 10 days before presentation with a metronidazole/spiramycin association (Stomorgyl®, Boehringer Ingelheim Animal Health), carprofen, wound cleaning and topical antiseptics.

Neurologic examination at RRV showed a normal, alert mental state. Gait evaluation showed spastic paraparesis and pelvic limb ataxia. Pelvic limb hypertonia was bilateral but clearly asymmetric, with the left pelvic limb more hypertonic than the right one. Postural reactions and proprioceptive placing were normal at TL and delayed at PL. Cranial nerve evaluation was normal except for the absence of menace response and pupillary light reflex (PLR) in the left eye. The animal showed signs of pain at palpation in the left peri-orbital area.

The absent left menace response and PLR together with the normal palpebral reflex suggested an intracranial lesion, affecting the left afferent pathway of vision anywhere from the ipsilateral retina or optic nerve and contralateral optic tract or pretectal region. The dog's gait and postural reaction deficits suggest a lesion caudal to T2 spinal cord segment, proprioceptive placing deficits would suggest a left-sided lesion. It was not possible to further neurolocalize the lesion causing paraparesis because spinal reflexes test results were not available in the video records and were not mentioned in the medical records, however, the asymmetric spastic paraparesis with unaltered TL function is highly suggestive of focal or diffuse spinal cord lesion between the T3 and L3 spinal cord segments, worse on the left side.

The available information pointed to a multifocal neuroanatomical localization, however a more diffuse lesion was also possible.

Thoracic radiograph was unremarkable. Intracranial post-contrast transverse T1-W MR images revealed curvilinear meningeal enhancement that did not extend to the *sulci* at the level of the *falx cerebri*, bilaterally at the *tentorium cerebelli membranaceum* and at the left temporal cortex and left piriform lobe (figures 9 and 10). The enhanced structure did not extend into the *sulci* and was compatible with an altered dura mater. No intracranial intraparenchymal

enhancement or mass effect was present. T2-W and FLAIR images did not show any abnormal imagiological features.

Figure 9. Case 2 pre-contrast (above) and post-contrast (below) T1-W images, left parasagittal (a, b) and transverse view at the caudal fossa (c, d) at presentation. Note the curvilinear enhancement at the left temporal cortex and left piriform lobe (black arrows), *tentorium cerebelli* (black arrow heads) and *falx cerebri* (white arrow head).

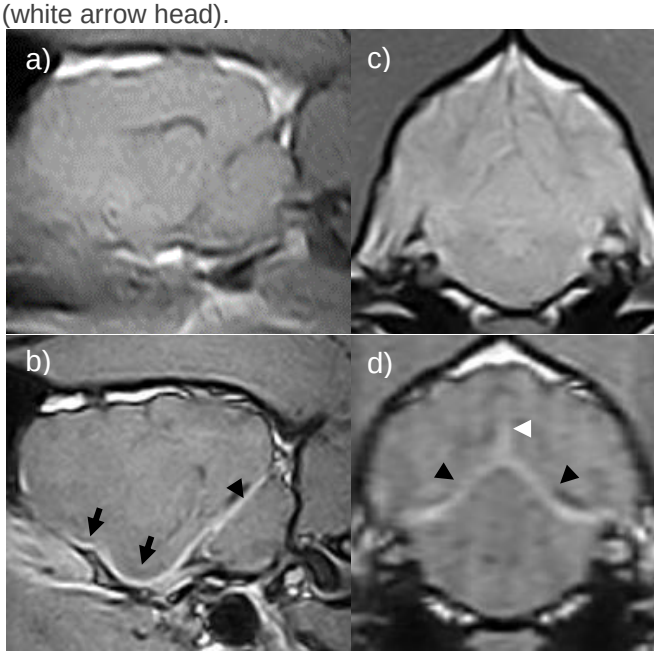
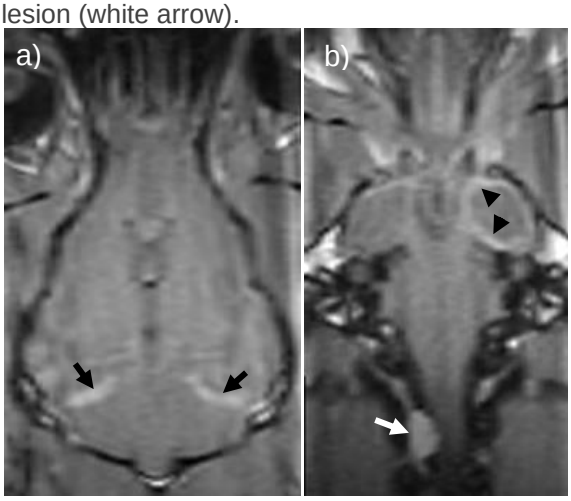


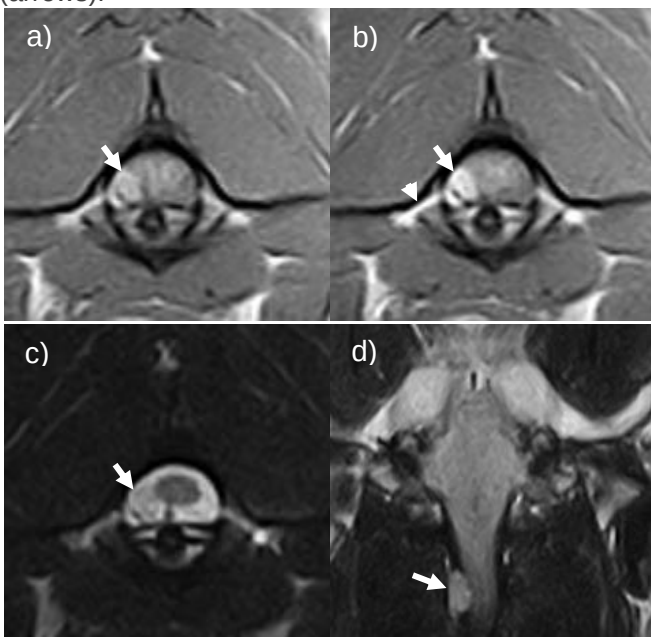
Figure 10. Case 2 post-contrast T1-W multiplanar reformation plain images, dorsal views at presentation. Note the curvilinear enhancement at the *tentorium cerebelli membranaceum* (black arrows) and at the outline of the left piriform lobe (black arrow heads) and the enhanced extramedular nodular lesion (white arrow).



A nodular lesion approximately with 5 mm of diameter with high signal intensity was observed in pre-contrast T1-W images on the right side of the spinal cord, located between the atlas and

axis, in an extramedullary position (figure 11). This lesion had high signal intensity in T2-W images (lower signal intensity compared to CSF) that did not supress in FLAIR sequence and suffered moderate enhancement after contrast administration on T1-W images (figure 11). Involvement of the dura mater adjacent to this extra-axial nodule was observed on FLAIR (figure 11), and in the dorsal post-contrast T1-W images, possibly corresponding to a dural tail sign (figure 10). Even though a mass effect was noted due to a displacement of the spinal cord to the left, no intraparenchymal lesions were observed in the spinal MR images collected. The right sided C2 spinal nerve also enhanced on post-contrast T1-W sequence.

Figure 11. Case 2 Transverse pre-contrast (a), post-contrast (b) T1-W images and T2-W images (c) at the transition between C1 and C2 vertebrae and dorsal FLAIR image (d), 6.5 weeks after presentation. Note the extramedullar nodular lesion (arrows).



CSF collected from cerebellomedullary cistern after the MRI exam was clear and colourless, showed more than 360 cells/µL with eosinophilic pleocytosis and occasional neutrophilic and mononuclear cells. Albumin detection with urine dipstrip was within normal limits (30 mg/dL). A CSF sample was also sent to an external laboratory for infectious disease testing.

Differential diagnoses included inflammatory diseases (including infectious meningitis and idiopathic eosinophilic meningoencephalitis) and neoplastic diseases (including lymphoma, meningioma, meningeal carcinomatosis, histiocytic sarcoma and plasma cell tumour).

The dog was treated initially with methylprednisolone 0.5 mg/kg SC q12h as an anti-inflammatory agent, as well as trimethoprim/sulphonamide association 15 mg/kg PO q12h for antibiotic coverage and gabapentin 12 mg/kg PO q8h for pain management.

Three days after presentation at RRV, methylprednisolone was substituted for prednisolone 0.6 mg/kg PO q12h and the animal was discharged from the referring hospital and re-evaluated

at RRV. Neurologic examination showed normal, alert mental status. Gait evaluation, postural reactions and proprioceptive placing test results were the same as at presentation. Cranial nerve evaluation was similar as the previous one except for the PLR in the left eye that improved from absent to decreased. The dog showed no signs of pain.

The results of infectious disease tests (i.e., enzyme-linked immunosorbent assay detection of antibodies against *C. neoformans*, detection of antigens of *D. immitis* and *A. vasorum* in CSF, detection of *N. caninum* by RT-PCR, detection of *Ehrlichia sp*p., *Mycoplasma spp*., *T. gondii*, canine distemper virus and *Aspergillus spp*. by PCR both in CSF and total blood and detection of parasites in a three-day faecal sample (by direct and floatage exams) were available two weeks after presentation at RRV and were all negative. By then, the patient was more active and alert, but started showing a stiff gait and paraparesis slightly progressed (worse on the left pelvic limb). However changes in medical treatment were postponed one week due to an episode of acute gastritis. Prednisolone dosage was increased to 1 mg/kg PO q12h to achieve an immunosuppressive effect and cyclosporine 5 mg/kg PO every 24 hours (q24h) was initiated as an adjuvant immunomodulator. Trimethoprim/sulphonamide association was discontinued. Gabapentin was maintained as well as the omeprazole 1.2 mg/kg PO q24h initiated during the gastritis episode.

Prednisolone dosage was reduced to 0.6 mg/kg PO q12h four weeks after presentation. At week five after presentation, the dog was re-observed at RRV showing signs of positive evolution in gait, general condition and in PLR in the left eye, dazzle reflex was present even tough menace response was still absent. At week six, prednisolone dosage was reduced to 0.6 mg/kg PO in the morning and 0.3 mg/kg PO after 12h.

At week nine, serum cyclosporine concentration was 60 ng/mL, which was inferior to the therapeutic range (100-300 ng/mL), therefore its dosage was increased to 6 mg/kg PO q24h. Prednisolone dosage was reduced to 0.6 mg/kg PO q24h at week 10 and to 0.3 mg/kg PO q24h at week thirteen. At week fourteen, serum cyclosporine concentration had not yet reached the therapeutic range (45 ng/mL), so its dosage was increased to 9 mg/kg PO q24h.

At week nineteen, serum cyclosporine concentration was 1166 ng/mL. The tutor of the dog reported changing the schedule of administration of cyclosporin from evening to morning. The referring hospital team recommended decreasing the dose of cyclosporin to 7 mg/kg PO q24h. The tutor further decreased the dose of cyclosporin to 5 mg/kg PO q24h two days after the referring hospitals recommendation.

At week twenty, the dog was re-observed at RRV, clinical state was stable, and the patient showed an increase in body mass. Cyclosporin dosage was reduced to 3 mg/kg PO q24h and it was recommended to continue with prednisolone withdrawal.

A week later, the dog started showing lameness of the left thoracic limb. At week twenty-three, paraparesis progressed and the dog presented kyphotic posture, low head carriage and episodes of spasms of the PL accompanied with fine, fast tremors in the scapular and thigh

regions compatible with cervical pain. Menace response was still absent on the left eye. The left lip commissure was drooping, there was less tone in the lips on the left side and the left ear did not move in response to noise or touch, pointing altogether to a left facial paralysis. The severe worsening of the clinical signs led the clinicians to increase the cyclosporin dosage to 5.5 mg/kg PO q24h and to re-introduce prednisolone 2 mg/kg PO q12h and trimethoprim/sulphonamide association. Five days later, prednisolone was reduced to 1 mg/kg PO q12h. At the twenty sixth week, re-evaluation at RRV showed slight improvement in gait and general condition, however, absent menace response and left facial paralysis were still present.

The clinical signs progressed rapidly in the following days and, at week twenty-seven the dog was observed at the referring hospital and showed ambulatory tetraparesis, a left head tilt, an a resting horizontal jerk nystagmus directed to the right. A left peripheral vestibular system disease was suspected, however a paradoxical vestibular disease with a lesion located in the caudal cerebellar peduncle was also possible, but less likely since all the central lesions observed with MRI were located in the left side of the brain and no neurological signs of cerebellar disease were apparent. Prednisolone was increased to 2 mg/kg PO q12h, gabapentin was increased to 18 mg/kg PO q8h and azathioprine 3 mg/kg PO q24h was introduced.

At the twenty ninth week, the dog was re-evaluated at the referring hospital and gait improved as well as head tilt and nystagmus that were no longer present. Five days after the latter re-evaluation, prednisolone was decreased to 2 mg/kg PO q24h. Two days after prednisolone decrease the patient started showing a depressed mental state, generalized weakness and the tutor opted for euthanasia four days later.

3 DISCUSSION

Post-contrast pachymeningeal enhancement is an imaging feature appreciated on a contrast-enhanced MRI. In human medicine PmE and thickening can occur with physiologic processes such as intracranial hypotension or it can be caused by infectious agents, inflammatory or auto-immune processes, neoplastic diseases and several other possible causes (Smirniotopoulos et al., 2007).

In small animal veterinary medicine, PmE has been observed in association with inflammatory process such as otitis interna, cryptococcal meningitis, bacterial meningitis, feline infectious peritonitis (Mellema et al., 2002), and neoplastic processes including histiocytic sarcoma, B-cell and T-cell lymphoma (Mellema et al., 2002), metastatic acute B-cell lymphoblastic leukaemia (Vernau et al., 2000), lymphocytic leukaemia associated with FeLV (Morozumi et al., 1993) and with pachymeningitis of unknown etiology (Roynard et al., 2012). It has also been found to occur in dogs with normal brains (Penning et al., 2008).

Even though meningeal contrast enhancement is recognized with acceptable repeatability in dogs (D'Anjou et al., 2012), PmE has been described and characterized in only a few veterinary reports, most on a limited number of patients (Cherubini, 2007; Mellema et al., 2002; Morozumi et al., 1993; Roynard et al., 2012; Vernau et al., 2000), resulting in a poorly understood imagiological feature in veterinary medicine. Similarly to human medicine, a better understanding of PmE in veterinary medicine may aid veterinarians to interpret images, direct further investigations based on differential diagnosis and formulate targeted management plans in neoplastic and nonneoplastic disorders affecting the meninges (Antony et al., 2015; D'Anjou et al., 2012).

Intracranial meningeal enhancement and thickening, that did not extend to the depths of the *sulci*, on multiple contiguous transverse images was the common feature between a 13 year-old spayed female Labrador retriever with suspected infectious meningoencephalomyelitis due to *T. gondii* and an 11 year-old spayed female crossbreed dog with presumed eosinophilic meningoencephalomyelitis. This imaging finding is characteristic of pachymeningeal enhancement and distinguishes it from leptomeningeal enhancement (Smirniotopoulos et al., 2007). Common sites for PmE in both dogs included the *falx cerebri* and *tentorium cerebelli membranaceum.* The *falx cerebri* was involved in four cases and the *tentorium cerebelli membranaceum* in three cases in the study of Roynard et al. (2012). PmE in the *falx cerebri* was also reported in a dog with B-cell lymphoma with leukemia (Mellema et al., 2002) and in a lymphocytic leukemic cat (Morozumi et al., 1993). Additionally, PmE was also observed bilateral and symmetrically at the dorso-lateral part of the frontal, parietal and temporal cortexes of dog 1 and at the ventral surface of the left temporal cortex and the left piriform lobe in dog 2. The regional distribution of PmE in dog 1 is in conformity with the study of Roynard et al. (2012) that concluded that the most common site for meningeal enhancement was the dorso-lateral part of each cerebral hemisphere, being present in all six dogs studied in either

the parietal or frontal cortex. The authors reported generalised PmE in two dogs and regional PmE affecting the right parietal lobe in one dog, the right parietal and temporal lobes in another dog and the left frontal lobe in another dog. PmE in the left parietal region was reported in a dog with subdural fluid accumulation (hygroma) in the study of Mellema et al. (2002). The distribution in the temporal region observed by Roynard et al. (2012) is similar to the one observed in dog 2. The additional presence of PmE in the piriform lobe has simillarities to what has been reported by Mellema et al. (2002) who observed the olphatory lobe as the primary affected site unilaterally by PmE in a dog with B-cell lymphoma and bilaterally in a dog with T-cell lymphoma. The same authors reported PmE in the cerebellum of a different dog with B-cell lymphoma, in a dog with histiocytic sarcoma and in a cat with otitis interna with presumptive extension. The meningeal and other significant lesions appeared to always involve only the cerebral cortex, although specific mention of the cerebellum and spinal cord in the other cases was not necessarily made or spinal cord was not always examined.

PmE enhancement in the brainstem is reported in five dogs and two cats presenting cranial nerve deficits and/or central vestibular signs in the study of Mellema et al. (2002). This was not observed in dog 2 even though his clinical progression was characterized by multiple cranial nerve deficits.

These intracranial lesions were isointense to grey matter in T1-W, T2-W and FLAIR in both cases at presentation. These findings differed from the iso to hypointense signal intensity in T1-W and hyperintense signal intensity in T2-W that did not supress in FLAIR observed in all dogs in the study of Roynard et al. (2012). However, Morozumi et al. (1993) also did not find any abnormality in both T1-W and T2-W images in a cat with diffuse enhancements of the margin of cerebral cortex and *falx cerebri* associated with lymphocytic leukemia. The two layers of contrast enhancement surrounding a hypointense centre observed by Roynard et al. (2012) in three of the six dogs studied, was not observed in our cases.

No focal, multifocal or diffuse parenchymal lesions were observed nor were any mass lesions anywhere inside the cranium, which is not compatible with the typical imaging presentation of the most commonly reported meningoencephalitides and intracranial neoplastic processes reported in dogs (Dewey & Da Costa, 2016; Granger et al., 2010; Kraft et al., 1997; Mai, 2018). Adjacent structure changes were also not observed on MRI, making the observed lesions less likely to be associated with an intracranial direct extension of an infectious (e.g. otitis interna/media or traumatic wounds) or neoplastic process (e.g. nasopharyngeal carcinoma, osteosarcoma, chondrosarcoma, and osteochondrosarcoma).

Isolated intracranial PmE is not usually detected in other meningoencephalitides in small animals, including canine toxoplasmosis (Falzone, Baroni, De Lorenzi, & Mandara, 2008; Lamb, Croson, Cappello, & Cherubini, 2005; Pfohl & Dewey, 2005; Terzo et al., 2012) or EME (Cowan, Bibevski, & Axlund, 2017; Henke, Vandevelde, Gorgas, Lang, & Oevermann, 2009; Salvadori, Baroni, Arispici, & Cantile, 2007; Windsor, Sturges, Vernau, & Vernau, 2009) and

T1-W, T2-W and FLAIR isointensity is not typical in canine IHP according to Roynard et al. (2012). Interestingly, our MRI findings are similar to human idiopathic hypertrophic cranial pachymeningitis where dural thickening is better appreciated on coronal (equivalent to transverse images in quadruped animals) and sagittal images in the interhemispheric fissure, *tentorium* and basal dura and the thickened dura appears isointense to hypointense on both T1 and T2-W images, with uniform dense enhancement on contrast study (Kupersmith et al., 2004).

The area of pachymeningeal involvement partially correlated with the clinical picture in dog 1, but not in dog 2.

The neurological syndrome in dog 1 was assessed clinically to be a multifocal (forebrain and T3-L3 spinal cord region) or diffuse lesion. Dog 1 tested positive for *T. gondii* (PCR) at presentation which, according to Schatzberg et al. (2003), is considered diagnostic for active infection. Both antigen (DNA) and antibody (immunofluorescent antibody assay for IgG) of *T. gondii* were not detected in CSF five weeks after starting clindamycin treatment suggesting that treatment against *T. gondii* succeeded which strengthens the probable diagnosis of protozoal meningitis. In this case, PmE was bilaterally involving the convexities of the frontal and parietal cortexes, as well as the right ventral occipital cortex (at the *tentorium cerbelli membranaceum*) at presentation. No signs of parenchymal abnormalities were observed, however the visual stimulus sensitive reflex myoclonus, together with the abnormal dural imaging signs, and positive response to anti-epileptic drug support the possibility of a symptomatic origin for the myoclonus due to cortical involvement - whether associated to a primary pathological process at the prosencephalic meninges (affecting concomitantly the cortical tissue underneath) or to a primary cortical process (affecting concomitantly the adjacent meninges). The cortex is also the most commonly proven source of the symptomatic myoclonic jerks in humans. In humans, cortical myoclonus is more often action – or stimulus sensitive, mostly to distal touch or stretch, and occasionally to visual stimuli. Brainstem myoclonus is more commonly provoked by auditory stimuli, or tactile stimuli around the face (Caviness & Brown, 2004). The acute onset of the episodes also points to the hypothesis of symptomatic myoclonus, since in humans, the acute or subacute onset of myoclonus should trigger the suspicion of symptomatic myoclonus due to infectious, post-infectious, paraneoplastic, demyelinating, and other autoimmune disorders (Caviness & Brown, 2004). Given the high probability of myoclonic episodes being symptomatic myoclonic seizures together with the progressive paraparesis makes all clinical signs compatible with toxoplasmosis in this case. However, as previously mentioned, imaging findings in this dog were not in agreement with the typical imaging features found in dogs and cats with toxoplasmosis. Diffuse meningoencephalitis and, less-commonly, focal mass-like granulomas secondary to toxoplasmosis have been reported in dogs and cats (Graham, Newell, Voges, Roberts, & Harrison, 1998; Greene, 2012; Marc Vandevelde et al., 2012). MRI features with

toxoplasmosis-associated diffuse meningoencephalitis include multifocal T2-W hyperintense, T1-W isointense, variably contrast-enhancing, and poorly marginated lesions of the cerebral hemispheres and brainstem (Lamb et al., 2005; Tarlow, Rudloff, Lichtenberger, & Kirby, 2005). MRI features with toxoplasmosis-associated granulomas are reported in two cats. Lesions are T2-W hyperintense, hypointense on T1-W images with strong and homogeneous contrast enhancement (Falzone et al., 2008; Pfohl & Dewey, 2005).

Toxoplasmosis with involvement of the spinal cord in canine and feline patients is very rare, and includes segmental myelopathy extending over several vertebral bodies, characterized by lateralized malacia of areas of white and gray matter with prominent perivascular cuffs and inflammatory infiltrates of the parenchyma, and focal severe mononuclear meningitis. Protozoal cysts can be seen within the parenchymal lesions. MRI features are non-specific and rarely reported in the literature and include focal or multifocal parenchymal lesions (Mai, 2018). Gerhold et al. (2014) described spinal cord swelling over T4-T6 and L2-L4 with assymetric parenchymal T2-W hyperintensity and contrast enhancement predominantly intramedullary in a dog with dual infection with *Sarcocystis neurona* and *T. gondii* in the spinal cord presenting with acute non-ambulatory UMN paraparesis with cranial lumbar pain. Changes in a cat were described in one case report and included a focal parenchymal lesion with spinal cord swelling over T6-T9. The lesion was hyperintense on T2-W and FLAIR images, hypointense on T1-W images, with strong diffuse contrast enhancement (Alves, Gorgas, Vandevelde, Gandini, & Henke, 2011). Similarly, in human patients, reports on isolated spinal toxoplasmosis, are also rare, and focused on cases of toxoplasmosis involving the spinal cord parenchyma. Nevertheless, two possible presentations can occur in spinal toxoplasmosis in humans toxoplasmic myelitis and toxoplasmic arachnoiditis. These constrict and compress the cord causing ischemia and sometimes atrophy of the spinal cord. An extremely rare presentation of an isolated spinal toxoplasmic arachnoiditis is described by Cosan et al. (2001). According to the authors, thoracic spinal MRI of a twenty-eight-year-old woman presenting with symptoms of chronic spastic paraparesis (that had begun thirteen years before admission) showed spinal cord atrophy, apparent enlargements of the subarachnoid space and small lesions in dorsal subarachnoid space at T7–T8. Clinical, immunologic, and pathologic examinations showed adhesive spinal arachnoiditis associated with osteoid formation caused by past toxoplasmic infection. These lesions, showing no contrast enhancement, were hypointense in T2-W images and isointense in T1-W images. There was no impairment of the immunologic defense system in the human patient with spinal toxoplasmic arachnoiditis associated with osteoid formation described by Cosan et al. (2001). Therefore *T. gondii* cannot be excluded as a possible cause for the lesions observed at the spinal cord level in dog 1.

No information about CBC, biochemical profile or ultrasound was available for review in this dog, which hinders a more complete interpretation of the case, especially since it does not

completely allow ruling out reactive seizures that may result from systemic metabolic disorders or from intoxications (De Risio et al., 2015).

Dog 1 CSF showed 120 cells/µL with mixed pleocytosis with predomination of lymphoid cells at presentation and 290 cells/µL with a mononuclear pleocytosis with vacuolized mononuclear cells and reactive lymphocytes and high globulin concentration. A mixed-cell pleocytosis, primarily composed of neutrophils and mononuclear cells, with increased protein levels, is generally present in the CSF of dogs and cats affected with toxoplasmosis (Greene, 2012). Nevertheless, CSF abnormalities in protozoal meningoencephalitides tend to be quite variable (Dewey & Da Costa, 2016) and the mixed pleocytosis with predomination of lymphoid cells observed in dog 1 at presentation was in agreement with the mixed pleocytosis with predominant lymphocytes reported in a golden retriever with probable diagnosis of toxoplasmosis (positive IgG titter and definite response by clinical improvement to drugs) (Tarlow et al., 2005), in a basset-beagle mix with definite diagnosis of encephalomyelitis associated with dual infection of *S. neurona* and *T. gondii* (Gerhold et al., 2014) and in a cat with definite diagnosis of segmental meningomyelitis associated with *T. gondii* (Alves et al., 2011). Reported CSF cytologic results in canine and/or feline toxoplasmosis include eosinophilic (Tarlow et al., 2005), neutrophilic (Alves et al., 2011), lymphocytic (Alves et al., 2011; Gerhold et al., 2014) and mixed (Greene, 2012)pleocytosis but, to our knowledge, not mononuclear pleocytosis with vacuolized mononuclear cells and reactive lymphocytes as observed six and a half weeks after presentation in dog 1. Unfortunately, protein content results were not available at presentation. However, Pandy reaction test revealed an increased globulin content six and a half weeks later which is likely to correspond to immunoglobulins of intrathecal origin since reactive lymphocytes were observed in CSF, however analysis of the ratio of CSF-serum immunoglobulin content would be necessary to confirm it (Di Terlizzi & Platt, 2009).

Myoclonic seizures cessed immediately after starting antiepileptic and corticosteroid therapy and did not reoccur along with therapy modifications including corticosteroid tapering, which supports the possibility of responding to phenobarbital. The remaining clinical signs in dog 1 also showed improvement one week after starting clindamycin therapy but started to worsen twenty-three days after. Available drugs usually suppress replication of *T. gondii* and are not completely effective in killing the parasite. Clindamycin hydrochloride or a trimethoprim sulfonamide combination has been used most frequently in dogs (Dubey, Lindsay, & Lappin, 2009). Clindamycin is the drug of choice for treating clinical toxoplasmosis in dogs and cats. Treatment with trimethoprim sulfonamide combination is preferred by some authors when neurologic signs are present, but development of adverse effects may require its discontinuation and resuming therapy with clindamycin (Tarlow et al., 2005). However, clindamycin has also shown to be effective in crossing the blood-brain and blood-vascular barriers in animals and humans with toxoplasmosis (Greene, 2012). Neurologic deficits

improve, but signs may not totally resolve because of permanent damage caused by CNS inflammation (Greene, 2012). Evidence of CNS inflammation was not observed by MRI that could explain the clinical signs of dog 1 and not only did the imaging characteristics show no signs of improvement, but additional lesions were observed after treatment. Clinical signs did improve after initiation of clindamycin treatment but worsened again twenty-three days later. During treatment, the glucocorticosteroid dose was being tapered until it was below the anti-inflammatory dose when clinical signs began to worsen, therefore, precise conclusions about clinical response to treatment cannot be drawn especially without Ig G and Ig M dynamics. PCR and IgG titters for *T. gondii* in CSF were negative five weeks after starting clindamycin. The pattern of canine *T. gondii* antibody production and persistence is still being studied. All dogs experimentally inoculated with a highly virulent strain of *T. gondii* in the study of Silva et al. (2002) had IgG seroconversion by immunofluorescence antibody test and enzyme-linked immunosorbent assay starting on the seventh day after infection, achieving the highest level around the 20th–40th days, with antibody titters ranging from 1:64 to 1:8192, and all dogs exhibited detectable IgG levels throughout the experiment (62 days). However, in the study of Lindsay, Dubey, Butler, and Blagburn (1996) in two dogs experimentally inoculated with tissue cysts of an avirulent strain of *T. gondii,* both dogs developed low antibody titters and became completely seronegative by the end of the study (112 days). The reason why Ig G titters were below 1:40 six and a half weeks after presentation is unclear. It is possible that a strong antibody response to infection with T. gondii may not have been mounted. Ig G and Ig M simultaneous testing was not performed at any moment in this patient, therefore, an adequate interpretation of the antibody dynamics is not possible.

The neurological syndrome in dog 2 was assessed clinically to be a multifocal (forebrain and T3-L3 spinal cord region) or diffuse lesion. CSF of dog 2 showed an eosinophilic pleocytosis indicative of EME. EME is an inflammatory condition of the brain and surrounding meninges characterised by an eosinophilic pleocytosis. Eosinophils are rarely identified in the CSF of dogs and other species and is classically associated with migrating helminth infection. Eosinophylic pleocytosis in dogs has been described in infectious diseases caused by *C. neoformans*, *T. gondii*, *N. caninum*, *Baylisascaris procyonis*, *Angiostrongylus cantonensis*, distemper virus, rabies, protothecal meningoencephalitis and bacterial infections, non-infectious diseases such as GME, infarction of the caudate nucleus, cerebrocortical infarction, intervertebral disc herniation, neoplastic diseases such as cerebral lymphosarcoma and tumours of the spinal and epidural tissues but eosinophilic meningoencephalomyelitis of undetermined etiology (idiopathic EME) is more commonly reported (Cowan et al., 2017; Henke et al., 2009; Salvadori et al., 2007; Smith-Maxie et al., 1989; M. Vandevelde & Spano, 1977; Williams et al., 2008; Windsor et al., 2009). Dog 2 received extensive diagnostic testing to exclude helminthic, protozoan, viral and mycotic infections. Historically, dog 2 was treated for a fracture of the right upper premolar tooth 10 days before presentation and to a quick oral

health assessment and cleaning treatment 226 days before presentation. Ophthalmic manifestations can occur from dental disease because of the close proximity of the caudal maxillary teeth and the orbit (Greene, 2012), however in dog 2 the fractured tooth was contralateral to the imaging abnormalities observed and to the eye with the absent menace response, and no abnormalities were observed in these structures, therefore is not likely to be part of the pathogenic process at least not as a local spread of the infection. Nevertheless, systemic complications of periodontal disease, including cerebral and myocardial infarction, systemic hypertension, and early mortality, have been linked in people and similar relationships of periodontal inflammation to heart, liver, and kidney disease have been made in dogs, even though controversy has been addressed in veterinary publications as to the role of oral cavity infections and systemic disease. Even so, bacteraemia associated with dental manipulations and fractures may be clinically asymptomatic, cause acute septicaemia, or subsequently result in bacterial endocarditis or localized embolic tissue infections (Greene, 2012), therefore the possibility of an infectious EME cannot be discarded but is unlikely since there was no history of abnormal body temperature, heart or respiratory rates or leucocyte count in order to suspect of a previous systemic inflammatory response (Greene, 2012). No bacterial agents where observed in the CSF cytology and even if aerobic and anaerobic bacterial cultures of CSF were performed to confirm these results it may have not been more helpful since positive bacterial culture results in confirmed cases of bacterial meningitis are extremely uncommon (Radaelli & Platt, 2002; Tipold, 1995).

Abdominal ultrasound, CBC and biochemical profile ultrasound were also performed on presentation to exclude extracranial causes and no consistent abnormalities were found. Blood glucose was slightly increased, however mild hyperglycaemia (<180 mg/dL) can occur in some dogs up to 2 hours after consumption of diets containing increased quantities of monosaccharides and disaccharides as well as in stressed, agitated, or excitable dogs (Nelson & Couto, 2014). Anxiety related to the clinical setting may also falsely increase blood pressure in some animals. Therefore no conclusions could be drawn out of the hyperglycaemia and high blood pressure observed in dog 2 without repeated measures of glucose after fasting for a period longer than 2 hours and without repeated measurements of blood pressure in a less stressed state, especially because this patient showed behavioural signs of anxiety/fear including several attempts to escape to the veterinary staff during the consultation, avoidance, lip licking, etc. (Horwitz & Mills, 2009) and no information about diet and timing of blood collection was available.

Because the etiology of the EME remained undetermined besides all ancillary tests performed, and that an intervertebral disk herniation in T3-L3 segment was possible but would not explain the neurological signs associated with intracranial disease in this case, an idiopathic EME was presumed. Idiopathic EME has been described in 32 dogs however MRI features were only described in 4 case reports and briefly in one retrospective study that includes 16 dogs with

idiopathic EME (Bennett, Allan, Guilford, Julian, & Johnston, 1997; Cowan et al., 2017; Henke et al., 2009; Olivier, Parkes, Flaherty, Kline, & Haynes, 2010; Salvadori et al., 2007; Smith-Maxie et al., 1989; Williams et al., 2008; Windsor et al., 2009). A type I hypersensitivity reaction may be suspected when no aetiological agents are detected and when partial or complete recovery is achieved in EME treated with corticosteroids. It is currently hypothesised that the disease pathogenesis may be linked to neurotoxic substances released by the eosinophils (Bennett et al., 1997; Schultze, Cribb, & Tvedten, 1986; Smith-Maxie et al., 1989). Peripheral eosinophilia was not present in this dog, thus a systemic allergic reaction is not likely to account for eosinophils in the CSF. Eosinophilia of peripheral blood did not appear to be a reliable indicator of eosinophilic meningoencephalomyelitis and the degree of peripheral eosinophilia did not correlate with the numbers of eosinophils in the CSF in the study of Smith-Maxie et al. (1989). However, an obvious clinical recovery was achieved with corticosteroids, and worsening of the clinical signs seemed to be associated with corticosteroid tapering. High immunosupressive doses of corticosteroids (2 mg/kg BID) appeared to be more effective in improving clinical signs than lower doses and the clinical picture remained stable with anti-inflammatory doses of corticosteroids combined with cyclosporin despite the fact that cyclosporine blood levels suffered strong variations and results failed to prove that they were within the therapeutic range at any time during treatment. These results, combined with the eosinophilic pleocytosis strongly support an immunomodulated component of the disease in this dog but the lack of peripheral eosinophilia could indicate a specific immune response by eosinophils in the cortical pachymeninges and cervical spinal meninges.

Idiopathic eosinophilic meningoencephalitis has been described in a number of dog breeds, most commonly in young to middle-aged (range 3 month – 13 years), large breed dogs (Cowan et al., 2017; Henke et al., 2009; Salvadori et al., 2007; Windsor et al., 2009) and the clinical outcome is more favourable than in dogs with parasitic, protozoal, or mycotic causes of CSF eosinophilia (Henke et al., 2009; Windsor et al., 2009). MRI appearance is quite variable and may include unremarkable MRI (Windsor et al., 2009), widening of the cerebral *sulci* indicating cortical atrophy (Salvadori et al., 2007; Windsor et al., 2009), intra-axial cerebral solitary or multifocal mass (Windsor et al., 2009) or ill-defined lesions (Cowan et al., 2017; Henke et al., 2009; Salvadori et al., 2007; Windsor et al., 2009), T2-W hyperintensity (Cowan et al., 2017; Henke et al., 2009; Windsor et al., 2009) (most commonly) or isointensity (Henke et al., 2009), T1-W hypointensity (Henke et al., 2009; Salvadori et al., 2007) or isointensity (Henke et al., 2009), FLAIR hypo- (Salvadori et al., 2007), iso- (Henke et al., 2009) or hyperintensity (Cowan et al., 2017; Henke et al., 2009) and patchy or diffuse contrast enhancement of the cerebral cortex or meninges (Henke et al., 2009; Windsor et al., 2009). All studies report intra-axial abnormalities and no study mentions the pattern of meningeal enhancement. The most similar reported MRI result in a case of canine idiopathic EME was diffuse post-contrast T1-W meningeal enhancement throughout the brain, with a focal T2-W hyperintensity centrally within

the spinal cord at the level of C3 but no intra-axial brain abnormality reported in a dog in the study of Windsor et al. (2009), but no further comparisons can be made since no more information on the case is provided by the authors.

Clinical signs of dog 2 at presentation have some similarities to the ones reported in idiopathic EME including pelvic limb weakness/ataxia, proprioceptive deficits in the PL, unilateral absent menace response, PLR deficits (Bennett et al., 1997; Henke et al., 2009; Olivier et al., 2010; Smith-Maxie et al., 1989; Williams et al., 2008). Additional frequently reported abnormal clinical findings in idiopathic EME include hyperthermia, depression, behavioural changes (loss of trained habits), seizures, circling, neck pain or hyperesthesia, loss of gag reflex, bilateral menace response deficits, decreased spinal reflexes (Bennett et al., 1997; Cowan et al., 2017; Henke et al., 2009; Olivier et al., 2010; Smith-Maxie et al., 1989; Williams et al., 2008; Windsor et al., 2009).

MRI findings did not correlate with the absence of the left menace response in dog 2 at presentation as no abnormalities were observed in afferent visual pathway of the left eye nor in the right occipital cortex. At presentation, no imaging abnormalities that could explain the cranial nerve deficits that occurred 21 weeks after, were observed. Vestibular signs and facial paralysis have also been reported in studies of idiopathic EME (Bennett et al., 1997; Cowan et al., 2017; Henke et al., 2009; Smith-Maxie et al., 1989). The motor and intermediate nerve fibres of the facial nerve and the ipsilateral vestibulocochlear nerve are surrounded together by a common sheath of dura mater in the short distance from the brainstem, at the level of the trapezoid body, through the internal acoustic meatus located in the medial surface of the petrosal part of the temporal bone (de Lahunta et al., 2015). The left facial paralysis and left peripheral vestibular deficits, without additional signs of otitis media/interna observed in dog 2, may have been consistent with lesions in the left facial and left vestibular nerves or to a compressive or infiltrative lesion in their shared dura mater.

A cranial-caudal progression of the lesion observed at presentation culminating in a left basolateral distribution on the skull floor affecting the left optic, facial and vestibulocochlear nerves could be hypothesised since it would explain all the cranial neurological deficits 21 weeks after diagnosis, but this could not be verified since MRI was only performed at presentation and histopathology was not performed.

The positive clinical response to corticosteroids in this dog is also reported in idiopathic EME. In the study of Bennett et al. (1997), one dog rapidly recovered with prednisolone (1.5 mg/kg PO q12h), showing a normal CBC and CSF after 8 weeks and discontinuing glucocorticoids after 16 weeks. Another dog in the same study showed a slow recovery in response to glucocorticoids (dexametasone 0.25 mg/kg IV q24h, substituted for prednisolone which was gradually reduced to 0.1 mg/kg every 48 h) but 4 months after treatment he had almost fully recovered. A relapse was observed in the latter dog but the signs (seizures and circling) responded to increased doses of prednisone (1.0 mg/kg daily) and phenobarbitone (4 mg/kg

every 12 h). No further follow up information of these two dogs is available. Two dogs with positive responses to dexametasone (0.25 mg/kg PO q8h, gradually decreased) were reported by Smith-Maxie et al. (1989) (one of them had a 10 month follow up). In the study of Windsor et al. (2009), clinical signs resolved in 12 of 16 (75%) dogs with idiopathic EME. Two dogs recovered without treatment and no further CSF analyses were performed. In 8 dogs with idiopathic EME, clinical signs resolved with prednisone treatment ranging from 0.33 to 1 mg/kg PO q12h (median 0.51 mg/kg, mean 0.54 mg/kg). All dogs were eventually tapered off prednisone without recurrence of signs after a median of 105 days (mean 109 days, range 60–165 days). Recheck CSF analysis was permitted in 5 dogs with idiopathic EME and all showed complete resolution of the eosinophilia at the first recheck (range 2 weeks to 3 months). One dog treated with prednisone (0.54 mg/kg PO q12h) died at home 2 days after diagnosis. Another dog improved with prednisone (0.67 mg/kg PO q12h) and clindamycin (5 mg/kg PO q8h) but began seizuring 6 weeks after diagnosis and was euthanized 3 months after initiating treatment. The last dog improved with prednisone (0.9 mg/kg PO q12h) for 3–4 months but was euthanized when clinical signs recurred after prednisone was discontinued (5.5 months after diagnosis).

Lack of response to glucocorticoids in idiopathic EME has also been reported. Four dogs with idiopathic EME died or were euthanized because of poor response to corticosteroid therapy in the study of Windsor et al. (2009). One dog was euthanized in the study of Bennett et al. (1997) due to no improvements after febendazol and six weeks of treatment with prednisolone (1.5 mg/kg PO q12h). CSF improved but seizures worsen in a dog treated with phenobarbital and dexamethasone in the study of Smith-Maxie et al. (1989). Chloramphenicol was effective in one dog, partially effective in another dog and ineffective in combination with glucorticoids after antibiotic therapy was attempted in one dog in the study of Smith-Maxie et al. (1989).

The glucocorticoid doses necessary for clinical improvement in dog 2 were higher and tapering was not as successful as in the study of Windsor et al. (2009). Relapse of clinical signs in dog 2 occurred 5.5 months after diagnosis which is in agreement with one of the cases in the study of Windsor, but differs from the case of Smith-Maxie et al. (1989), where no relapses were described in a 10 month follow-up and the case reported by Cowan et al. (2017) where acute recurrence of neurological dysfunction was observed three months after diagnosis and a second MRI showed recurrence of active meningoencephalitis (diffuse hyperintensity on T2W and FLAIR sequences throughout right cerebral cortex) and a cerebral biopsy showed eosinophils within and around the cerebral vasculature. In the same study, at the nine-month follow-up, the patient's clinical status was overall improved with some residual neurological deficits but an MRI scan revealed evidence of cerebral atrophy (severe right ventriculomegaly) and the CSF analysis was unremarkable, supporting the hypothesis that MRI changes (ventriculomegaly secondary to cerebral atrophy) at that time were not due to active inflammation. The latter study demonstrated that complete resolution of the EME was not

observed on MRI despite clinical improvement. Lowrie, Smith, and Garosi (2013) also identified persistent abnormalities on MRI in MUE cases despite clinical resolution. The same authors also observed that an abnormal CSF analysis at three-month re-examination was found to be associated with an increased risk of relapse.

Serial MRI and CSF analysis might have been useful for better understanding the clinical outcome in our case, even though MRI findings showed no evidence of active inflammation. Nevertheless, persistent neurological deficits can be seen without active inflammation likely secondary to cerebral atrophy, creation of seizure foci or other architectural changes initially caused by inflammatory disease as was observed in the case studied by Cowan et al. (2017) where histopathology five years after diagnosis of idiopathic EME confirmed resolution of the inflammatory lesions at the necropsy. According to this study, cerebral atrophy appears to be a long-term consequence of idiopathic EME and can lead to persistent neurological dysfunction, despite eradication of active inflammation. The authors suggested that prompt treatment may limit the extent of atrophy or rate at which it progresses but, even thought permanent neurologic damage may have happened in dog 2, it was not possible to provide evidence in our study to confirm it.

The extra-axial spinal lesion observed in dog 2 has not been previously reported in EME studies where spinal lesions are described as intra-axial or associated with intervertebral disk disease (Henke et al., 2009; Windsor et al., 2009).

Other possible etiologic agents for extra-axial spinal cord lesions observed in both dogs include neoplastic meningioma, disseminated histiocytic sarcoma, lymphoma, granular cell tumours as the neoplastic processes can present as single or multifocal extra-axial spinal cord masses, with variable MRI characteristics. These lesions could be related or not to the intracranial lesions. Intraspinal peripheral nerve sheath tumours are reported to affect the nerve roots of caudal cervical and cranial thoracic regions (Mai, 2018), thus it is also an etiologic plausible explanation for the extra-axial spinal lesions observed, especially in dog 1 due to its localisation in the caudal cervical spinal cord region.

Both dogs showed a positive response to treatment but relapse was recorded 34 and 153 days after presentation at RRV likely due to corticosteroid tapering and corticosteroid/cyclosporine tapering in dog 1 and dog 2 respectively. Paraparesis progressed to non-ambulatory tetraparesis within 46 days in dog 1 and within 190 days in dog 2. Tetraparesis was apparently determinant for the election of euthanasia in both cases since the interval between these was relatively short (6 and 21 days in dog 1 and 2 respectively).

The main limitations of this study include its retrospective design, single-centre site, small sample size, lack of histopathologic analysis, lack of serial MRI scans and CSF analysis in one of the cases. The scarce literature on veterinary PmE and the lack of layer discrimination on affected meninges in the studies mentioning meningeal lesions also limited the interpretation of the results of this study.

4 CONCLUSION

PmE can be noted in contrast enhanced MRI in dogs with normal brains, as well as, in association with processes of infectious or non-infectious inflammatory and neoplastic aetiologies.

Even though a definitive diagnosis was lacking in our study, toxoplasmosis should be tested and eosinophilic meningoencephalitis should be included in the differential diagnoses when considering regional or diffuse intracranial PmE in dogs without the presence of any other intra-axial abnormalities. Concomitant extra-axial spinal abnormalities may exist and should be investigated in case spinal lesion is clinically suspected.

Histopathological analysis should be performed to obtain a definitive diagnosis and serial MRI scans and CSF should be performed to monitor treatment and determine prognosis since improvement in clinical signs does not always reflect improvements in neuroimaging and in CSF.

Knowledge of normal and abnormal pachymeningeal enhancement and thickening can assist imagiology and neurology veterinary specialists to better interpret images and direct further management in the pathologic cases. Further studies are necessary.

PART IV: REFERENCES

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ANNEXES

Annex 1. Summary of clinical and histologic characteristics of granulomatous meningoencephalomyelitis and necrotizing encephalitis (Adapted from Charalambous, Danourdis, Hatzis, and Polizopoulou (2013), Coates and Jeffery (2014) and Dewey and Da Costa (2016))

	GME	NME	NLE
Clinical presentation	Any breed of dog but most often occurs in young to middle-aged (mean age 5 years) small breed dogs, more commonly terrier and toy breeds and in Poodles.	Most often occurs in young (mean age 2 years) small breed dogs, more commonly Pug, Maltese, Chihuahua, Pekingese, French Bulldog, Shi-Tzu, and Lhasa Apso.	Most often occurs in young (mean age 4.5 years) small breed dogs, more commonly Yorkshire Terrier, French Bulldog, Maltese, Shih-Tzu.
Anatomic distribution	**Forebrain**, **brainstem**, **spinal cord**. Three GME possible distribution patterns: 1) **Multifocal** – Diffuse lesions involving cerebrum, caudal brainstem, cerebellum or the cervical spinal cord. 2) **Focal** – Represented by a true mass lesion in the cerebral hemispheres, brainstem or spinal cord. 3) **Ocular** – Lesions localized in the optic nerves and optic chiasm.	**Focal** or **multifocal forebrain lesions**. Commonly affects the cerebral hemispheres and subcortical white matter, with profound inflammation extending from the leptomeninges through the cerebral cortex into the corona radiata. NME in Pugs is usually limited to the forebrain, however in Chihuahuas and French Bulldogs additional brainstem involvement seems more common.	**Focal forebrain lesions** (asymmetric bilateral) confined to hemispheric white matter. Characteristically the overlying cortex and meninges are not involved. The midbrain and brainstem including the cerebellum may also be affected (usually with multifocal lesions).
Clinical signs	Acute-onset, progressive multifocal disease. Subacute or chronic onsets are also possible. Forebrain signs or forebrain associated with brainstem signs. Central vestibular syndrome and cervical pain are commonly present. Visual impairment and abnormal pupillary reflexes in the ocular form.	Acute-onset, rapidly progressive, multifocal disease. Forebrain signs or forebrain associated with brainstem signs. Cervical pain is commonly present.	Acute-onset, rapidly progressive, focal or multifocal disease. Forebrain signs or forebrain associated with brainstem signs. Cervical pain is commonly present.

Annex 8 (continued). Summary of clinical and histologic characteristics of granulomatous meningoencephalomyelitis and necrotizing encephalitis

CSF	Mild to pronounced mononuclear pleocytosis, mainly lymphocytes (60-90%) and monocytes (10-20%). In rare occasions neutrophils can predominate. Protein concentration mildly or moderately increased.	Mild to pronounced mononuclear pleocytosis, (usually lymphocytic). Protein concentration mildly or moderately increased.	Mild to moderate mononuclear pleocytosis, (usually lymphocytic). Protein concentration mildly or moderately increased.
Neuroimaging	MRI: Lesions are typically hyperintense on T2-W and FLAIR images, iso- to hypointense on T1-W images and variably contrast enhancing. Meningeal enhancement may or may not be observed. In ocular GME, MRI may show enlargement of the optic chiasm and fairly symmetric contrast enhancement of the optic nerves, optic chiasm, and visual pathways.	MRI: Diffuse asymmetric cerebral lesions which are nonuniformly hyperintense on T2-W images, isointense to hypointense on T1-W images, and affect gray and white matter resulting in loss of gray/white matter distinction. Additional findings include variable degrees of contrast enhancement of brain and leptomeninges, enlarged and asymmetric lateral ventricles, mass effect, brain herniation, and T2/FLAIR hyperintensity associated with the hippocampus and piriform lobes.	MRI: Asymmetric cerebral white matter and brainstem lesions. Lesions appear iso- to hypointense on T1-W and hyperintense on T2-W and FLAIR sequences; minimal contrast enhancement of parenchymal lesions; lack of meningeal enhancement and mass effect; varying ventriculomegaly possible. In almost all cases of NLE reported in Yorkshire Terriers, lesions are located in the cerebrum and brainstem and show variable contrast enhancement.
Histopathological findings	Whorling perivascular mononuclear cell infiltrates; white matter, meninges, spinal cord; acute lesions in gray and white matter; chronic lesions in white matter.	Asymmetric extensive necrosis and cavitation; mononuclear infiltrates involve cerebral cortex, corona radiata, subcortical white matter; prominent reactive astrogliosis effacing areas of cavitation; inflammation can occur in brainstem and cerebellum; extensive leptomeningeal inflammation	Asymmetric extensive necrosis and cavitation; mononuclear infiltrate and prominent reactive astrogliosis effacing areas of cavitation; predominantly white matter; meninges minimally affected.

Annex 8 (continued). Summary of clinical and histologic characteristics of granulomatous meningoencephalomyelitis and necrotizing encephalitis

Prognosis	Poor without aggressive immunosuppression. Most dogs die or are euthanised within a few weeks to months after diagnosis, despite treatment.	Poor even with aggressive anti-inflammatory, immunosuppressive and/or chemotherapy. Most dogs die or are euthanized within 6 months of the start of their clinical signs.	Poor even with aggressive anti-inflammatory, immunosuppressive and/or chemotherapy. Most dogs die or are euthanized within 6 months of the start of their clinical signs.

Legend: GME. Granulomatous meningoencephalomyelitis; NME. Necrotizing meningoencephalitis; NLE. Necrotizing leukoencephalitis; MRI. Magnetic resonance imaging; T1-W. T1 – weighted magnetic resonance images; T2-W. T2 – weighted magnetic resonance images; FLAIR. Fluid-attenuated inversion recovery magnetic resonance images.

Annex 2. Summary of clinical and histologic characteristics of idiopathic eosinophilic meningoencephalitis and steroid-responsive meningo-arteritis (Adapted from de Lahunta et al. (2015), Tipold and Schatzberg (2010) and Williams et al. (2008))

	EME	SRMA
Clinical presentation	Any age from 4 months to 10 years but most young to middle-aged dogs. Large dogs appear to be predisposed, with Golden Retrievers and Rottweilers being overrepresented.	Usually younger than 2 years. Any breed but Beagles, Boxers, Bernese mountain dogs, Weimaraners and Nova scotia duck tolling retrievers are over-represented.
Anatomic distribution	Focal or multifocal. Forebrain, hindbrain or spinal cord.	Meninges of the spinal cord (mainly cervical) and to a lesser degree in the brain.
Clinical signs	Acute or subacute onset.	Acute onset and progressive (acute form) or waxing and waning course over a period of weeks or months (chronic form). Cervical spinal hyperaesthesia (90% of the affected dogs). Reluctance to move, stiff gait, pain on mouth opening, muscle rigidity and anorexia Neurologic deficits are uncommon but may be seen, particularly in dogs for which diagnosis and treatment are delayed. Possible concurrent polyarthritis.
Haematological findings	Eosinophilia may be evident.	Neutrophilic leucocytosis with a left shift (two thirds of the affected dogs). Mild hypoalbuminemia and elevated α2-globulin. Elevated IgA levels.
CSF	Eosinophilic pleocytosis.	Elevated IgA levels. Acute form: marked neutrophilic pleocytosis; elevated total protein; variable red blood cells and erythrophagocytosis. Chronic form: variable. Predominantly mononuclear cells or mixed cell population; normal or mildly elevated total protein.
Neuroimaging	MRI: May be normal or may be characterized by focal or multifocal lesions. The lesions consist of patchy regions of T2-W hyperintensity and contrast enhancement in the cerebral cortex, solitary or multiple masses, meningeal enhancement, or enlargement and contrast enhancement of cranial nerves.	MRI: Meningeal enhancement in T1-W post contrast and enlarged vessels in severe cases. In a boxer with cerebral extension of SRMA, MRI findings included symmetrical multifocal to diffuse T2-W/FLAIR hyperintense changes of the cerebral gray matter and ependymal lining, increased periventricular signal and a large amount of intraventricular sediment.

Annex 7 (continued). Summary of clinical and histologic characteristics of idiopathic eosinophilic meningoencephalitis and steroid-responsive meningo-arteritis

Histopathological findings	Due to the limited number of dogs with EME histopathologic results reported, there is no defined histopathologic pattern upon which to base a definitive diagnosis. Histopathologic findings have included diffuse cerebrocortical subarachnoidal meningitis and sub meningeal encephalitis, the exudate containing variable numbers of eosinophils together with neutrophils and mononuclear cells as well as parenchymal granulomas, necrosis, malacia, axonal loss, and demyelination.	Necrotizing arteritis of medium- and small-sized arteries and associated purulent leptomeningitis. In chronic lesions, proliferative changes of the vascular intima with stenosis and adventitial fibrosis occur together with Meningeal fibrosis. In rare cases vessels may be thrombosed and secondary spinal cord infarction can occur as well as nerve root degeneration.
Prognosis	Variable. Better that infectious eosinophilic meningoencephalitis.	Acute form: good in treated dogs. Chronic form: guarded.

Legend: EME. Idiopathic eosinophilic meningoencephalitis; SRMA. Steroid-responsive meningo-arteritis; MRI. Magnetic resonance imaging; T1-W. T1 – weighted magnetic resonance images; T2-W. T2 – weighted magnetic resonance images; FLAIR. Fluid-attenuated inversion recovery magnetic